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EDITORIAL

RESEARCH PAPERS

CASE REPORT

COMMENTARY

Overview and Challenges of Implantable Auditory Prostheses

Until the middle of 20th century it was believed that a full understanding of the anatomy and physiology of the ear and the neural system is required for achieving successful auditory prostheses devices that can substitute the clever and subtle functions of the middle and inner ears and the associated neural structures. Surprisingly however very quickly auditory implants happened to become successful enough to be an accepted clinical treatment for restoring hearing and communication abilities to individuals with profound and severe deafness and have been used by around 200,000 people worldwide to date. Auditory implants bypass the ear and stimulate the auditory system electrically via tiny electrodes inserted in close contact with or directly inside the auditory processing structures of the brain, from where neural activity transmits to cortical areas and produces auditory perception. The first demonstrations that electric stimulation, instead of sound, can induce auditory sensations likely started more than two centuries ago with an unpleasant sound when the Italian scientist Alessandro Volta (1745–1827) placed the two ends of a 50-volt battery to his ears. Electronic hearing was brought into clinical practice in 1960's and has considerably improved in both safety and performance since the early trials that electrically activated a wire simply inserted into the ear of the deaf volunteers.

Cochlear Implants

Cochlear implants, mimicking the processing that takes place in a normal ear, transform acoustic energy to auditory nerve activity and thus bypass the malfunctioning or missing middle and inner ears in severe deafness. Figure 1 shows a typical modern cochlear implant system. The behind ear segment is a micro-computer that processes and converts into RF (Radio Frequency) the environmental sounds picked up by a microphone. The surgically placed internal part receives and decodes the RF signals and generates electrical current pulses. The electrical activity transmits to the auditory nerve fibers via contacts of a linear array of electrodes that the surgeon carefully inserts inside the cochlea through the round window in the inner ear. Auditory nerve, similar to the neural structures in the other stages of auditory processing is tonotopically organized. In other words the electrodes along the cochlea from cochlear base to apex are located in descending order of the frequency percept they produce when electrically activated. Cochlear implant processing takes advantage of this tonotopicity and encodes the energy of each narrow spectral band of the signal by the level of electrical activity on the implant electrode whose tonotopic location is estimated to be associated to the band's center frequency. Thus the electrical signal delivered to the auditory nerve is a coarse representation of the input sound's spectrogram obtained with a fixed number of frequency bins equal to the number of electrodes. However the auditory processing system of most cochlear implant users is not able to fully receive or optimally decode the information presented by the electrodes. The average speech perception performance of cochlear implant users is poorer than that of normal hearing listeners presented with a similarly spectrally degraded signal.

Auditory Brainstem Implants

The design of auditory brainstem implants is similar to that of cochlear implants except that their matrix array of electrodes is located more centrally on the surface of Cochlear Nucleus, one of the first stages of the auditory brainstem. Auditory brainstem implants are now clinically used in the conditions that cannot be alleviated via cochlear implantation, i.e. where auditory nerve is absent or damaged (via congenital non-development, tumor damage, or surgical damage resulting from tumor removal) or where the cochlea has malformations that prevent viable electrode array insertion. The current brainstem implants use the same speech processors as cochlear implants. The stimulation site of brainstem implants is complex and contains different types of neurons with differing roles in auditory processing. This is perhaps the reason why performance of the users of these devices is usually poorer than the average users of cochlear implants.

Challenges of electronic hearing

Despite not having access to much of the speech spectral and temporal details, most users of auditory implants are able to communicate well specially when using the device in conjunction with lip reading. However there is a huge individual variability in the outcome with the implant alone. To date there is no clear expla-

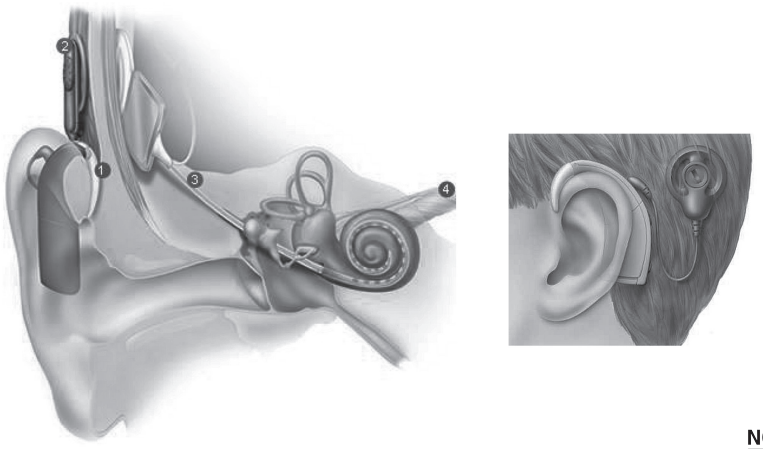

Figure 1. The external and the surgically implanted segments of a modern cochlear implant (www.cochlear.com).

nation why with the same device, some implant users have zero speech recognition in natural environments and some do perform much closer to normally hearing listeners. There is also no reliable method of predicting the outcome before prescribing the implant and performing the surgery. There are certainly inevitable differences among implant users in how the electrodes were inserted during the surgery and how the implant signal is coded by the neural system. The location of the electrode array within the stimulation site may introduce in some implantees a large mismatch between the tonotopic map of the electrodes and the center frequencies to which the electrodes are assigned in the signal processor. The spread of electrical current along the tonotopic axis, influenced by both the distance of electrodes from the neural fibers and the distribution of the functioning neurons, causes overlap in the neural fibers stimulated by different electrodes and thus may blur the spectral representations in some implant users. The ability of neural responses to follow and transmit to the higher processing stages the temporal variations of electrical stimulation might be another source of variability of implant users. Although using simple psychophysical and electrophysiological methods huge differences have been observed among implant users in their abilities to encode spectral and temporal information of the implant signal, none of these factors has been consistently known to play a considerable role in speech perception with an auditory implant.

Age and etiology of deafness play some role in the outcome with implants. In implanted adults there is a trend that those who were prelingually deafened or were not hearing for a long period before implantation are the poorer performers. However it is not known how the representation of speech information is affected by these factors. It has been speculated that the auditory system may lose some of its functioning neurons at different processing levels or be taken over by the other sensory modalities such as vision or touch if doesn't get stimulated for a long period.

Future Directions

Respecting the individual differences, development of auditory implants requires a better understanding of how the brain of each individual implant user decodes the spectral and temporal information presented by implant electrodes. In addition to peripheral processing, implant studies should consider brain plasticity and the outstanding ability of the speech processing system to adapt to the novel situations and extract from the redundant complex signal the reliable information for communication. Central auditory processes may play an important role in compensating for or interacting with the limitations of cochlear implant stimulation and the misrepresentation of information at the peripheral neural system.

Implants that stimulate more central auditory system bypass more of the neural structures and can theoretically restore hearing to a wider range of deaf people affected by neuroplogical disorders. The future development of these implants should take into account the functional anatomy of the stimulation sites to devise new processing strategies.

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Combination Therapy with A1 Receptor Agonist and Vitamin C Improved Working Memory in a Mouse Model of Global Ischemia-Reperfusion

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ABSTRACT

Introduction: Stroke is one of the most important reasons of death. Hence, trials to prevent or lessen the complications originated by stroke are a goal of public health worldwide. The ischemia-reperfusion causes hypoxia, hypoglycemia and incomplete repel of metabolic waste products and leads to accumulation of free radicals triggering neuronal death. The A1 adenosine receptoras an endogenous ligand of adenosine is known to improve cell resistance to destructive agentsby preventing apoptosis. Vitamin C as a cellular antioxidant is also known as an effective factor to reduce damages initiated by free radicals. We studied the protective effects of A1 receptor agonist in combination with vitamin C against ischemia-reperfusion.

Methods: Ischemia was induced by common carotid artery occlusion in bulb-c mice (20-30 gr). Y-Maze was employed to scale the short-term memory and Nissl staining was used to count the cells in hippocampus.

Results: We found that concurrent treatment of A1 receptor agonist and vitamin C significantly reduced neuronal death in CA1. The Memory scores were also significantly improved ($P<0.05$).

Discussion: Our data point to the therapeutic effects of CPA/vitamin C co-administration and highlight the beneficial role of A1 adenosine receptor signaling in the context of stroke.

1. Introduction

Cerebral ischemia, after heart attack and cancer, is the third cause of mortality and disability in people older than65 years in the world with no satisfactory cure (Camarata, Heros, & Latchaw, 1994; Organization, 2004).

Hippocampus which is known to be involved in memory formation and spatial information processing, is among the first areas of the brain affected by degenerative diseases like Alzheimer's, Huntington's, and Parkinson's diseases and injuries caused by trauma and ischemia Hippocampus is very sensitive to hypoxia and free radicals formed during ischemic conditions. During ischemia reperfusion, released oxygen free radicals cause severe damage to cells. Rapid medical interventions may reduce ischemic necrosis and apoptosis (Bast, Haenen, & Doelman, 1991; Parman, Wiley, & Wells, 1999).

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It has already found that the model of global cerebral ischemia leads to neurodegenerative lesions inCA1 area of hippocampus, stratum and the neocortex. It is also known that global cerebral ischemia can cause neuronal death in CA1 pyramidal hippocampus and reduces the spatial learning and memory in rats (McBean & Kelly, 1998).

Antioxidants are substances that remove free radicals, prevent damage to cell membranes and DNA, and reduce cell death. The usage of antioxidants has been recommended to prevent free radicals damaging the cells especially to lessen the destructive effects of ischemia. Vitamin C is a powerful antioxidant that is accessible in a proper diet (Iwata, Okazaki, Kamiuchi, & Hibino, 2010; Miura et al., 2009; Sato & Hall, 1992). Neuroprotective role of vitamin C as a powerful and available antioxidant was verified in several animal models (Iwata, et al., 2010).

A1 receptor, a member of purinergic receptors family, is distributed widely throughout the body including CA1 region (Deckert & Jorgensen, 1988). One of the important functions of this receptor is enhancing cell resistance to various environmental stresses preventing the process of programmed cell death, apoptosis (Becker et al., 2004; Drury & Szent-Györgyi, 1929; Hatfield, Belikoff, Lukashev, Sitkovsky, & Ohta, 2009; Kulinsky, Minakina, & Usov, 2001; Londos, Cooper, & Wolff, 1980). It has been approved that the accumulation of A1 receptors in the hippocampus (CA1) is correlated to neuroprotection against brain degenerative disease (Dirnagl, Iadecola, & Moskowitz, 1999; Velazquez, Frantseva, & Carlen, 1997).

Due to less information on administration of A1 receptor agonist, N6-cyclopentyladenosine (CPA) in combination with vitamin C on hypoxia complications, we designed this study to evaluate the effects of co-administration of vitamin C and CPA on ischemia-reperfusion induced cell death in hippocampus.

2. Methods

2.1. Animals

Fifty six adult bulb-c mice (20-30 gr) were obtained from Iranian Razzi Institute, Iran. Mice were maintained in Specific pathogen free unit at 21 ± 1C (50 ± 10% humidity) on a 12-h light/12-h dark cycle with access to water and food ad libitum.

2.2. Experimental Design

Mice were assigned to 8 experimental groups (n=7/group) as follows:

1. Intact group: no ischemia, no treatment; 2. Ischemia control group: ischemia without any treatment; 3. Vehicle group: received treatment with vehicle from one week after ischemia to the end (second week after ischemia); 4.Pretreatment group: received vitamin C (100mg/kg) from one week before ischemia to the end; 5. A1 receptor agonist treatment: received CPA (1mg/kg); 6.Combination treatment with vitamin C/CPA: received vitamin C (100mg/kg)/A1receptor agonist (1mg/kg); 7. A1 receptor antagonist (DPCPX) treatment: received DPCPX (2.25mg/kg); 8. Combination treatment with vitamin C and DPCPX: received vitamin C (100mg/kg)/DPCPX (2.25mg/kg). Animals in groups 5 to 8 received their treatments from one week after ischemia to the end.

2.3. Ischemia Procedure

Animals in 2-8groups were subjected to 15 min of global brain ischemia induced by clamping the common carotid artery. Treatments were injected intraperitoneally after one week following reduction of inflammation in ischemic zone.

In order to evaluate the protective effects of vitamin C pretreatment, it was started one week prior to ischemia induction. The y-maze memory test performed two weeks following ischemia and then brains prepared for histological studies.

2.4. Y-maze Test

This working memory test is based on spontaneous exploration and alternations between arms with neither training nor food restriction (Lees, K.R., et al. 2006). Three identical arms are mounted symmetrically on an equilateral triangular center. In the test each mouse was placed at the end of one arm a permitted to walk through the maze for 300 second.

The ability to alternate requires that the animals know which arm they have already visited. In the task, each mouse was placed at the end of one arm and allowed to move through the maze for eight minutes. The percentage of alternation (defined as consecutive entries into all three arms without repetitions in overlapping triplet sets, to all possible alternations × 100%) was counted. For example, if the arms were marked as X, Y and Z

and the animal entered the arms in the following order XYZXZYZXYXYZXZ, the actual alternation would be seven, and total number of arm entries would be fourteen and the percent alternation would be 58.33%.

2.5. Nissl Staining

This staining was used for identifying the basic neuronal structure from necrotic neurons in CA1 region.

Mice were deeply anesthetized with ketamine/xylazine (5/1) and perfused with cold PBS. Brains were removed and immersed in 4% paraformaldehyde for 72 hours. Fixed tissues were paraffin-embedded and 5µm sections were prepared from each brain (from a minimum of three animals per group). Sections were then deparaffinized in xylene and hydrated through series of alcohol and rinsed in distilled water. Sections were then incubated with 0.1% cresyl violet solution for 3-10 minutes. Then slides were rinsed in distilled water, dehydrated in series of alcohol, cleared in xylene and finally mounted with permanent mounting medium and prepared for microscopy.

2.6. Data Analysis

Data are expressed as means ± SEM. Comparisons of data were performed by one-way ANOVA. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

Co-administration of CPA and vitamin C inhibited short-term memory disruption

Y-maze behavioral test showed severe damage to short-term memory following ischemia with a significant difference compared to intact group($P<0.05$, Fig.1). Treatment withCPA or vitamin C significantly reduced short-term memory disruption following ischemia compared to the ischemia group ($P<0.05$, Fig.1).

Co-adminisration of CPA and vitamin C significantly improved memory scores compared to single administration of CPA or vitamin C ($P<0.05$, Fig.1).DPCPX showed a decrease in short memory with no significant difference compared to the ischemia group (Fig.1).

Co-administration of CPA and vitamin C reduced number of dead neurons in CA1 region

Cresyl violet (Nissl) staining showed the situation of normal and necrotic cells in tissue sections (Fig.2). Ischemia reduced the density of normal cells in the CA1 area whereas animals treated with CPA or vitamin Chad less dead cells and a significant increased cell density ($P<0.05$, Fig.3).

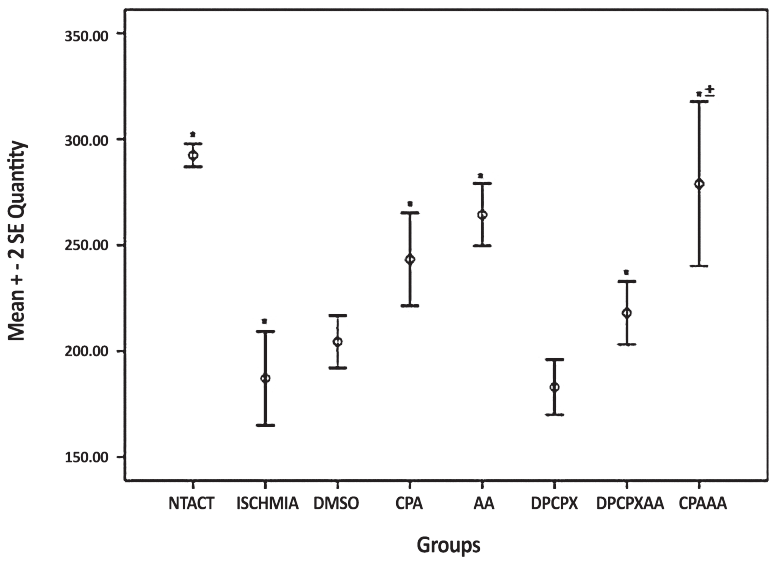

Figure 1. Comparison of short term memory status among the experimental groups

Short term memory status was measured using Y maze. Values are shown as mean ± SEM. Ischemic group represents significant difference compare to the intact group (*$P<0.05$). Treatment groups, except DMSO and DPCPX treated groups represent significant difference compared to ischemic group (*$P<0.05$).The group named CPA/AA treated with CPA and vitamin C showed a significant increase in Y-Maze results, compared to the CPA or vitamin C groups (±$P<0.05$).

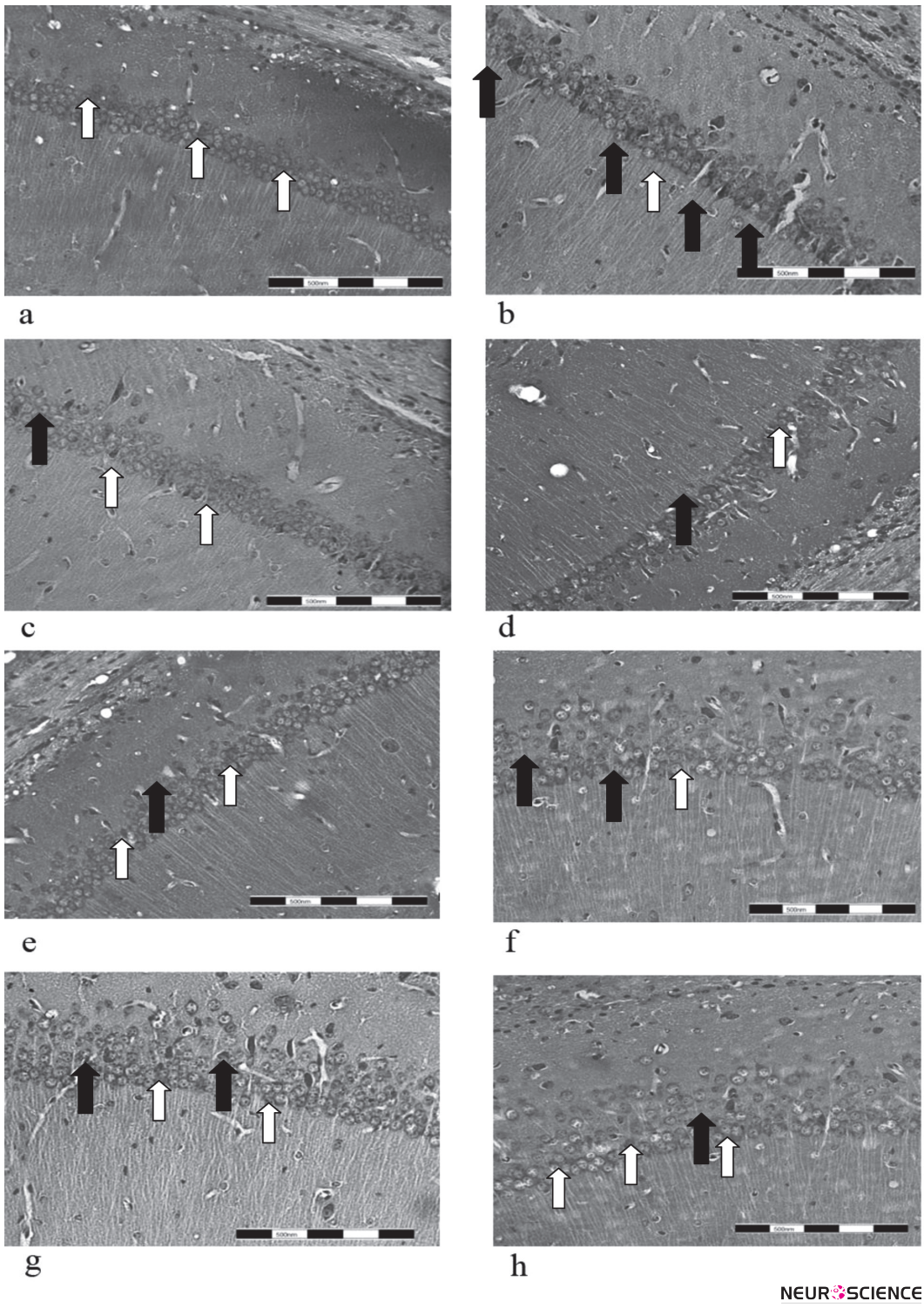

Figure 2. Neuronal death in CA1 region of hippocampus

Cresyl violet staining of brain sections from experimental groups was performed at the end of treatment used to evaluate the neuronal density and structure. The Animals treated with CPA (f) or vitamin C (g) had more cell density which is representative of less dead neurons compared to the ischemia group (b). Co-administration of both CPA and vitamin C reduces cell death (h) compared to ischemia groups and groups received CPA (f) or vitamin C (g). Administration of A1 receptor antagonist (DPCPX) intensified the cell death among ischemic neurons and reduced cell density (d). White and black arrows are representative of normal and dead cells, respectively. Experimental groups including Intact, Ischemia, DMSO, DPCPX, DPCPX/AA, CPA, AA (vitamin C) and CPA/AA are shown a, b, c, d, e, f, g, h digital images prepared at 40 x magnifications, respectively. Scale bars: 200 µm.

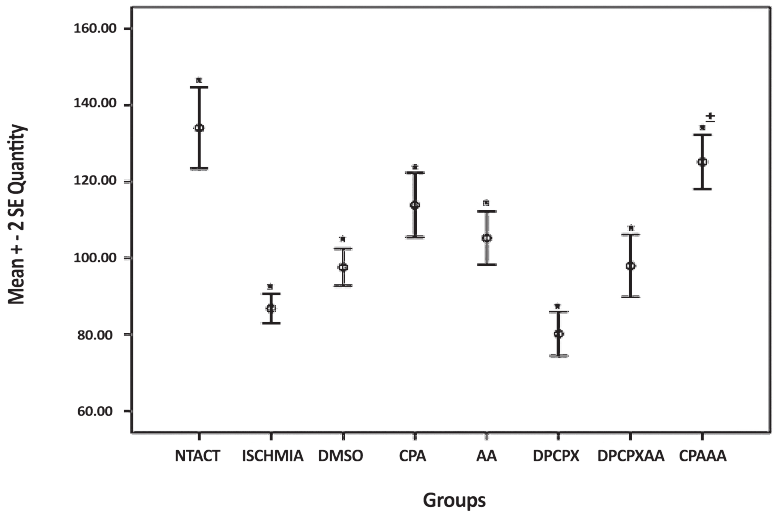

Figure 3. Comparison of the normal cells density in the CA1 region of hippocampus

Values are shown as mean ± SEM. Cell density was measured by counting normal neurons in CA1 region using cresyl violet staining. Cell density in groups treated with CPA or vitamin C (shown with AA) is significantly increased compared to the ischemic group (*P<0.05). The group named CPA/ AA, treated with both CPA and vitamin C showed a significant increase in density of normal cells in the CA1 region compared to the CPA or vitamin C (AA) groups (±P<0.05).

Co-adminisration of CPA and vitamin C caused a significant decreased in number of dead neurons compared to single administration of CPA or vitamin C (P<0.05, Fig.3).DPCPX intensified cell death among ischemic neurons and reduced cell density significantly compared to the ischemia group (P<0.05, Fig.3)

4. Discussion

The results of this study demonstrate that co-administration of CPA and vitamin C can reduce destructive effects of ischemia reperfusion such as spatial memory loss and neuronal death. Less number of dead cells in CA1 region of hippocampus in CPA and vitamin C treatment group suggests neuroprotective effects for combination therapy against tissue destruction following ischemia.

Our study reveals the protective effects of CPA/Vitamin C co-administration for the first time. The results of this study showed that improvement of memory status in treatment groups has been closely correlated with the effects of therapeutic strategy on neuronal death. This study showed that vitamin C and CPA, as protective and/or therapeutic agents, can increase the survival of hipocampal neurons in the brain and thus improves hipocampal function by reducing damage to neurons caused by free radicals in stressful conditions. But also introduce CPA/vitamin C as a successful approach containing both vitamin C and CPA positive effects.

A previous study by Miura, 2009 demonstrated that treatment with vitamin C during hypoxia in newborn animals' brain can reduces the number of both necrotic and apoptotic cells in cortex, caudate putamen, thalamus and hippocampus (Miura, et al., 2009). Their results showed that vitamin C is neuroprotective after hypoxic ischemia in immature rat brain.

Moreover, protective effects of CPA has been demonstrated on heart and cardiovascular system before by Urmaliya, with improved post-ischemic contractility, left ventricular developed pressure, end diastolic pressure and reduced infarct size (Urmaliya et al., 2010; Urmaliya, Pouton, Ledent, Short, & White, 2010). Other studies revealed that activation of adenosine receptors in neuronal membranes prevents the onset of enzymatic cascade responsible for neuronal apoptosis and gives them enough time to repair their structures and resume normal activities (Dirnagl, et al., 1999; Regan et al., 2003; Velazquez, et al., 1997). Thus using a combination of these two components as shown in our study can be more favorable than taking each medication alone. This can be due to the fact that vitamin C decrease neuronal vulnerability to ischemia and if it fails and neurons are damaged, adenosine A1 receptor agonist postpones the onset of apoptosis and gives them time to repair which results is reducing ischemic complications.

In conclusion, concurrent treatment with vitamin C and adenosine A1 Receptor agonist (CPA) can be tested

as a pharmaceutical approach to lessen destructive effects of ischemia reperfusion on hippocampus.

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Prediction of Quality of life by Self-Efficacy, Pain Intensity and Pain Duration in Patient with Pain Disorders

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ABSTRACT

The quality of life (QOL) has been defined as "a person's sense of well-being that stems from satisfaction or dissatisfaction with the areas of life that are important to him/her". It is generally accepted that pain intensity and duration have a negative impact on the QOL. One specific type of control is "self-efficacy", or the belief that one has the ability to successfully engage in specific actions. The ability to adapt to pain may play an important role in maintaining the QOL. In this study, we investigated the role of self-efficacy, pain intensity, and pain duration in various domains of quality of life such as physical, psychological, social and environmental domains. In this study, 290 adult patients (146 men, 144 women) completed coping self-efficacy and the WHOQOL-BREF Questionnaire. Moreover, we illustrated numerical rating scale for pain intensity. The results were analyzed using SPSS version of 19.0 and means, descriptive correlation, and regression were calculated. Our data revealed that self-efficacy but not the pain duration could significantly anticipate the QOL and its four related domains ($P<0.001$). In addition, it is noticeable that the effect of self-efficacy on the prediction of QOL is much more obvious in the psychological domain. However, the pain intensity could predict all of the QOL domains ($P< 0.001$) except social and environmental ones. In conclusion, to predict the quality of life (QOL) in person suffering from chronic pain, self-efficacy and pain intensity are more important factors than the pain duration and demographic variables.

1. Introduction

In the last decade, the importance of patients' quality of life (QOL) in relation to return to work and psychological well-being has been recognized. It is generally accepted that chronic pain has a negative impact on quality of life (Kempen, Ormel, Brilman, & Relyveld, 1997; Schlenk et al., 1998; Stewart et al., 1989).Chronic pain has negative consequences for general health (Becker et al., 1998) and for social and psychological well-being (Gureje, Von Korff, Simon, & Gater, 1998; Lame, Peters, Vlaeyen, Kleef, & Patijn, 2005). QOL is often viewed as a multidimensional construct that encompasses several domains (e.g., health, physical functioning, psychological status, spiritual well-being, social functioning). Although several studies have documented that pain can have negative effects on QOL, (Esnaola et al., 2002; Ferrell, Grant, Padilla, Vemuri, & Rhiner, 1991; Rustoen, Moum, Padilla, Paul, & Miaskowski, 2005), But at the present time it is necessary to more investigate about the probably negative effect of pain intensity and pain duration on QOL.

On the other hand, biopsychosocial models of chronic pain hypothesize that psychological and social factors play a key role in the adjustment to chronic pain. Pain self-efficacy that is, the belief or confidence in one's ability to engage in a specific behavior or other action to achieve desired goals despite pain (Arnstein, 2000; Bandura, 1977; Geisser, Robinson, Miller, & Bade, 2003; Nicholas, 2007). It has been one of the factors

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thought to mediate the impact of pain on disability. (Arnstein, 2000; Costa Lda, Maher, McAuley, Hancock, & Smeets, 2011; Denison, Asenlof, & Lindberg, 2004; Saunders, 2004; Ferreira-Valente, Pais-Ribeiro, & Jensen, 2011) To maintain a high quality of life, participants reported that they took control of their illness and advocated for themselves. Since the self-efficacy is the person's confidence in his ability to keep functioning despite pain and Individuals with higher self-efficacy can better cope with the pain. (Asghari-Moghadam, Karami & Rezaee, 2002).Also, pain coping strategies have been shown to be associated with severity of pain well as physical and psychological functioning among patients with chronic pain. Assessment of coping strategies has received increasing attention and several measures of cognitive and behavioral coping. (Asghari-Moghadam, Golak, 2005).

Taking control and self-advocacy are consistent with self-efficacy, which refers to the perception of one's ability and capability to successfully achieve valued behavioral outcomes (Bandura, 1977). Individuals with high self-efficacy gain an increased sense of confidence in their ability to control and manage the symptoms associated with their chronic disease (Daltroy, 1993). They also demonstrate long-term adherence in managing their disease, which significantly enhances quality of life (Han, Lee, Lee, & Park, 2003; Rosenstock, 1985).

Self-efficacy appears to be a modifiable variable that can affect health status, influence mood and motivation, and maintain participation in daily routines and roles (Bodenheimer, Lorig, Holman, & Grumbach, 2002; Buck, Poole, & Mendelson, 2010; Marks, Allegrante, & Lorig, 2005).The importance of positive pain self-efficacy for successful adaptation to chronic pain is well documented and chronic pain are more affected by self-efficacy (Arnstein, 2000) (Turk & Okifuji, 2002). One study demonstrates that social, psychological and part of the physical domain of QOL did not show significant correlation with pain duration (Tjakkes, Reinders, Tenvergert, & Stegenga, 2010). On the other hand, Gutierrez et al in 2007 showed that higher levels of shoulder pain were associated with lower subjective QOL scores (Gutierrez, Thompson, Kemp, & Mulroy, 2007). Also, Women on long-term sick-leave have more difficulties in focusing attention, making decisions, and carrying out tasks, as well as reduced quality of life in the dimensions of vitality, social functioning, emotional role, and mental health (Jansen, Linder, Ekholm, & Ekholm, 2011). The purpose of this study was the role of self-efficacy, pain intensity & pain duration on quality of life in patient with pain disorder.

2. Methods

2.1. Participants

A cross-sectional study was performed in a population of the patient's clinic for Pain and Pain management of the Tehran hospitals. This population (290 adult: 146 men and 144 women) is a heterogeneous group of chronic pain patients with different localizations of pain, such as low back pain, Arthritis, Rheumatoid, Foot pain, hand pain and migraine. The age of all patients was between 18 and 65 that who were selected through stratified random sampling. All participants were paid $2 for completing the study.

2.2. Measurement

WHOQOL-BREF: Before being asked the subjects to participate and fill out quality of life (QOL) questionnaire, a formal consent was obtained from all of the participants. With some modification, world health organization (WHOQOL-BREF) was used to measure QOL in the chronic pain patients. Each question had an equal value and the QOL was quantified as the sum of the scores for all domains. The higher scores on this scale represent a better QOL.

Coping Self-Efficacy: Participants were administered a truncated 13 item version of the coping self-efficacy scale (CSE) which is an accepted modification of the original 26 item scale (16). The goal of this measure is to assess how confident or certain someone is that they can do certain behaviors when faced with life challenges. Ratings are based on an 11 point scale ranging from 0 ('cannot do at all') to 10 ('certainly can do'). The 13 items are broken up into 3 different subscales which include one's perceived ability to a) use problem focused coping ("break an unpleasant problem down into smaller parts"), b) stop unpleasant thoughts and emotions ("keep from feeling sad"), and c) get emotional support from friends and family ("get friends to help with the things you need"). Each category contains 6, 4, and 3 items respectively. A self-efficacy score is created for each of the 3 domains by adding the items in each category together. For the purpose of this study, we solely focus on a caregiver's ability to use problem-focused coping (α= .87) which has been shown to be Predictive of decreased psychological distress and an augmented sense of psychological Well-being.

Numerical rating scale: Using this scale, the health care provider asks patients to rate their pain intensity on a numerical scale that usually ranges from 0 (indicating

"No pain") to 10 (indicating "The worst pain imaginable"). In addition, an author developed demographic information questionnaire was completed by patient with chronic pain, assessing information such as pain duration, age

3. Results

3.1. Descriptive Statistics

The results were analyzed by using SPSS software version of 19.0. Consequently, correlation and regression (Linear Method) for inferential statistics and Descriptive statistics were computed for frequency patient. Basic summary statistics (e.g. mean, standard deviation, minimum, and maximum,) were calculated for each variable in Table 1 & 2.

Table 1. Frequency table by sex, age and education

	Sex		Age		Education	
	M	F	≤40	>41	Bachelor and upper	Under of bachelor
Number	146	144	102	188	97	193
Total	290		290		290	

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Table 2. Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Self	290	13	125	64.67	22.839	.149	.143	-.027	.285
Intensity	290	1	10	5.94	1.998	-.209	.143	-.633	.285
Duration	290	1	420	67.04	66.804	2.323	.143	7.161	.285
Quality of life	290	35	126	79.84	16.739	-.415	.143	.021	.285
Physical	290	7	34	21.28	5.678	-.359	.143	-.357	.285
Psychological	290	1	31	18.30	4.778	-.398	.143	.323	.285
Social	290	3	22	9.19	3.002	.149	.143	.843	.285
Environment	290	10	40	24.90	5.613	-.324	.143	.114	.285
Valid N (listwise)	290								

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3.1. Prediction of Quality of Life from Self-Efficacy, Pain Intensity and Pain Duration in Patients with Pain Disorders

Our data revealed that pain duration could not anticipate the QOL, but self-efficacy (P<0.001) and pain intensity (P<0.001) could significantly anticipate the QOL. Forty-two percent of the variance in QOL total score was predicted by self-efficacy and pain intensity (R: 0.65 R2: 0.42 F: 69.70). The analysis indicated that high self-efficacy score could positively anticipate the QOL, but the relationship between pain intensity and QOL was negative. Accordingly, a model was formulated as a regression equation:

Y: 0.418 (Self-Efficacy) – 1.312 (Pain Intensity) + 61.397 (Fig. 1).

3.2. Prediction of Physical Aspect of QOL by Self-Efficacy, Pain Intensity and Pain Duration in Patients with Pain Disorders

Our data showed that there was an important relationship between self-efficacy (P<0.001) and pain intensity (P<0.001) but duration unable to predict physical aspect of QOL. Patients with higher levels of self-efficacy have better QOL; however, pain intensity could negatively predict this domain. Self-efficacy and pain intensity predicted 31 percent of the variance in this domain (R: 0.55 R2:0.31 F: 42.96).

Accordingly, a model was formulated as a regression equation:

Y: 0.10 (Self-Efficacy) - 0.71 (Pain Intensity) + 18.99 (Fig. 2).

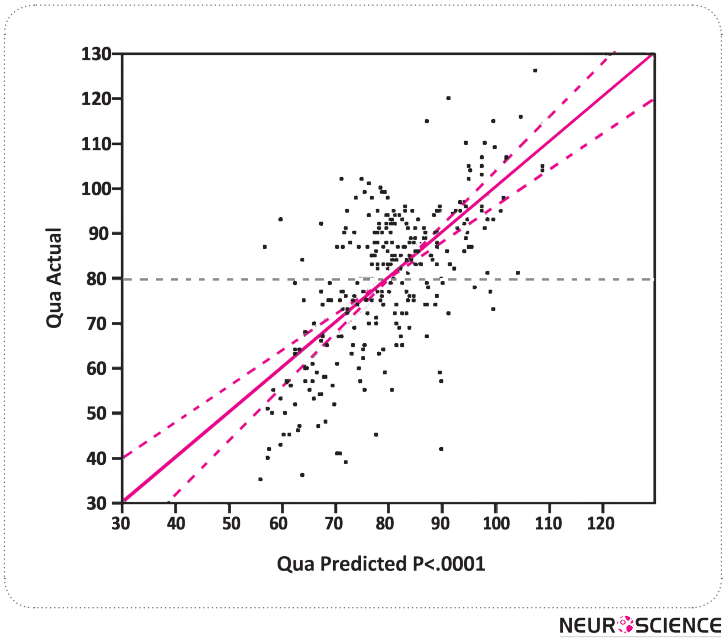

Figure 1. Prediction of quality of life from Self-Efficacy, pain intensity and pain duration in patients with pain disorders

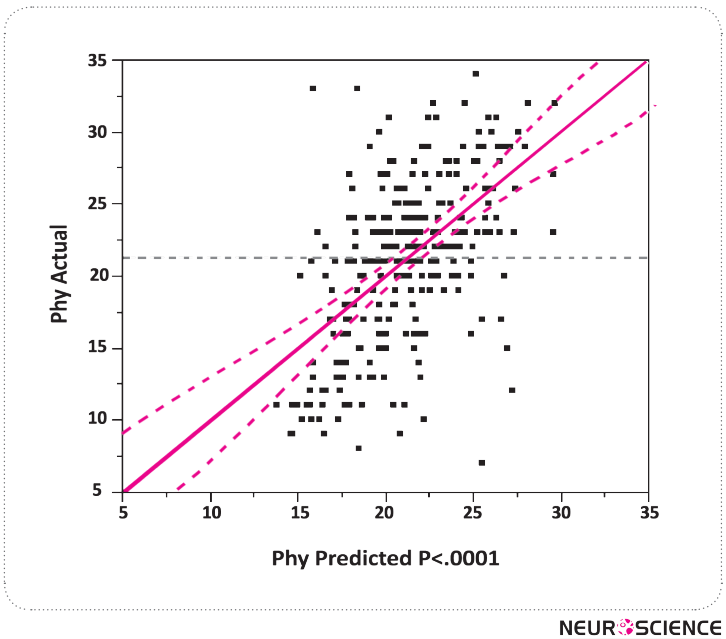

Figure 2. Prediction of physical aspect of QOL by Self-Efficacy, pain intensity and pain duration in patients with pain disorders

3.3. Prediction of Psychological Aspect of QOL by Self-Efficacy, Pain Intensity and Pain Duration in Patients with Pain Disorders

In this aim, the results showed that self-efficacy and pain intensity but not the pain duration could significantly anticipated the psychological aspect (P<0.001) (P<0.017). Self-efficacy and pain intensity predicted 38 percent of variance psychological domain (R: 0.62 R2:0.38 F: 59.84). Patients with high levels of self-efficacy have significantly higher scores on psychological aspect of quality of life subscales. In addition, it is noticeable that the effects of self-efficacy and pain intensity on the prediction of QOL are much more obvious in the psychological domain. Accordingly, a model was formulated as a regression equation:

Y: 0.16 (Self-Efficacy) - 0.28 (Pain Intensity) + 12.82 (Fig. 3).

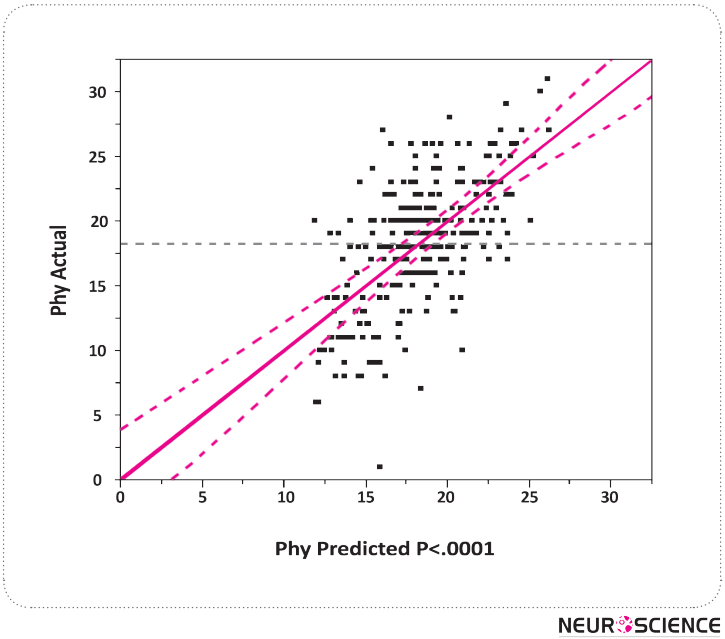

Figure 3. Prediction of psychological aspect of QOL by Self-Efficacy, pain intensity and pain duration in patients with pain disorders

3.4. Prediction of Social Aspect of QOL by Self-Efficacy, Pain Intensity and Pain Duration in Patients with Pain Disorders

Our result revealed that self-efficacy could anticipate the social aspect of quality of life (P<0.001) and pain duration and pain intensity could not significantly predicts the social domain. High levels of self-efficacy scale were predicted greater levels of social aspect of quality of life (R: 0.54 R2:0.29 F: 40.70). Accordingly, a model was formulated as a regression equation:

Y: 0.068 (Self-Efficacy) + 5.39 (Fig. 4).

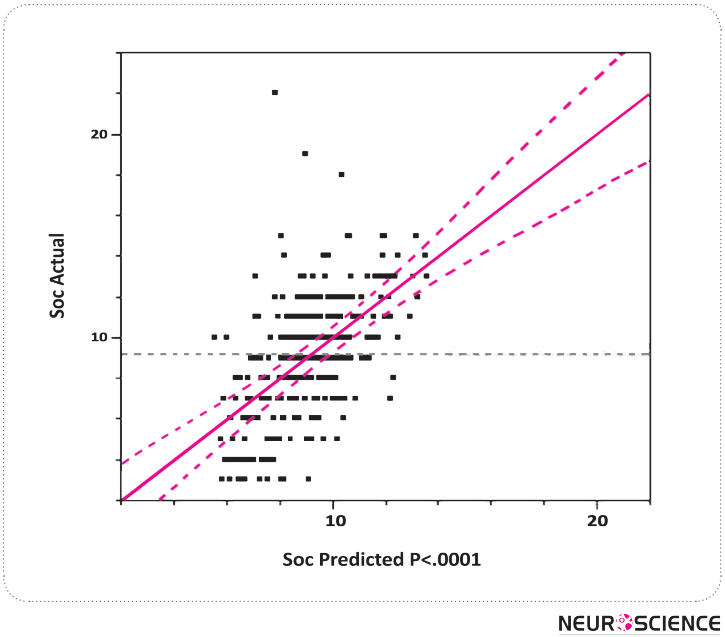

Figure 4. Prediction of social aspect of QOL by Self-Efficacy, pain intensity and pain duration in patients with pain disorders

3.5. Prediction of Environmental Aspect of QOL by Self-Efficacy, Pain Intensity and Pain Duration in Patients with Pain Disorders

Self-efficacy could significantly predict the environmental domain (P<0.001) and pain intensity and pain duration could not anticipate this domain. It is noticeable that the effect of Self-efficacy on the prediction of QOL is at a low level in the environmental domain(R: 0.43 R2:0.18 F: 21.98). Accordingly, a model was formulated as a regression equation:

Y: 0.10 (Self-efficacy) + 18.63 (Fig. 5).

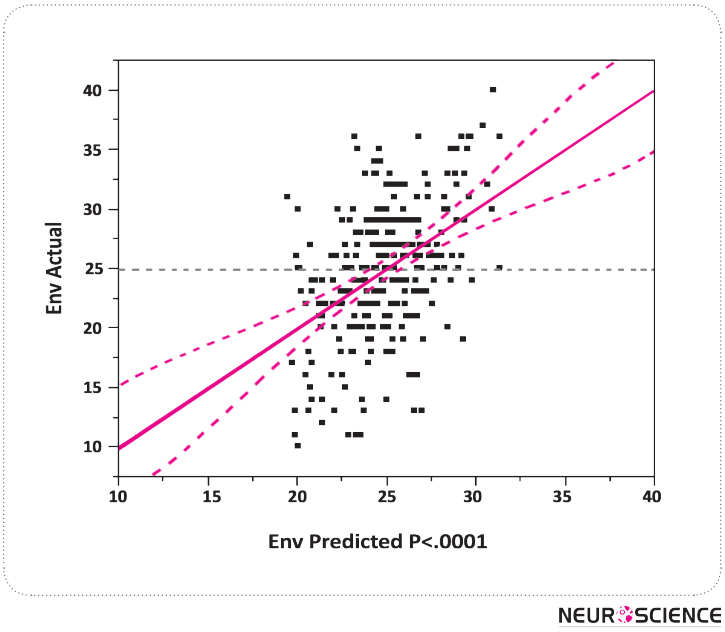

Figure 5. Prediction of environmental aspect of QOL Self-Efficacy, pain intensity and pain duration in patients with pain disorders

4. Discussion

The primary goal of this study was to examine the effects of self-efficacy, pain intensity & pain duration on quality of life in patient with pain disorder. Our results found that the significant association between self-efficacy and all of the domains of QOL. Also our data revealed that there was an important relationship between pain intensity and QOL and physical, psychological aspect of QOL, But pain duration could not anticipate the QOL and for all quality of life domains. Our findings confirmed the results of previous study, that chronic pain has negative impacts on many aspects of patient's life, including social and recreational activities as well as job and marital satisfaction (Asghari-Moghadam, 2004). While several studies show that increased self-efficacy promotes a higher sense of psychological well-being (DeWitz SJ, 2002; Yue, 1996), our current findings illustrate that increased self-efficacy can also have positive effect on prediction of high QOL. Alternatively, low self-efficacy has been shown to have a negative impact on disease management behaviors, resulting in poorer health outcomes, more pain, poorer psychological well-being, and decreased physical health status (Shifren, Park, Bennett, & Morrell, 1999). Specifically, Bandura (Bandura, 1997) conceptualizes that self-efficacy beliefs determine whether or not individuals think in self-aiding or self-debilitating ways, their emotional well-being, Therefore, self-efficacy in large part, determines whether problems appear manageable or overwhelming (Harmell et al., 2011), and it support that the Motivational Model of Pain Self-Management perceived the importance of self-efficacy for a particular pain management behavior. (Kratz, Molton, Jensen, Ehde, & Nielson, 2011).

Gutierrez et al., showed that the relationship between pain intensity and physical activity. The study indicates that persons were experiencing greater pain had lower levels of physical activity. Haghighat in 2011 provided empirical evidence supporting bio-psycho-social model and shows that psychological factors influence the per-

ception and experience of pain intensity (Haghighat, Zadhoosh, Tabatabaei, Etemadifar, 2011). Moreover evidences showed the chronic pain contributes to greater psychological distress (Ehde, Osborne, Hanley, Jensen, & Kraft, 2006; Hadjimichael, Kerns, Rizzo, Cutter, & Vollmer, 2007), health care utilization, physical disability, and reduced quality of life. (Khan & Pallant, 2007; O'Connor, Schwid, Herrmann, Markman, & Dworkin, 2008; O'Connor, et al., 2008; Thompson, 1998). Lame et al., in 2005, found that the pain intensity explains the relatively low scores on all quality of life domains (Lame, et al., 2005). In addition, the correlation between pain intensity, Health related quality of life (HRQL) and disease severity significantly reduced HRQOL(Afendy et al., 2009). In agreement with our result Lame et al., in 2005 showed that there are no significant relation between pain duration and quality of life (Lame, et al., 2005), despite in long-term disease, patients experience a considerable decrease in quality of life especially in physical and psychosocial domain, (Van Aken et al., 2005). Our findings propose the important role of self-efficacy in prediction of quality of life, and influence of pain intensity in health-related quality of life.

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Basic and Clinical NEUROSCIENCE

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Evaluation of the Effect of Jobelyn® on Chemoconvulsants-Induced Seizure in Mice

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ABSTRACT

Introduction: Epilepsy is a common central nervous system (CNS) disorder characterized by seizures resulting from episodic neuronal discharges. The incidence of toxicity and refractoriness has compromised the clinical efficacy of the drugs currently used for the treatment of convulsions. Thus, there is a need to search for new medicines from plant origin that are readily available and safer for the control of seizures. Jobelyn® (JB) is a unique African polyherbal preparation used by the natives to treat seizures in children. This investigation was carried out to evaluate whether JB has anti-seizure property in mice.

Methods: The animals received JB (5, 10 and 20 mg/kg, p.o) 30 min before induction of convulsions with intraperitoneal (i.p.) injection of picotoxin (6 mg/kg), strychnine (2 mg/kg) and pentylenetetrazole (85 mg/kg) respectively. Diazepam (2 mg/kg, p.o.) was used as the reference drug. Anti-seizure activities were assessed based on the ability of test drugs to prevent convulsions, death or to delay the onset of seizures in mice.

Results: JB (5, 10 and 20 mg/kg, p.o) could only delay the onset of seizures induced by pentylenetetrazole (85 mg/kg, i.p.) in mice. However, it did not did not offer any protection against seizure episodes, as it failed to prevent the animals, from exhibiting tonic-clonic convulsions caused by pentylenetetrazole (85 mg/kg, i.p.), strychnine (2 mg/kg) or picrotoxin (6 mg/kg, i.p.). On the other hand, diazepam (2 mg/kg, i.p.), offered 100% protection against convulsive seizures, induced by pentylenetetrazole (85 mg/kg, i.p.). However, it failed to prevent seizures produced by strychnine (2 mg/kg, i.p.) or picrotoxin (6 mg/kg, i.p.).

Discussion: Our results suggest that JB could not prevent the examined chemoconvulsants-induced convulsions. However, its ability to delay the latency to seizures induced by pentylenetetrazole suggests that JB might be effective in the control of the seizure spread in epileptic brains.

Key Words:
Anti-Seizure,
Jobelyn®,
Picrotoxin,
Strychnine,
Pentylenetetrazole.

1. Introduction

Epilepsy is a common central nervous system (CNS) disorder characterized by seizures which take diverse forms and result from episodic neuronal discharges. Meanwhile, the form of the seizure depends on the part of the brain affected (Rang et al., 2000; Leonard, 2000). Epilepsy affects 0.5-1% of the population globally (Rang et al., 2000). Often, there is no recognizable cause, although it may develop after brain insults, such as trauma, infection, tumor growth, or other kinds of neurological disorders (Leonard, 2000).

Current anticonvulsant drugs are effective in controlling seizures in about 70% of cases; however, their use is often limited by severe side effects (Rang et al., 2000). As alternatives to these existing drugs, a number of compounds from plants that are readily available and well tolerated are being developed as potential medica-

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tions for the management of seizures (Lobo et al., 2008; WHO, 1996). Jobelyn® (JB), manufactured by Health Forever Products Ltd, Lagos, Nigeria, is a commercial herbal preparation which has recently made its way into the medicine market in Nigeria. It is available as a powdered preparation specially formulated into capsules and suspensions for the treatment of anemia (Erah et al., 2003; Okochi et al., 2003). JB is made from three notable medicinal plants; Parquetina nigrescens, Harungana madagascariensis and Sorghum bicolor. Parquetina nigrescens (family: Periplocaceae) is a shrub found in the equatorial region of West Africa (Mabberley, 1987). Harungana madascariensis (family: Clusiaceae) is locally cultivated in Madagascar, Mauritius and Tropical Africa for its valuable medicinal properties (Hutchinson & Dalziel, 1954). Sorghum bicolor or Sorghum (family: Gramineae) is an important staple food crop in Africa, South Asia and Central America, and is also grown in some developed nations for its medicinal values (Erah et al., 2003; Okochi et al., 2003). Most of the compounds in JB including apigenin, luteolin and naringenin, have been reported to exhibit a wide range of CNS activities (Okochi et al., 2003; Olsen et al., 2008; Yi et al., 2010). Apigenin, luteolin and naringenin in particular have been shown to exhibit neuroprotection, anti-oxidation and to reduce neuroinflammation, suggesting their therapeutic efficacy in CNS disorders (Yi et al., 2010; Awika & Rooney, 2004).

Jobelyn® is claimed to be helpful in stress related ailments and has gained international recognition as energizer, immune enhancer and antioxidant supplement (Erah et al., 2003; Okochi et al., 2003). It has also gained popularity as a remedy for ensuring good joint health and relieving rheumatoid arthritis (Erah et al., 2003; Okochi et al., 2003). A recent survey carried out in Lagos, Nigeria, listed JB as a remedy used by the populace for the treatment of convulsive seizures in children (Oshikoya, 2008). However, no experimental studies have been carried out to confirm the efficacy of JB, as an anticonvulsant agent. Thus, the present study was designed to investigate the effect of JB on chemoconvulsants-induced seizure in mice.

2. Methods

2.1. Experimental Animals

Male albino Swiss mice (20-24 g) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature with 12:12 h light-dark cycle. They had free access to commercial food pellets and water ad libitum. They were acclimatized for at least one week before use for all experiments. The study was carried out in accordance with the ethical guidelines of the University of Ibadan for the care and use of laboratory animals for experimental studies.

2.2. Drugs and Treatment

Diazepam (Sigma, USA), Jobelyln® (Health Forever Products Ltd, Lagos, Nigeria), picrotoxin (Sigma-Aldrich, St. Louis, USA), strychnine (Sigma, USA) and pentylenetetrazole (Sigma-Aldrich, St. Louis, USA) were used in the current investigation. All drugs were dissolved in distilled water immediately before administration. The doses of 5, 10 and 20 mg/kg of JB used in the study were selected based on the results obtained from preliminary investigations.

2.3. Experimental Procedures

2.3.1. Effect of Jobelyn® on Picrotoxin-Induced Convulsion

Picrotoxin-induced convulsion test was carried out according to the method previously described (Das et al., 2010). Mice were randomly distributed into treatment groups (6 per group) and were given JB (5, 10, 20 mg/kg, p.o.), diazepam (2 mg/kg, p.o.) or distilled water (10 ml/kg; p.o.), 30 min prior to the administration of picrotoxin (6 mg/kg, i.p.). Animals were immediately placed individually in a transparent observation chamber and observed for the expression of convulsions for 30 min after picrotoxin injection.

2.3.2. Effect of Jobelyn® on strychnine-Induced Convulsion

The activity of JB against seizures was also assessed on strychnine-induced convulsions as described earlier (McAllister, 1992). Mice (5 per group) were given JB (5, 10 and 20 mg/kg, p.o.), diazepam (2 mg/kg; p.o.) or distilled water (10 ml/kg, p.o.), 30 min before strychnine (2 mg/kg, i.p.) injection. The time required for the expression of convulsive seizures was assessed by an observer as described before.

2.3.3. Effect of Jobelyn® on Pentylenetetrazole-Induced Convulsion

Pentylenetetrazole-induced convulsion test was employed to further evaluate the anti-convulsant activity of JB as previously described (Loscher et al., 1991). Mice (5 per group) received JB (5, 10 and 20 mg/kg, p.o), diazepam (2 mg/kg; p.o.) or distilled water (10 ml/

Table 1. Effect of Jobelyn® on picrotoxin-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	6.60±0.24	100	100
JB (5 mg/kg)	6.80±0.37	100	100
JB (10 mg/kg)	7.00±0.44	100	100
JB (20 mg/kg)	9.60±0.93*	100	100
DZ (2 mg/kg)	12.80±0.86*	100	100

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Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

Table 2. Effect of Jobelyn® on strychnine-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	2.40±0.25	100	100
JB (5 mg/kg)	3.70±0.37	100	100
JB (10 mg/kg)	2.40±0.51	100	100
JB (20 mg/kg)	3.60±0.40	100	100
DZ (2 mg/kg)	3.20±0.58	100	100

NEUROSCIENCE

Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

Table 3. Effect of Jobelyn® on pentylenetetrazole-induced convulsive seizures in mice

Treatment	Onset of seizures (min)	Seizures (%)	Death (%)
Control	1.60±0.25	100	100
JB (5 mg/kg)	3.00±0.32*	100	100
JB (10 mg/kg)	3.40±0.51	100	40
JB (20 mg/kg)	2.80±0.20*	100	60
DZ (2 mg/kg)	-	0	0

NEUROSCIENCE

Values represent the mean ± S.E.M for 5 animals per group. *p < 0.05 compared to control group (ANOVA followed by Dunnett's post-hoc test).

kg, p.o.), 30 min before pentylenetetrazole (85 mg/kg, i.p.) injection. The animals were immediately placed individually in a transparent observation chamber and observed for a period of 30 min for the onset of convulsions after pentylenetetrazole injection.

2.3.4. Statistical Analysis

The data were expressed as mean ± S.E.M. The data were analyzed with Graph Pad Prism Software version 4.03. Statistical analysis of data was done by One-way ANOVA, followed by Dunnett's post-hoc test. A level of $p < 0.05$ was considered as statistically significant.

3. Results

The effects of JB (5-20 mg/kg; p.o.) on convulsions induced by picrotoxin (6 mg/kg, i.p.), strychnine (2 mg/kg, i.p) and pentylenetetrazole (85 mg/kg, i.p) in mice are shown in Tables 1, 2 and 3, respectively. As shown

in the Tables, JB (5-20 mg/kg, p.o) did not provide any protection against convulsions induced by the chemoconvulsant agents in mice. However, JB (5, 10, 20 mg/kg, p.o.) significantly delayed the onset of pentylenetetrazole- induced seizures (85 mg/kg, i.p). In contrast, while diazepam (2 mg/kg, p.o.) prevented the convulsive seizures produced by pentylenetetrazole (85 mg/kg, i.p), it was ineffective against picrotoxin- (6 mg/kg, i.p.) or strychnine (2 mg/kg, i.p.)-induced seizure in mice (Tables 1-3).

4. Discussion

The results of this study showed that JB did not did not provide any protection against the development of seizure episodes induced by pentylenetetrazole, strychnine or picrotoxin in mice. However, it delayed the onset of seizures and also reduced the mortality rate in pentylenetetrazole-treated animals. These chemoconvulsants are widely used to induce convulsions in experimental animals (McNamara, 1999). They also served as useful animal models for the development of potential anticonvulsant drugs as well as and in exploring the underlying mechanism(s) for their actions (McNamara, 1999). Convulsions may ensue from either a decreased inhibitory synaptic or enhanced excitatory synaptic neurotransmission (McNamara, 1994). The most common type of seizure is febrile convulsions, which occurs frequently in children (Gokhan & Ercument, 2010). It is an acute symptomatic convulsion triggered by fever without the presence of any form of known CNS abnormalities. Febrile seizure it is ranked among prevalent neurological disorders in the pediatric age (Gokhan & Ercument, 2010). Synaptic neurotransmitters implicated in convulsions are mostly glutamate, glycine and gamma-aminobutyric acid (GABA). These transmitter substances are linked to ion channels which regulate the rate of neuronal excitation (McNamara, 1999).

According to Kendall et al., 1981, the anticonvulsant activity of a novel compound is not measured only by its ability to prevent convulsions but also to delay the onset of seizures or to reduce death rate and/or to decrease the frequency of the episodes. Thus, the ability of JB to delay the onset of seizures, and to reduce death rate in pentylenetetrazole-treated animals suggests its possible effectiveness to control convulsive episodes. Previous studies have shown that compounds that could delay the onset of convulsions or reduce the frequency of the episodes in experimental animals are capable of halting the spread of seizures in epileptic brains (Corda et al., 1982). However, more detailed studies are required to verify how JB may be acting to arrest convulsive seizures in epileptic brains.

The differential effects of diazepam on convulsions induced by picrotoxin, strychnine or pentylenetetrazole, may be related to the dissimilarity in their mode of action. Pentylenetetrazole produced convulsions by blocking GABAA receptors thereby impairing GABA-mediated inhibitory neurotransmission (Gnyther, 1986; Oni et al., 2009). On the other hand, picrotoxin acts through blockade of chloride ion channel which is known to be resistant to most anticonvulsant agents (Corda et al., 1982; Zetler, 1981). However, strychnine acts by antagonizing glycine receptors, thereby increasing the rate of neuronal excitability (Sayin et al., 1993). Thus, the effectiveness of diazepam against pentylenetetrazole-induced seizures may be related to its well known action of potentiation of GABA-mediated inhibitory neurotransmission (De Sarro, et al., 1999). However, its failures to prevent seizures induced by picrotoxin may be related to the inability of diazepam to reopen the closed chloride ion gates (Corda et al., 1982; Zetler, 1981).

Jobelyn® has been shown to possess various bioactive elements including the well- characterized compounds, such as apigenin, luteolin and naringenin (Erah et al., 2003; Okochi et al., 2003; Yi et al., 2010; Awika & Rooney, 2004). These compounds have been reported to show antidepressant, anti-amnesic, anti-inflammatory and membrane stabilizing properties (Yi et al., 2010; Awika & Rooney, 2004; Heo et al., 2004; Weichiel et al., 1999). However, the role of these compounds in convulsive disorders remains to be confirmed experimentally.

5. Conclusion

The data obtained from this study suggest that JB delayed the onset of seizures produced by pentylenetetrazole supporting the hypothesis of its relevance in controlling the spread of seizure in epileptic brains.

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Evaluating the Effects of Oral Morphine on Embryonic Development of Cerebellum in Wistar Rats

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ABSTRACT

In the present research, the effect of morphine consumption during pregnancy on the development of the embryo's spinal cord was studied in Wistar rat.

Female Wistar rats (Wt: 250-300 g) were mated with males. The test group received morphine (0.01 mg/ml) in their drinking water. Pregnant rats were later killed with chloroform on the 12th, 13th and 14th days of pregnancy, and the embryos were taken out surgically. The embryos were fixed in formalin 10% for 2 weeks. Then, the weight of fixed embryos was calculated by a scale. In addition, several animals' sizes including fronto-posterior and lateral length were measured by a caliper. Tissue processing, sectioning and hematoxylin and eosin (H&E) staining were applied for the embryos. The sections were examined for spinal cord development by light microscope and MOTIC software.

Significant decrease was observed in the fronto-posterior and lateral length and the weight of the embryos in the test groups. The thickness of the white matter layer decreased on the 12th, 13th and 14th embryonic days. The thickness of the spine's grey layer was also less than the control group, on the same days. Increase in the length of the ependimal duct observed as well. Number of grey substance cells decreased compared to the control group within the same days. Meanwhile, thickness of the germinal layer reduced in comparison to the control group on the mentioned days.

In conclusion, morphine consumption during pregnancy causes defects in growth and completion of the spinal cord.

1. Introduction

Problems caused by addiction do not only end to the addict but the aftermath of addiction is also detrimental to the health of the children. Studies have shown that taking narcotics during pregnancy can delay fetal distinction and can also cause symptoms such as weight loss and neurological defects. For example, studies of the animal models have shown that daily morphine injection can lead to activity decline in chickens (Schmidt, 1983). Morphine administration can also reduce brain, kidney, and liver weight as well as a reduction of head-tail length in rat embryos (Erikson and Ronnback, 1989). Morphine consumption can change the ovary cycle and sexual periods of the female rats (Dohler, 1991). On the other hand, studies have shown that morphine can easily pass through biological barriers such as blood-placenta barrier and affect the embryonic cells (Kazemi et al., 2011; Levitt, 1998; Behravan and Piduette-Miller, 2007).

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In previous researches, the influence of the oral morphine on rats' neural tube (Nasiraei, et al., 2005) and frontal cortex (Sadraei et al., 2008) completion has been tested and proved to be effective. Abnormal completion of the spinal cord may induce certain abnormalities in locomotion. On the other hand, opioid usage during pregnancy delays nervous system development (Orony et al., 1996; Ray et al., 1977; Kazemi and Sahraei, 2011). On the basis of these findings, this research has studied the effects of oral morphine consumption on development of spinal cord in Wistar rat embryo.

2. Experimental Procedures

2.1. Animals

In this study, Wistar rats with an average weight of 250-300g were used. Two rats were housed per cage at a temperature of 24 ± 1ºC with natural light periods (12 hours light/dark). Enough food and water were available for rats during the experiment. Rats were kept in an animal house at Baqyiatallah Medical University. Animal experiments were carried out ethically.

2.2. Drugs

The morphine sulfate (TEMAD-Iran) was used in this research. The drug (0.01 mg/ml) was dissolved in tap water and was offered to the animals in a volume of 100 ml/rat/cage.

2.3. Procedure

The rats were divided in four groups each containing 6 rats (n=6).

24 healthy rats were mated in dual groups with two adult male rats. Once the pregnancy was definite (observation of the vaginal plague and sperm observation in vaginal smear), they were separated from the male ones and kept in groups of two at embryonic day 0 (E0). The experimental group received 0.01 mg of morphine in each ml of their drinking water. In the previous research the suitable morphine dosage in order to induce addiction was determined to be 0.1 mg/ml (Nasiraei et al., 2005). But an important study has indicated that 0.01 of morphine has the highest impact in causing malfunctions in the embryo, therefore this dosage was used as the effective amount (Nasiraei et al., 2005). The amount of morphine in 100 ml of water was measured, but it was tried to provide animal with enough water. The first group was killed on E12, second group on E13 and third group on E14 (5,6), with chloroform and the embryos as well as the uterus were taken out surgically and transferred into 10% formalin solution for 2 weeks. At this stage the embryos were separated from the uterus and were weighed with digital scale (Sartorius, Germany) with 0.0001 g precision and their fronto-posterior length was measured with caliper with 0.05 mm precision. The embryos were then underwent tissue processing procedure and stained with Hematoxilin &vEozin (H&E) (Wilson and Gamble, 2002) and were examined by a light microscope and MOTIC software after staining. In this research, thickness of the grey and white matter layers and the epandimal duct were measured as three main factors in spinal cord evolution. Also, the thickness of the ventricular layer and number of the cells in the grey matter layer were counted. In order to count these cells, in each group, different areas were randomly chosen and were counted in a square area of 625 micrometer. Since the animals did not complete the morphine consumption period (Khalili et al., 1997), the morphine dependency test was not carried out.

2.4. Blood Sampling and Corticosterone Assessment

One ml blood was collected in an Eppendorf tube containing 5 µl heparin (5000 IU/ml) and centrifuged at 3000×g for 5 min. Plasma was removed and kept at -74 °C for measuring the corticosterone. Plasma corticosterone was analyzed by the corticosterone Eliza kit (Bio Activa, Germany).

2.5. Statistical Analysis

Data were reported as mean± SEM. The differences between means were assessed by one way analysis of variance (ANOVA) and unpaired sample T-test using SPSS (version 18.0). P-value <0.05 was considered as significant.

3. Results

3.1. Effects of Oral Morphine Consumption on Plasma Corticosterone

Plasma corticosterone level increased significantly in the experimental group (1850± 102.73 ng/l, day 12; 1995.85 ± 63.96 ng/l, day 13; and 1860. 23 ± 123 ng/l, day 14) compared to the control (735.48 ± 98.83 ng/l, day 12; 536.50 ± 89.27 ng/l, day 13; 632.67 ± 68.5 ng/l, day 14) (P<0.001) (Fig.1)

In this research, the fronto-occipital length of the embryos was measured as a pattern for their length and was measured in millimeter (Fig. 2). Examinations showed

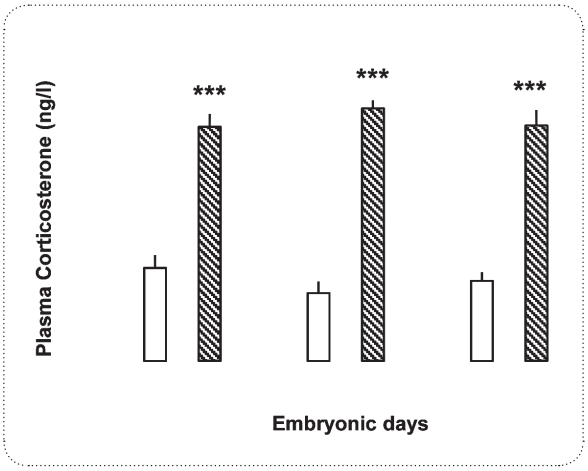

Figure 1. It is suggested that micrograph of section of spinal cord of 12-days-old embryo in control group(A). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: and section of spinal cord of 12-days-old embryo in experimental group(B). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: Ependimal channel (×100).

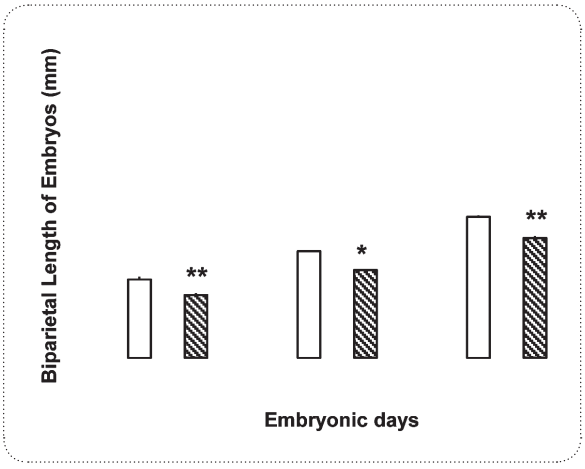

Figure 2. It is suggested that micrograph of section of spinal cord of 13-days-old embryo in control group(A). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: and section of spinal cord of 13-days-old embryo in experimental group(B). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: Ependimal channel (×100).

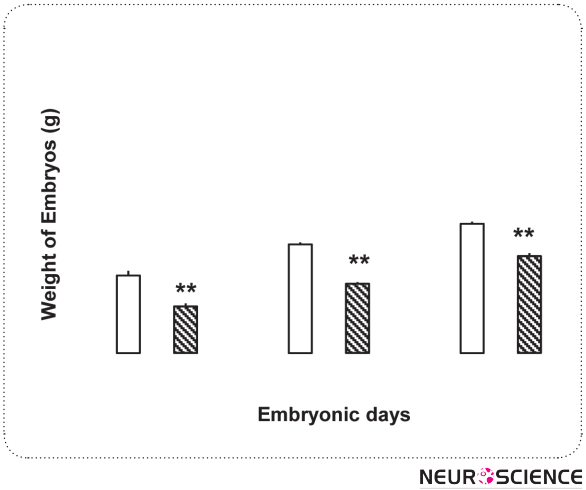

Figure 3. It is suggested that Micrograph of section of spinal cord of 14-days-old embryo in control group(A). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: section of spinal cord of 12-days-old embryo in experimental group(B). W.M: White matter, G.M: Gray matter, Vent: Ventricular layer, E.C: Ependimal channel (×100).

that morphine consumption in pregnant rats leads to decrease of embryos fronto-occipital length. The differences were statistically significant in all three days ($P<0.01$) (Fig.1). The weight of the embryos in control and test groups was measured in milligram and it was found that morphine can cause weight loss in embryo in all three test groups ($P<0.05$) (Fig. 3).

3.2. Microscopic Observations

3.2.1. Differences Observed on 12th Embryonic Day (E12)

Observations showed that both grey and white layer were formed in control and test groups and theses layers can be differentiated. The thickness of all three pos-

terior dorsal, abdominal ventral and lateral parts in the white substance, grey substance and ventricular layer in the control group was larger than the test group, whose variation was statistically significant. Also the length of the epandimal duct in the control group was less than the test group, which indicates better completion of the spinal cord in the control group. The number of grey cells was also more than the test group (Fig. 4A and B).

3.2.2. Differences Observed on 13th Embryonic Day (E13)

Both grey and white substances in control and test groups on this day have more thickness and a keratinous position was observable in both groups. The thickness of the white substance, grey substance and ventricular layer in control group is more than the test group. Also, the length of the epandimal duct in control group is less than the test group. The number of cells in the grey substance in the test group is less than the control group (Fig. 5A and B).

3.2.3. Differences Observed on 14th Embryonic Day (E12)

As shown in pictures 5 and 6, the thickness of white layer, grey layer and ventricular layer in the control group is more than the test group. Also, the length of epandimal duct in control group was less than test group which shows a more complete spine in control group. The number of grey cells was also more in comparison to the test group (Fig. 6A and B).

4. Discussion

This study showed that oral morphine consumption during pregnancy can delay spinal cord development. In addition, our findings show that oral morphine can excites corticosterone plasma level increment in pregnant rats. The results of this research are compatible with several other researches showing the effect of both oral and injection opioid prescription in inducing delay in embryonic development (Sadraei et al., 2008; Nasiraei et al., 2005; Ramazanyet al., 2009; Zagon and McLaughlin, 1985). The importance of these studies is that they all indicate that morphine consumption, even at the beginning of pregnancy, can cause defects in development of the embryos, a result which has been specifically shown in this research on spinal cord development. Since spinal development in rats begins on 12th embryonic day (E12) and is completed on the 14th day (E14), this period is chosen to examine the influence of morphine on spinal cord development.

Today, it is known that in certain periods of pregnancy, the embryos show more sensitivity to hexogen substances (such as narcotics) and these certain periods have been studied in previous researches (For review see: Ornoy et al., 1996). In one of the researches of Ornoy et al., it was realized that morphine shows its impact from the beginning of the formation of the fetus nervous system (Nasiraei et al., 2005; Kazemi and Sahraei, 2012; Sadraei et al., 2008; Kazemi and Sahraei, 2011). This research too, shows that one of the effects of morphine on the embryo is to control the development of the spinal cord. The importance of which, lies in the fact that the natural function of the spinal cord is essential for many of the sensory and movement activities and morphine delaying the spinal cord development can cause deficit in spinal cord function and thus the person may show extraordinary reactions in movement responses or sensory receptions.

The information gathered in this research stated that the thickness of the grey and white substances as well as the ventricular layer which is the germinal layer of the spinal cord decreased in the test groups. On the other hand, counting the number of cells in the gray substance showed that the number of these cells in test group in comparison with the control group is less (Kazemi and Sahraei, 2011). Meanwhile, the length of the Epandimal duct in the control group was more than the test group, which suggests that in morphine recipients the spinal cord is less complete. Thus, we can come to the conclusion that oral morphine consumption can delay spinal cord development in all morphine recipient groups. Such deficiency was observable in all test groups and no compensation was detected (Kazemi et al., 2011). In addition, embryos' weight and height were reduced in the morphine treated group. Therefore, it seems that morphine prescription has destructive effects on embryo's development in general and spinal cord completion of the rats in particular. In this regard there are a few explanations: first, morphine affects all types of opioid receptors and the number of these opioid receptors is different both on embryo and placental tissues. Second, the opioid receptors on the placental tissue have different sensitivity in reaction to morphine (Kazemi et al., 2012). Therefore, it is possible that morphine affects one embryonic tissue more than the other.

The mechanism(s) by which morphine exerts its effects on the embryo is not well known yet. Previous studies have shown that morphine can easily pass through the placental barrier and reach the embryo (Levitt, 1998; Kazemi et al., 2011) and thus affect the embryonic cells. The presence of the kappa-opioid receptors on the vil-

lous and placental veins has also been shown (Ahmad et al., 1989). Once these receptors become active, the blood vessels contract and less blood is delivered to the fetus (Ahmad et al., 1989). Defect in oxygen delivery and loss of nutrition will delay the fetal growth (Ray et al., 1977; Fowden et al., 2006; Fowden et al., 2008). It is important to consider that certain opioid receptors have been found (Ray and Wadhwa, 1999; Leslie et al., 1998; Zhu and Barr, 2001) on embryonic tissue, whose function is not yet known. Such receptors might be responsible for morphine's delaying effects on embryonic cells. Therefore, future studies should examine morphine's impact zones.

On the other hand, our findings showed that corticosterone plasma level is also elevated in morphine treated pregnant rats. The effects of high plasma corticosterone level on fetal developmental delay are well known (For review see: Van den Bergh et al., 2005). Studies indicated that different stresses applied to the pregnant animals could lead to distinct nervous system developmental delay (Meaney et al., 2002). However, our finding is in consistence with the results that indicate single morphine injection can increases corticosterone plasma level in rats (Prinik et al., 2001; Kazemi et al., 2011; Buckingham and Cooper, 1984). Our results also indicated that chronic oral morphine consumption can increases plasma corticosterone level in pregnant rat. However, the source of corticosterone release in our study may be the mother or fetus adrenal and/or the placenta itself. The results of this research can open new horizons in behavioral complications of the human and livestock newborns whose parents have taken opioid during pregnancy. For example it is known that the rats, who have received heroin during embryonic period, are more active (Ramazany et al., 2009). Also, newborns of the rats who have received morphine are more sensitive against morphine (Erikson and Ronnback, 1989; Zagon and McLaughlin, 1985). In human subjects too, it has been realized that opioids during pregnancy can cause behavioral and movement abnormalities in newborns (Wilson et al., 1979; Kazemi et al., 20012), which could be due to the delay in spinal cord development.

In conclusion, the results show that oral morphine consumption can delay spinal cord completion in rats and the same can be true in humans as well. Such delay can also cause behavioral malfunctions in children, necessitating further studies.

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Effects of Neonatal C-Fiber Depletion on Interaction between Neocortical Short-Term and Long-Term Plasticity

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ABSTRACT

Introduction: The primary somatosensory cortex has an important role in nociceptive sensory-discriminative processing. Altered peripheral inputs produced by deafferentation or by long-term changes in levels of afferent stimulation can result in plasticity of cortex. Capsaicin-induced depletion of C-fiber afferents results in plasticity of the somatosensory system. Plasticity includes short-term and long-term changes in synaptic strength. We studied the interaction between paired-pulse facilitation, as one form of short-term plasticity, with long-term potentiation (LTP) in the neocortex of normal and C-fiber depleted freely moving rat.

Methods: Neonatally capsaicin-treated rats and their controls were allowed to mature until they reached a weight between 250 and 300g. Then animals were anesthetized with ketamine and xylazine. For recording and stimulation, twisted teflon-coated stainless steel wires were implanted into somatosensory cortex or corpus callusom. In experiments for LTP induction, after two weeks of recovery period, 30 high frequency pulse trains were delivered once per day for 12 days. Paired-pulse ratio (PPR) was monitored before and after the induction of LTP in capsaicin-treated and control rats.

Results: Paired-pulse stimulation affected all field potential components at intervals < 200 ms. The largest changes occurred at intervals between 20- 30 ms. C-fiber depletion postponed the development of LTP, whereas it had no effect on PPR.

Discussion: This finding provides further evidence that the expression of this form of LTP is postsynaptic. Furthermore, these results suggest that the effect of C-fiber depletion on cortical LTP is also postsynaptic and, therefore, is not caused by a decrease in neurotransmitter release.

1. Introduction

Neurons in the mammalian cerebral cortex are connected into networks by synapses whose strengths can change as a function of recent activity (Buhl et al., 1997; Galarreta & Hestrin, 1998; Goriounova & Mansvelder, 2012; Varela & Sen & Gibson & Abbott & Nelson, 1997). Plasticity includes short-term [e.g., paired-pulse facilitation (PPF) and paired-pulse depression (PPD)] and long-term changes in synaptic strength [e.g., long-term potentiation (LTP) and long-term depression (LTD)] (Ziakopoulos & Tillett & Brown & Bashir, 1999). LTP and LTD of synaptic transmission have been extensively studied in the rat brain and are defined as an increase and a decrease in synaptic efficacy respectively, following either high-frequency stimulation (HFS) in the case of LTP, or low-frequency

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stimulation (LFS) in the case of LTD (Bear & Malenka, 1994; Bliss & Lomo, 1973; Howland & Wang, 2008; Goriounova & Mansvelder, 2012; Hunt & Castillo 2012; Lee & Kirkwood, 2011). Both LTP and LTD are thought to be biologically plausible models for the processes engaged during memory formation and storage (Bear & Malenka, 1994; Howland & Wang, 2008; Hunt & Castillo 2012; Lamont & Weber,2012).

It is widely accepted that the facilitatory effect observed with PPF is related to presynaptic mechanisms (Manita & Suzuki & Inoue & Kudo & Miyakawa, 2007; M¨uller & Felmy & Schneggenburger, 2008; Wu & Saggau, 1994; Zucker, 1973, 1989). This phenomenon is attributed to an increase in the amount of transmitter release in response to the second stimulus, and there is strong evidence that facilitation is caused by residual Ca2+ that remains in the nerve terminal from the Ca2+ influx during a previous action potential (Debanne & Gurineau & Ghwiler & Thompson, 1996; Hess & Kuhnt & Voronin, 1987; M¨uller et al., 2008; M¨uller & Felmy & Schwaller & Schneggenburger, 2007; Zucker, 1989; Zucker & Regehr, 2002). Therefore, PPF is considered to be an example of purely presynaptic plasticity (Angela, 1999; M¨uller et al., 2007; M¨uller et al., 2008; Zucker, 1989).

The mechanisms underlying PPD are poorly understood. Although postsynaptic receptor desensitization can contribute to PPD at some specialized synapses (Otis & Zhang & Trussell, 1996; Trussell & Zhang & Raman, 1993) or under some experimental conditions, most synapses exhibit a strong presynaptic component of PPD (He, 2002; Zucker, 1989, 1999).

A possible complication in understanding LTP is that there might be an interaction between PPF and LTP (Craig & Commins, 2005; Kawashima & Izaki & Grace & Takita, 2006; Maruki & Izaki & Nomura & Yamauchi, 2001 ; Schulz & Cook & Johnston, 1994; Schulz & Cook & Johnston, 1995). The presynaptic expression of paired-pulse plasticity is often used as an analytic tool for interpreting changes in behavior of presynaptic terminals associated with long-term plasticity (Akopian & Musleh & Smith & Walsh, 2000; Wang & Kelly, 1997). Therefore, a change in PPF would provide strong evidence for presynaptic involvement in LTP (Schultz, 1994).

Capsaicin (Cap)-induced depletion of C-fiber afferents result in plasticity of the somatosensory system which is manifested as a functional alteration at different levels of the somatosensory pathway (Komaki & Esteky, 2005; Kwan & Hu & Sessle, 1996; Nussbaumer & Wall, 1985; Sheibani & Shamsizadeh & Afarinesh & Rezvani, 2010; Wall & Fitzgerald & Nussbaumer & Van der Loos & Devor, 1982). Some subclass of capsaicin-sensitive C-fibers provides a primary source for the masking inhibition that normally limits the extent of the receptive fields (RF) of cortical neurons (Calford & Tweedale, 1991). Capsaicin-induced C-fiber depletion causes expansion of excitatory RFs and alters neuronal properties in the somatosensory cortex (Nussbaumer & Wall, 1985). Consistent with these findings it has been shown that C-fiber depletion alters the balance of the excitation and inhibition and expands the RF size of rat barrel cortex cells (Farazifard & Kiani, & Esteky, 2005; Farazifard & Kiani &Noorbakhsh &Esteky, 2005). Also, it has been reported that neonatal capsaicin-induced C-fiber depletion modulates experience-dependent plasticity in the rat barrel cortex (Sheibani & Shamsizadeh & Afarinesh & Rezvani, 2010). Previously, it has been reported that C-fiber depletion using neonatal Cap treatment postponed the development of the neocortical LTP (Komaki & Esteky, 2005). The aim of the present study was to address the question of whether the change acutely induced by Cap takes place in presynaptic sites, postsynaptic sites or both. To address this question, we analyzed the paired-pulse ratio (PPR) of two responses evoked by two successive stimuli at given intervals, because any change in presynaptic sites is expected to accompany changes in PPR (Jiang et al., 2004; Manita et al., 2007; Salazar-Weber & Smith, 2011; Zucker, 1989). PPR is the ratio of the amplitude of the second response to that of the first, depends on the probability of vesicular release at the synapse, and PPR has been used as an easy measure of the release probability (Manita et al., 2007).

2. Methods

2.1. Animals

This study was based on data from 27 male Sprague-Dawley rats. Nine rats were neonatally treated with Cap, and the remainders were vehicle-treated.

Capsaicin treatment: Neonatal albino rats were treated by Cap (50 mg/kg intraperitoneal [i.p.] Sigma; dissolved in saline containing 10% Tween 80 and 10% ethyl alcohol) within 48 h of birth. Control neonates were given an equal volume of the vehicle (only the capsaicin solvent). Treatment of neonatal rats with Cap effectively destroys the majority (95%) of C-fibers (Fitzgerald, 1983; Hiura, 2000; Holzer, 1991; Kiani& Farazifard & Noorbakhsh & Esteky, 2004 Komaki & Esteky, 2005; Sheibani et al., 2010; Kwan et al., 1996; Toldi & Joo & Wolfe, 1992).

Efficacy of Cap treatment in depleting C-fibers was assessed in the current study by corneal chemosensitivity test (Krahl & Senanayake & Handforth, 2001; Sheibani et al., 2010). Corneal chemosensitivity is principally mediated by C-fibers (Holzer, 1991) and its significant reduction is used as a measure of C-fibers depletion. One drop of 1% ammonium hydroxide was applied to the right eye of adult animals and the number of times they wiped their right eye in the first 10 s after application was counted. The corneal chemosensitivity was significantly reduced in the capsaicin-treated (3.9± 0.92) compared with the vehicle-treated (13.1±1.27) (t-test, P<0.001).

2.2. Surgery

Neonatally treated rats and their controls were allowed to mature until they reached a weight between 250 and 300g. Then the animals were anesthetized with ketamine and xylazine (i.p.). The procedure for implantation of recording and stimulating electrodes was as previous experiments (Komaki & Esteky, 2005; Komaki & Shahidi & Lashgari & Haghparast & Malakouti & Noorbakhsh, 2007). In brief, for recording and stimulation, twisted Teflon-coated stainless steel wire (120 m diameter ; Advent) were implanted into somatosensory cortex [AP= -1 to -2, ML= 3.5-4 , DV=2 mm] or corpus callusom (AP= -1 to -2, ML= 2 , DV=3 mm) in the same coronal plane respectively (Paxinous & Watson, 2005) (Fig. 1). Two weeks recovery period preceded experimental testing. All experiments were done in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1996). Rats were housed individually on a 12:12 h light/ dark cycle and tested during the light cycle.

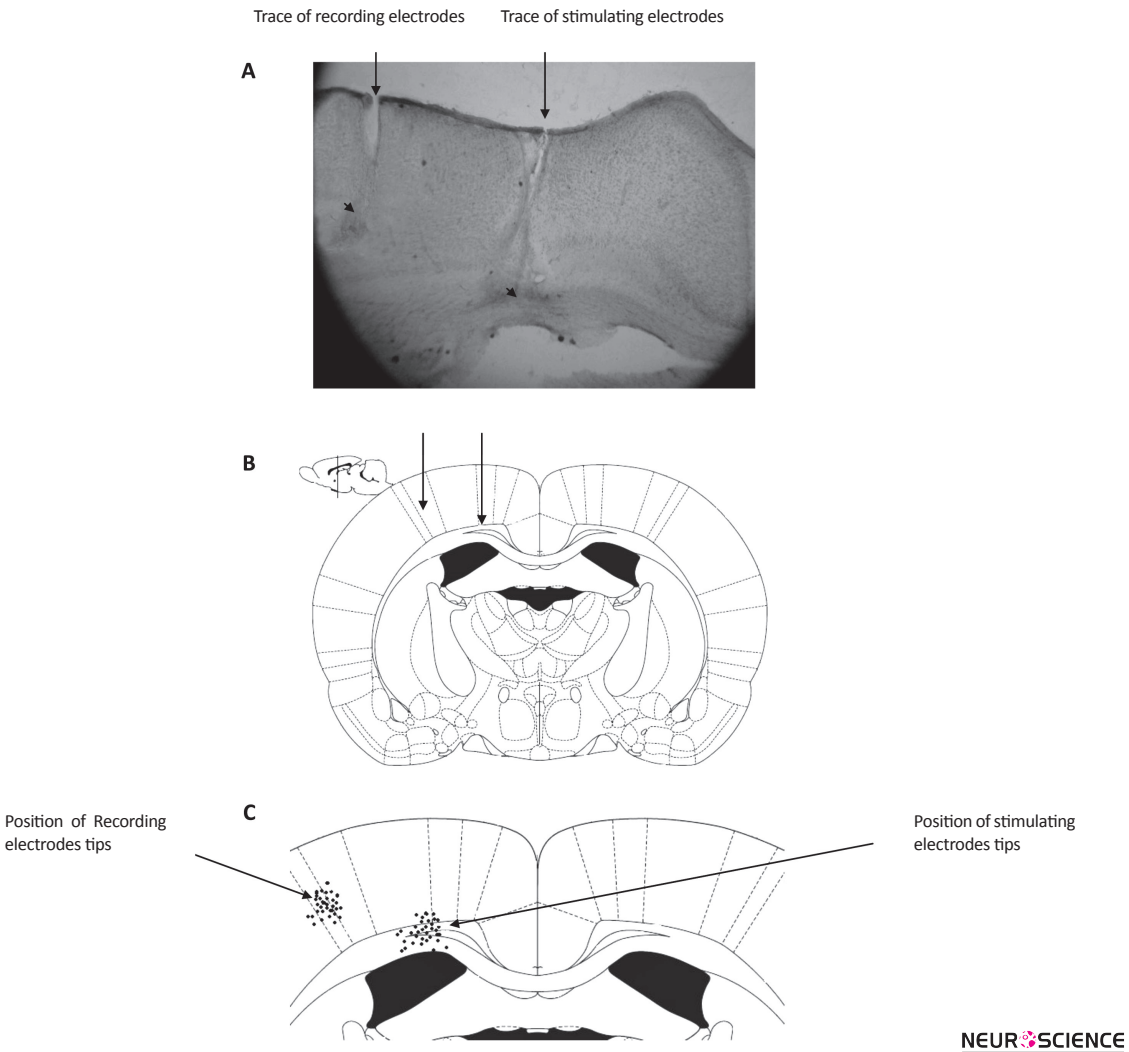

Figure 1. Histology; Electrodes placements in coronal section. A: Photomicrograph scan of a coronal section (50 µm) showing the electrodes trace. The trace of stimulating and recording electrodes can be seen at the right and left side, respectively (arrow). Arrowheads represent the place of stimulating and recording electrodes tips. B and C: Position of stimulating electrodes in corpus callosum and recording electrodes in layers V-VI of neocortical area SI are shown [adapted from Paxinos and Watson (2005)]. Scale bar represents 0.5 mm.

2.3. Input – Output Tests

Input – output (I-O) tests were similar to those described previously (Chapman, et al., 1998; Komaki & Esteky, 2005; Komaki et al., 2007; Trepel & Racine, 1998). Briefly, stimulation pulses were delivered at varying intensities to the appropriate stimulation site (see below) and the resulting cortical evoked field potentials were monitored. Single 0.1 ms biphasic square wave pulses were delivered through constant current isolation units (Pulsemaster: model A300 WPI and Isolator: model A365 WPI) at a frequency of 0.1 Hz. The I-O test included ten responses evoked at each of 10 logarithmically spaced intensities (16, 32, 64, 100, 160, 250, 500, 795, 1000, and 1260 A). The responses were filtered (0.1 Hz to 3 kHz), amplified (Differential amplifier DAM 80 WPI), digitized at 10 kHz, and stored on a computer hard drive. I-O tests were conducted every 2 days during a 1 week baseline period (Before applying HFS) and a 12 days LTP induction period.

2.4. Paired-Pulse Stimulation

Paired-pulse stimuli were used to investigate short-term plasticity (Torii & Tsumoto & Uno & Astreline & Voronin, 1997). PPS was delivered every 10 sec to the corpus callusom at 13 interpulse intervals. In every experiment, the interstimulus interval (ISI) was varied systematically from 20 ms up to 400 ms. Thus, a complete series consisted of a sequence of paired pulses with ISIs of 20, 30, 40, 50, 60, 70, 80, 120, 160, 200, 250, 300 and 400 ms. To compute PPR, the amplitude of the response to the second pulse of the pair was divided by the amplitude of the response to the first pulse in the pair. In each animal, at least three consecutive ISI series were run and averaged (Manita et al., 2007; Rozas et al., 2001).

Paired-pulse tests were conducted on the last baseline day and one day after LTP induction. Pulse intensity was set to evoke an early component at approximately 75% of the maximum amplitude (Chapman et al., 1998; Racine & Milgram, 1983).

2.5. High Frequency Stimulation for LTP Induction

In experiments for LTP induction, 30 high frequency pulse trains were delivered once per day for 12 days (Chapman et al., 1998; Komaki & Esteky, 2005; Komaki et al., 2007). Each 24 ms train consisted of 8 pulses at 300 Hz, and the trains were delivered once every 10 sec. Pulse intensity and duration were 1260 A, and 0.1 ms, respectively.

2.6. Data Analysis

The analysis of the early monosynaptic and late polysynaptic components of field potentials were similar to those described previously (Chapman et al., 1998; Komaki & Esteky, 2005; Trepel & Racine 1998). Changes in the field potentials over PPF, PPD and LTP induction and decay sessions were measured by subtracting the final baseline responses from all other baseline and potentiated responses at a single I/O intensity that best reflected potentiation or depression. The intensity that evoked a response that was approximately half the maximum amplitude was chosen for analysis. This was the sixth intensity in the I/O (250 µA) in almost all cases. The criterion for labeling a response as potentiated was that its change from the baseline average exceed that seen in any of the control responses (Racine & Teskey & Wilson & Seidlitz & Milgram, 1994; Racine & Chapman & Teskey & Milgram, 1995). Changes in response amplitudes were analyzed using mixed design ANOVAs and the Tukey post-hoc test.

2.7. Histology

Rats were deeply anesthetized with urethane (2.0 mg/kg) and perfused through the heart with formol-salin. Brain sections were cut at 50 µm and stained with tionine to verify electrode placements.

3. Results

3.1. Baseline Field Responses

In general, neocortical field potentials were similar to those described previously (Chapman et al., 1998; Komaki et al., 2007; Trepel & Racine 1998). Briefly, they appeared to have two main components: an early surface-negative response (average latency-to-peak 8.8 ms, range 7.0-11.0 ms), and a larger late response (average latency-to-peak 18.8 ms, range 17.0-25.5 ms) (Fig. 2A). (All data are reported as means ± SEM.)

Figure 2 shows neocortical field potentials evoked by paired-pulse stimulation at intervals of 20 (B), 30 (C) and 40 (D) ms, before the HFS application. Paired-pulse stimulation of the Corpus Callusom (20-400 ms ISIs) produced a facilitation of the late component to 134ms ± 10% of the control peak amplitude (Fig. 2B). PPF maximizes between 20 and 30 ms and declines as the ISIs are increased or decreased. The peak of the enhanced response occurred at a longer latency (20.8 ± 2.5 ms). Paired-pulse stimulation of the Corpus Callusom produced a depression of the early component to 20ms ± 10% of the control peak amplitude (Fig. 2B).

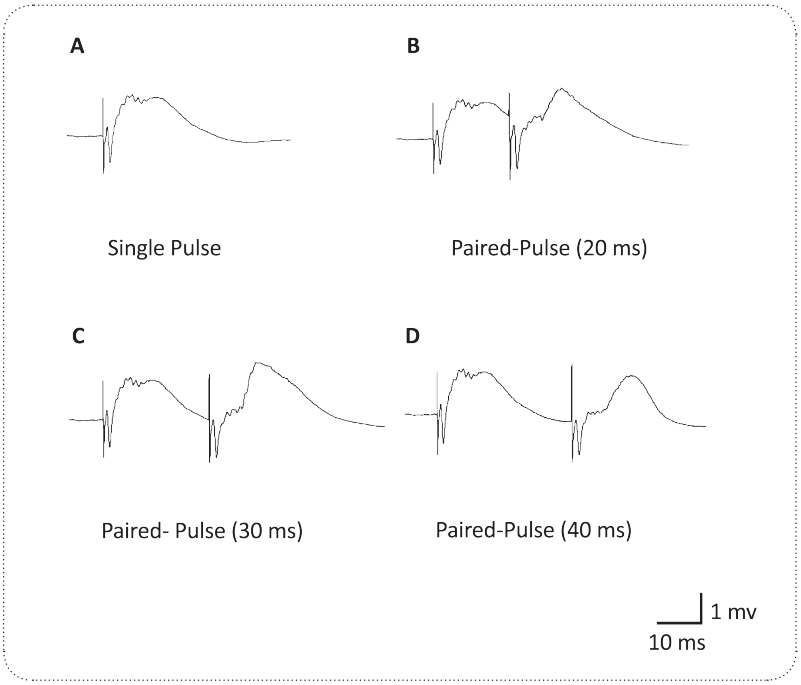

Figure 2. A representative example of filed potential evoked by paired-pulse stimulation at intervals of 20, 30 and 40 ms, before the HFS application. The stimulation intensity was 250 µA.

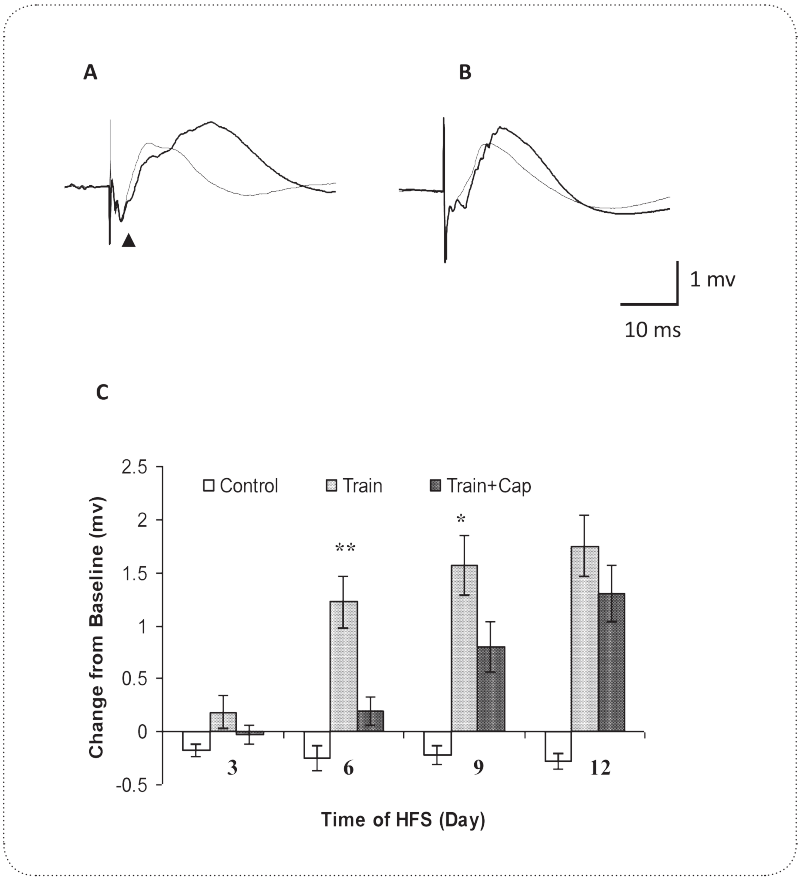

Figure 3. Potentiation effects induced by high-frequency (300 Hz) stimulation. The thin line represents an unpotentiated evoked field potential, while thick line represents an evoked field potential following 6 days of HFS in vehicle-treated (A) and Cap-treated (B) animal. This kind of stimulation caused an enhancement in the repetitive population spike (Arrowhead) activity associated with an apparent reduction in the amplitude of the early monosynaptic component and the enhancement of a longer latency polysynaptic component. The stimulation intensity was 250 µA. C: The effects of Cap on the induction of long-term potentiation in the late component of somatosensory field potential. The mean change from baseline amplitude (±SEMs) of the late polysynaptic components are shown over days. After baseline test session, the train and train + Cap groups received 12 days of high-frequency stimulation trains. Control animals did not receive HFS. Values indicate differences (mV) between the last baseline and all other sweeps for the LTP induction periods. *P < 0.05 and **P < 0.01 compare train + Cap group. High Frequency Stimulation (HFS), Capsaicin (Cap).

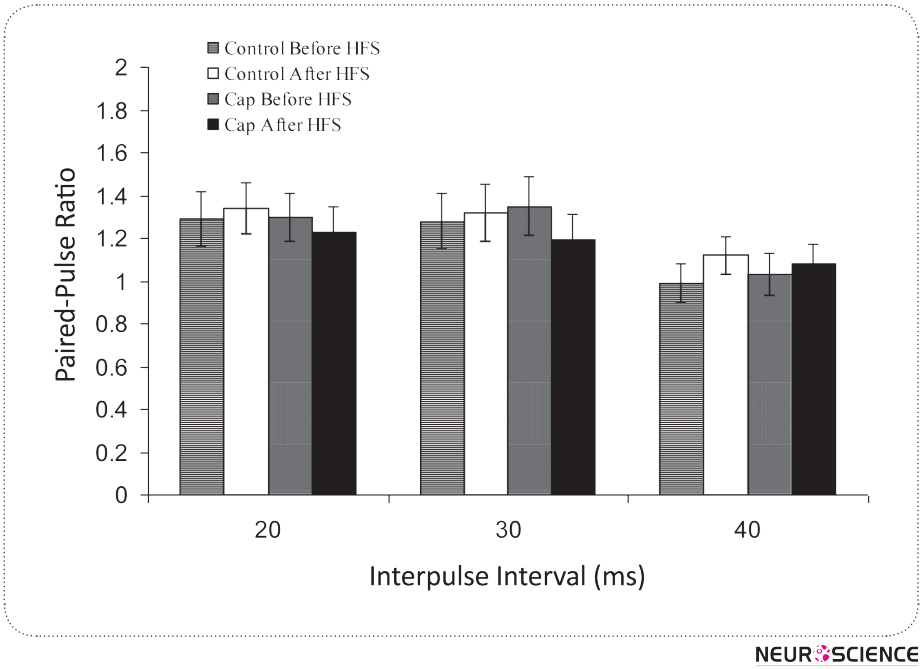

Figure 4. Paired-pulse ratio of evoked field potential was not changed by HFS. In the graph, ratios of the amplitude of the second to first field potentials evoked by paired stimulation at intervals of 20, 30 and 40 ms are plotted for all groups. Values obtained before and after the induction of LTP by 6 days of HFS. All data are expressed as means ± SEM.

High Frequency Stimulation (HFS), Capsaicin (Cap).

3.2. Effects of C-fiber Depletion on LTP Induction

Figure 3 shows representative sweeps taken from vehicle- and Cap-treated animal that received HFS for 6 days. Induction was characterized by an increase in the repetitive population spike activity which masked the enhancement of the early monosynaptic field excitatory postsynaptic potential (fEPSP) and a potentiation of longer latency polysynaptic component (Chapman, et al., 1998; Trepel & Racine, 1998). There was a significant interaction effect between session and group for late component (ANOVA; $P<0.001$), the potential magnitude of both train (n=10) and Cap (n=9) groups were significantly higher compared to the control group (n=8) at the end of the stimulation period (Tukey HSD; $P<0.005$). The train group showed significantly greater potentiation than the Cap-treated group in the 6th day of induction period ($P<0.01$).

3.3. Effects of LTP Induction on PPR of Evoked Potential

In next set of experiments, the ratios of the amplitude of the second to the first field potentials evoked by paired stimulation at intervals of 20, 30 and 40 ms are plotted for each animal (Fig. 4). Paired-pulse stimulation affected all field potential components at intervals < 200 ms. The largest changes occurred at intervals between 20- 30 ms. No significant effects of LTP induction on PPF were observed. In other words, PPR of evoked field potential was not changed by HFS ($P>0.05$). The most remarkable observation in our study was that C-fiber depletion postponed the development of LTP, whereas it had no effect on PPR.

4. Discussion

The LTP characteristics observed following HFS in our study were similar to those observed previously using 300 Hz trains (Chapman, et al., 1998; Komaki et al., 2007; Trepel & Racine, 1998). In summary, the present study has revealed that the LTP induction had no effect on PPR in our control group. Previous study has shown that C-fiber depletion using neonatal Cap treatment postponed the development of the LTP of neocortical late component. Also, it has been reported that there is no effect of C-fiber depletion on the magnitude and time course of the LTP of early components (Komaki & Esteky, 2005). Taken together, this evidence supports claims that the first component is monosynaptic and the later component is polysynaptic (Chapman, et al., 1998; Trepel & Racine, 1998). In addition, the present study has shown that C-fiber depletion using neonatal capsaicin treatment has no effect on PPR.

Findings in previous studies have provided evidence for a role of C-fibers in normal synaptic potentiation and depression in the somatosensory system which supports the notion that the selective inactivation of C-fibers can induce long-evolving shifts in the balance of inhibition and excitation in the somatosensory system (Katz & Simon & Moody & Nicolelis 1999; Komaki & Esteky, 2005). The Cap effects on cortical responses observed in previous experiments have suggested that the effect of Cap on central somatosensory responses is mainly exerted by destruction of C-fibers and not by non-specific systemic actions of Cap (Farazifard & Kiani & Noorbakhsh & Esteky 2005; Kiani et al., 2004; Komaki & Esteky, 2005). Furthermore, the selective impact of C-fiber depletion on the late evoked components suggests that this phenomenon may have a cortical origin (Chapman, et al., 1998; Komaki & Esteky, 2005 Trepel & Racine, 1998).

To explore the place of LTP in the neocortex of freely moving rat, we have examined the effects of neocortical long-term synaptic plasticity on neocortical short-term plasticity following paired-pulse stimulation. The results of our experiments strongly suggest that there is a complex interaction between the expressions of LTP. The change in PPF with LTP was obscured when viewed over all experiments because there were both significant increases and decreases in PPF in individual experiments. The magnitude and sign of the change in PPF appears to be dependent on the initial state of the synapses, and there is a significant correlation between the magnitude of LTP and the change in PPF associated with that particular amount of LTP, comparable to results from others experiments (e.g., Schulz et al., 1995). Synaptic strength is a dynamic property such that a postsynaptic potential exhibits short- and long-term changes in amplitude after different patterns of use. Long-term changes are believed to underlie learning and memory formation (Bear & Malenka, 1994: Howland & Wang, 2008; Hunt & Castillo 2012; Lamont & Weber,2012), whereas those that are shorter lasting and that occur on the time scale of seconds or less play an important role in the dynamic function of neural networks (Waldeck & Pereda & Faber, 2000). Synaptic short-term facilitation is a Ca2+ dependent elevation of transmitter release probability during repetitive stimulation of synapses that influences the information transfer between neurons (Abbott & Regehr, 2004; M¨uller et al., 2008; M¨uller et al., 2007; Zucker, 1989; Zucker & Regehr, 2002).

The second finding of this study was that C-fiber depletion had no effect on PPR. To properly understand what underlies variation in paired-pulse plasticity among synapses, as well as to use it as a tool, one would have to know what factors determine release probability and how these factors may be altered after a first stimulus (Hanse & Gustafsson, 2001; Salazar-Weber & Smith, 2011). The presynaptic expression of paired-pulse plasticity is often used as an analytic tool for interpreting changes in behavior of presynaptic terminals associated with long-term plasticity (Akopian et al., 2000; Kuhnt & Voronin, 1994; Liao & Jones & Malinow, 1992; Malinow & Tsien, 1990; Salazar-Weber & Smith, 2011; Wang & Kelly, 1997). Postsynaptic factors like the addition or removal of AMPA receptors can influence measures of short- and long-term plasticity (Lee & Kirkwood, 2011; Selig & Hjelmastad & Herron & Nicoll & Malenka, 1995). In support of this view, it has been reported that changes in the percentages of AMPA and NMDA receptors can also influence paired-pulse plasticity due to difference in their voltage dependence of activation (Akopian & Walsh, 2002; Lee & Kirkwood, 2011; Salazar-Weber & Smith, 2011). Also, it has been reported that, a change in PPF would provide strong evidence for presynaptic involvement in LTP (Schultz et al., 1994). More recent study has shown that copper significantly enhanced the PPR in the CA1 region and decreased the PPR in the CA3 region in an LTP-dependent manner. Thus, LTP caused the appearance of a copper-sensitive factor which modulated the PPR. This suggests the involvement of a presynaptic mechanism in the modulation of synaptic plasticity by copper. Inhibition of the copper-dependent changes in the PPR with cyclothiazide suggested that this may involve an interaction with the presynaptic AMPA receptors that regulate neurotransmitter release (Salazar-Weber & Smith, 2011).

The findings presented here provides further evidence that the expression of this form of LTP is postsynaptic and, therefore, is not caused by an increase in neurotransmitter release. Compared with previous studies, the novel aspect of this work is the use of a chronic preparation of freely moving animal to be able to examine the time course of PPF induction and decay and the longer-lasting cortical LTP induced by multiple stimulation sessions in the neocortex.

A major difficulty in the present study was to identify changes to individual synaptic potentials that were contained within a complex synaptic response. Since there is significant temporal overlap among the potentials, changes in one could produce apparent (but not real) changes in another. In conclusion, our findings suggest that the effect of C-fiber depletion on LTP is postsyn-

aptic and, therefore, is not caused by a decrease in neurotransmitter release.

5. Conclusion

Our finding suggests that the effects of C-fiber depletion on LTP is postsynaptic and, therefore, is not caused by a decrease in neurotransmitter release.

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Gene Expression Profile of Calcium/Calmodulin-Dependent Protein Kinase IIα in Rat's Hippocampus during Morphine Withdrawal

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ABSTRACT

Introduction: Calcium/calmodulin-dependent protein kinase II (CaMKII) which is highly expressed in the hippocampus is known to play a pivotal role in reward-related memories and morphine dependence.

Methods: In the present study, repeated morphine injections once daily for 7 days was done to induce morphine tolerance in male Wistar rats, after which gene expression profile of α-isoform of CaMKII (CaMKIIα) in the hippocampus was evaluated upon discontinuation of morphine injection over 21 days of morphine withdrawal. Control groups received saline for 7 consecutive days. For gene expression study, rats' brains were removed and the hippocampus was dissected in separate groups on days 1, 3, 7, 14, and 21 since discontinuation of of morphine injection. A semi-quantitative RT-PCR method was used to evaluate the gene expression profile.

Results: Tolerance to morphine was verified by a significant decrease in morphine analgesia in a hotplate test on day 8 (one day after the final repeated morphine injections). Results showed that gene expression of CaMKIIα at mRNA level on day 1, 3, 7, 14 and 21 of morphine withdrawal was significantly altered as compared to the saline control group. Post hoc Tukey's test revealed a significantly enhanced CaMKIIα gene expression on day 14.

Discussion: It can be concluded that CaMKIIα gene expression during repeated injections of morphine is increased and this increase continues up to 14 days of withdrawal then settles at a new set point. Therefore, the strong morphine reward-related memory in morphine abstinent animals may, at least partly be attributed to, the up-regulation of CaMKIIα in the hippocampus over 14 days of morphine withdrawal.

1. Introduction

Morphine has analgesic and rewarding effects, which is mediated through μ-opioid receptors (Benyamin et al., 2008; Biala & Weglinska, 2006; He, Kim & Whistler, 2009; Walwyn, Miotto & Evans, 2010). Meanwhile, repeated administration of morphine results in the development of tolerance and dependence (Benyamin et al., 2008; Silverman, 2009). The chronic use of morphine gives rise to long-lasting changes in the brain which underlie the behavioral abnormalities associated with morphine dependence and withdrawal syndrome (Nestler, 2002; 2004b). This form of long-term plasticity in the brain requires relative stable changes in gene expression, which may alter neurotransmission and the structure of target neurons (McClung & Nestler, 2008; Robinson & Kolb, 2004). Recent studies have shown that the altered gene expression due to morphine exposure is responsible for behavioral changes in drug addicts (Prenus, Luscar, Zhu, Badisa & Goodman, 2012; Zhu, Badisa, Palm & Goodman, 2012). It has been shown that expression of many receptors and second messengers are affected by chron-

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ic morphine treatment. Among them Ca2+/calmodulin-dependent protein kinase 2 (CaMKII) is of great interest (Chen et al., 2008; Colbran, 2004; Nestler, 2004b; a). However, the underlying molecular and cellular mechanisms in morphine addiction and withdrawal syndrome are yet to be fully understood.

CaMKII is a serine/threonine protein kinase with its α and β isoforms highly expressed in the central nervous system and enriched at synaptic structures especially in the hippocampus (Chen et al., 2008; Martin & Arthur, 2012; Wayman, Lee, Tokumitsu, Silva & Soderling, 2008). CaMKII regulates number of important neuronal functions especially the neurotransmitter synthesis and release, modulation of ion channel activity, synaptic plasticity, learning, memory, and gene expression (Hudmon & Schulman, 2002). The α-isoform of CaMKII (CaMKIIα) plays a pivotal role in learning and memory [for review see (Wayman, Lee, Tokumitsu, Silva & Soderling, 2008)]. It has been reported that transgenic mice lacking the CaMKIIα are defective in long-term potentiation (LTP) and spatial learning (Yamauchi, 2005).

In a sence, opioid tolerance and addiction is a mode of neuronal plasticity similar to learning and memory (Nestler, 2004b). Evidence has shown that CaMKII inhibitors suppress the development and formation of tolerance to morphine when selectively administered to rats' brain (Fan, Wang, Qiu, Ma & Pei, 1999). Furthermore, inhibition of CaMKIIα in the locus coeruleus significantly attenuates some signs of morphine withdrawal (Navidhamidi et al., 2012). It has also been demonstrated that CaMKII activity in the NAc shell is essential for relapse in morphine-seeking behaviors (Liu, Liu, Zhang & Yu, 2012; Liu, Zhang, Liu & Yu, 2012). Investigation on the adaptive change of the level of gene expression caused by chronic morphine treatment is crucial for further understanding of mechanisms underlying morphine addiction (Ammon-Treiber & Hollt, 2005; Zhu, Badisa, Palm & Goodman, 2012). Nevertheless, changes in expression of CaMKIIα- in morphine withdrawal have not been carefully examined. The aim of the present study was to examine gene expression profile of CaMKIIα at mRNA level during 21 days of morphine withdrawal.

2. Methods

2.1. Subjects

Male Wistar rats initially weighing 220-250 g (Pasteur Institute, Iran) were used. They were housed in groups of 4 per cage, and kept in an animal house at a constant temperature (22 ± 2 °C) under a 12 h light/dark cycle (light on at 7:00 a.m.). Animals had free access to food and water except during the experiments. All animals were handled for 5 min/day during the five days adaptation period before the experiments began. All experiments were carried out during the light phase between 8:00 and 12:00. Experimental groups consisted of either eight rats in behavioral experiments or six rats in gene expression experiments. Each animal was tested only once. All procedures were performed in accordance with international guidelines for animal care and use (NIH publication #85−23, revised in 1985).

2.2. Drugs

The drug used in this study was morphine sulfate (Temad Co., Tehran, Iran). The drug was dissolved in 0.9% saline and administered 1 ml/kg through an intraperitoneal (i.p.) route.

2.3. Induction of Morphine Tolerance

Morphine tolerance was induced by daily i.p. injection of morphine (10 mg/kg) for 7 consecutive days in rats. Twenty four hour after the last injection, morphine tolerance was examined by a hotplate test of analgesia.

2.4. Antinociception Test

The temperature of hot plate was maintained at 52± 0.1°C. Animals were placed in a glass square on the hotplate apparatus (Armaghan Co., Iran) to prevent escaping from the hotplate. Then, the latency for licking of the hind paws or first jump was recorded as an index for pain reaction latency. A cutoff time of 80 s was defined as complete analgesia. Eight groups of either saline- or morphine- treated animals (n=8) were used to assess morphine analgesia. Four groups of these animals as controls (naïve) received repeated injections of saline (1 ml/kg) , and the other four groups received repeated injections of morphine (10 mg/kg) for seven consecutive days. One day after the final repeated injections, all four groups of animals received saline (1 ml/kg) or different doses of morphine (5, 7.5 and 10 mg/kg). In 30 min, they were submitted to the hotplate test to have their pain reaction latencies examined.

2.5. Tissue Extraction and Preparation

To examine the gene expression profile during 21 days of withdrawal, in different groups of rats (n=5), brain excision were done on on days 1, 3, 7, 14 and 21 after the final repeated injection of morphine (induction

of morphine tolerance). Assessment of CaMKIIα mRNA levels in the hippocampus was performed using 80-100 mg of the intended tissue. In brief, each rat was decapitated, the whole brain was quickly removed from the skull, the hippocampus was immediately separated from the whole brain on an ice-chilled sterile surface according to a method previously described (Chiu, Lau, Lau, So & Chang, 2007). After harvesting, the tissues were immediately submerged in RNAlater RNA stabilization reagent (QIAGEN) and, incubated overnight in the reagent at 4˚C, then stored at –70°C until further analysis.

2.6. Total RNA Extraction and cDNA Synthesis

Total RNA extraction was performed using a Trizol method with some modification. Each tissue sample was transferred to a new tube containing 1 ml lysis buffer (RNX+ reagent, Cinagen, Tehran, Iran). The tissue was then homogenized for 60 sec (Silent Crusher S., Heidolph, Germany), and subjected to total RNA extraction by a high pure tissue RNA extraction protocol. Total RNA was separated through a 1% agarose gel to assess quality of 28s and 18s ribosomal RNA. The concentrations of total RNA were also measured by a Biophotometer (Ependorph, Germany). Then, a semi-quantitative RT-PCR method was used to assess gene expression (Marone, Mozzetti, De Ritis, Pierelli & Scambia, 2001). RT reaction was performed using Viva 2-step RT-PCR Kit according to manufacturer's protocol (Vivantis, Malaysia). In brief, the RT reaction was performed using 0.3 µg/µl of total RNA as a template, random hexamer primer and M-MuLV reverse transcriptase enzyme. The reactions were incubated at 42˚C for 60 min, and then inactivated at 85˚C for 5 min.

The polymerase chain reaction (PCR) was used for amplification of β-actin (as an internal standard) and CaMKIIα genes. Primers for both genes were designed at exon-exon junctions with the following sequences:

β-actin forward primer, 5□-

CTGGGTATGGAATCCTGTGGC-3□;β-actinreverse primer, 5□-

CAGGAGGAGCAATGATCTTGATC-3□; CaMKIIα forward primer, 5□-

AGGAGGAAACTGAAGGGAG -3□; CaMKIIα reverse primer, 5□-

CAGGGTCGCACATCTTCGTG -3□.

PCR was carried out in a reaction volume of 20 µl consisting of 10 µl PCR Master Mix (Fermentase), 2 µl cDNA, 1 µl upstream and downstream mix of CaMKIIα primers (10 µM), 1 µl upstream and downstream mix of β-actin primers (10 µM) and nuclease free water up to 20 µl. The reaction parameters were adjusted to obtain a condition with a linear relation between the number of PCR cycles and PCR products, and with linear relation between the initial amount of cDNA template and PCR product. Therefore, thermal cycling was initiated with a first denaturation step of 95˚C for 3 min, followed by 29 cycles of thermal cycling of 94˚C for 30 sec (denaturation), 60˚C for 30 sec (annealing), 72˚C for 30 sec (extension), followed by 10 min final extension at 72˚C (C1000 Thermal Cycler, Biorad, USA). The PCR products were subsequently analyzed on 2% agarose gel electrophoresis, and respective bands were quantified by a densitometry method using an Image J software. Then, ratio of band density of CaMKIIα/β-actin was used as an index of gene expression.

2.7. Profiling mRNA Level of CaMKIIα in the hippocampus on days 1, 3, 7, 14 and 21 days of Morphine Withdrawal in the Hippocampus

Six groups of rats (n=6 per group) were used to assess CaMKIIα gene expression in the hippocampus. The first group as control received saline (1ml/kg) for 7 days, and their brains were excised one day after the final repeated injection. Other five groups received a daily injection of morphine (10 mg/kg) for 7 consecutive days, and were submitted to the brain excision on days 1, 3, 7, 14 and 21 since the final injection, respectively.

2.8. Statistical Analysis

For the hotplate experiment data, one-way ANOVA was used to determine the analgesic effect of morphine in different groups. Two-way ANOVA was used to determine the main effect of morphine tolerance (as Factor A) and group (as Factor B), and their interaction in the hotplate test. Further post hoc comparisons were made using Tukey's test. To analyze the semi-quantitative RT-PCR data, the intensity of nucleic acid bands on agarose gel electrophoresis was converted to quantitative values using an Image J program. Subsequently, the results were analyzed by one-way ANOVA to investigate overall differences between groups. Upon presence of a significant F value, Tukey's test was used to assess 'between groups' paired comparisons. $P<0.05$ was considered as a significant statistical level throughout.

3. Results

3.1. Antinociception Test Revealed that Repeated Administrations of Morphine for 7 Days Induced Morphine Analgesic Tolerance

Based on one-way ANOVA, morphine altered pain reaction latency in control rats which received 7 days pretreatment of repeated administrations of saline [$F(3, 28) = 13.51$, $P<0.001$]. Post hoc analysis with Tukey's test showed that morphine at the dose of 10 mg/kg, significantly induced analgesia in naïve rats as revealed by an increase in pain reaction latency as compared to saline control group. One-way ANOVA also showed that morphine could not alter pain reaction latency in rats which had received pretreatment of 7 days repeated administrations of morphine (10 mg/kg) [$F(3, 28) = 0.5$, $P>0.05$]. Post hoc analysis with Tukey's test showed that the analgesic effect of morphine at the dose of 10 mg/kg in rats which received repeated injections of the drug was significantly attenuated as compared to the analgesic effect of the same dose of morphine in naïve rats which received saline during the repeated injections. The later results indicated morphine-induced analgesic tolerance (Fig. 1).

3.2. Effect of Morphine Tolerance on mRNA level of CaMKIIα in the Hippocampus During 21 Days of Withdrawal

Analysis of the results with one-way ANOVA revealed that gene expression of CaMKIIα in the hippocampus was significantly altered during 21 days of morphine withdrawal [one-way ANOVA, $F(5, 24) = 2.7$, $P<0.05$]. Post hoc analysis with Tukey's test also revealed that gene expression of CaMKIIα in the hippocampus on day 14 of withdrawal was significantly increased ($P<0.05$). Meanwhile, there were no significant changes in CaMKIIα gene expression on days 1, 3, 7 and 21 of withdrawal period compared to the control saline- treated group (Fig. 2).

4. Discussion

The results of the present study showed that repeated injections of morphine for seven days induced morphine tolerance as revealed by a decrease in morphine analgesia in a hotplate test. This is similar to what has previously been reported (Sepehrizadeh et al., 2008a; Sepehrizadeh et al., 2008b). It has been proposed that repeated morphine injections can enhance agonist potency, hence increase receptor desensitization and promote tolerance (Bohn, Lefkowitz & Caron, 2002; Ingram, Macey, Fossum & Morgan, 2008). On the contrary, some studies have suggested that morphine tolerance may derive from a lack of rapid desensitization, resulting in other adaptive, and potentially slowly reversible changes becoming dominant [for review see (Martini & Whistler, 2007)].

In the present study, after induction of morphine tolerance, we examined the gene expression profile of CaMKIIα during 21 days of morphine withdrawal. According to the results, mRNA level of CaMKIIα in the hippocampus was significantly increased on day 14 of morphine withdrawal. Our results indicated no significant difference in gene expression of CaMKIIα in the hippocampus on days 1, 3, 7 and 21 of morphine withdrawal as compared to the saline control group. A return to a new set point in expression of CaMKIIα after 21 days was observed. It is possible that the gene expression of CaMKIIα in the hippocampus during repeated morphine injections which began to increase, would continue up to 14 days of morphine withdrawal, and then return to a new set point. Chen et, al. (2008) has reported that chronic morphine treatment (20 mg/kg) for 6 days, but not a single injection of morphine, increased CaMKIIα expression in the hippocampus (Chen et al., 2008). In contrast to this, we administered morphine at the dose of 10 mg/kg for 7 days, and did not observe a significant change in CaMKIIα expression in the hippocampus on day 1 after the last morphine injection. A possible explanation for different results may be the difference in applied doses of morphine during repeated injections. Explanation of the variability in the hippocampal CaMKIIα expression after morphine injection on days 1, 2, 3, 4, 5, 6 and 7 may be an interesting issue for which more experiments should be designed.

Chronic morphine administration has been shown to induce a down-regulation of mu-opioid receptors and number of receptors available (Bernstein & Welch, 1998; Lopez-Gimenez, Vilaro & Milligan, 2008; Takasaki, Nojima, Shiraki & Kuraishi, 2006; Zhu, Badisa, Palm & Goodman, 2012). Opioids have also been shown to modulate excitatory and inhibitory synaptic transmission in the hippocampus (McQuiston, 2007; Salmanzadeh, Fathollahi, Semnanian & Shafizadeh, 2003). Additionally, chronic treatment of three-week with methadone or morphine had no significant changes in phosphorylated CaMKII in the hippocampus at one hour and one week after treatment (Andersen, Klykken & Morland, 2012). It has been reported that subcutaneous implantation of morphine pellets for 6 days in mice increases the levels of CaMKIIα mRNA in spinal cord tissue, which may suggest that CaMKIIα expression in the spinal cord tissue might have contributed to

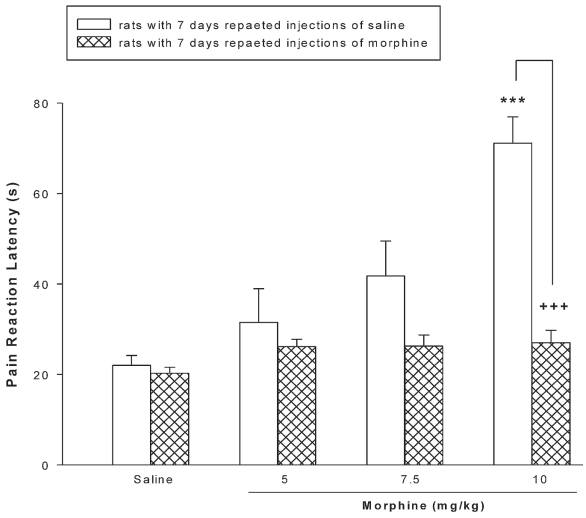

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Figure 1. Repeated injections of morphine induced analgesic tolerance to the drug. Eight groups of animals were used. Four groups of them received 7 days repeated administrations of saline, and 1 day after the final injection (day 8) they received saline (1 ml/kg) or one dose of morphine (5, 7.5 and 10 mg/kg) 30 min before the hotplate test. The other four groups of the animals received 7 days repeated administrations of morphine (10 mg/kg), and 1 day after the final injection (day 8) they received saline (1 ml/kg) or one dose of morphine (5, 7.5 and 10 mg/kg) 30 min before the hotplate test. Each bar represents mean±S.E.M. of pain reaction latency related to eight rats per group. Two-way ANOVA revealed significant effects of repeated injections of morphine (factor A) and groups (factor B), and their interaction [for factor A, $F(1, 56) = 17.59$, $P<0.001$; for factor B, [$F(3, 56) = 14.16$, $P<0.001$; for factor A * factor B, [$F(3, 56) = 10.83$, $P<0.001$]. ***$P<0.001$ compared to control group that received saline on the test day. +++$P<0.001$ compared to respective group of saline pretreated that received morphine 10 mg/kg on the test day.

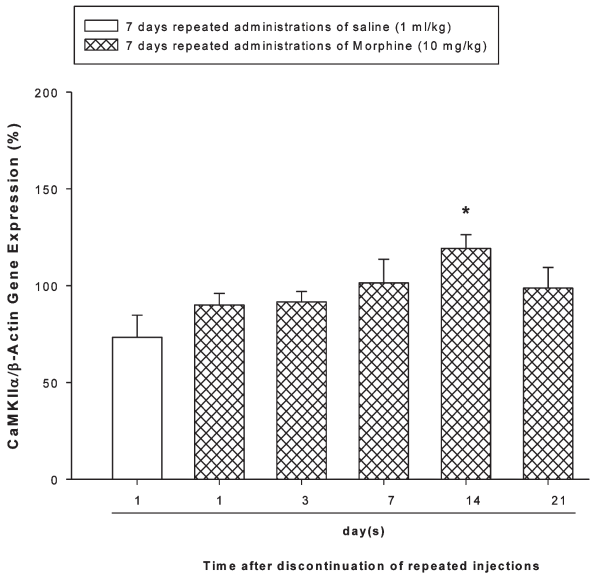

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Figure 2. Profiling the ratio of CamKII/β-actin mRNA expression in the hippocampus during 21 days of morphine withdrawal. Six groups of rats were used. One group of rats (n=6) as control group received seven days repeated injections of saline (1 ml/kg), and their brain were excised 1 day after the final injection to examine gene expression in the hippocampus. The other five groups of rats received seven days repeated injections of morphine (10 mg/kg), and their brains were excised on days 1, 3, 7, 14 and 21 after the final injection of morphine to examine gene expression profile in the hippocampus. Each bar represents mean±S.E.M. related to the ratio of CamKII/ β-actin band density of 5 rats per group. *$P<0.05$ compared to the saline treated control group.

the development of morphine tolerance in mice (Liang, Li & Clark, 2004). Based on the prespecified goals of the current investigation, we profiled gene expression of CaMKIIα during 21 days of withdrawal, with the results showing a significant increase in CaMKIIα gene expression at 14 days of withdrawal and no significant changes on days 1, 3 and 7. It is possible that CaMKIIα expression in the hippocampus significantly increases after a time window (14 days) since the chronic morphine treatment ceased. In support of this result, studies using a chronic administration of morphine (20 mg/kg for 9 consecutive days) showed that this treatment did not significantly affect mRNA of CaMKIIα in the spinal cord, brain stem and hippocampus one day after discontinuation of morphine treatment (Lou, Zhou, Wang & Pei, 1999). ,On the other hand, the results of the present study suggest that CaMKIIα expression and subsequent changes in the hippocampus may contribute to behavioral signs of morphine withdrawal.

Of the CaM kinases, CaMKII has particularly been proposed as a candidate molecule for the long-term storage of information because of its ability to remain phosphorylated in the absence of CaM (Lisman, Schulman & Cline, 2002). Once activated, CaMKII can phosphorylate a number of intracellular targets including AMPA receptors (Poncer, Esteban & Malinow, 2002), NMDA receptors (Bayer, De Koninck, Leonard, Hell & Schulman, 2001; Bayer & Schulman, 2001), and L-type calcium channels (Dzhura, Wu, Colbran, Balser & Anderson, 2000). Based on the role of AMPA, NMDA, and L-type calcium channels in the development of be-

havioral sensitization, it is possible that an increase in the expression of CaMKIIα during morphine injections and subsequently during withdrawal may activate these target molecules, and subsequently result in development of dependence and reward-related memory in the hippocampus. In support of the above suggestion, it has been reported that among four subunits of CaMKII namely α, β, γ and δ; , the α-subunit plays a crucial role in learning and memory, LTP and neuronal plasticity (Hudmon & Schulman, 2002). Numerous studies have also indicated that the stimulation of Ca2+ permeable NMDA receptors in the hippocampal neurons results in translocation of CaMKII to the postsynaptic density in the spine head (Colbran, 2004). Moreover, CaMKII association with the postsynaptic density can be either transient or prolonged (Shen, Teruel, Connor, Shenolikar & Meyer, 2000). Therefore, chronic morphine treatment may increase gene expression of CaMKIIα and its levels at postsynaptic density, which would in turn, improve or result in the rewarding memory for morphine up to 14 days of withdrawal. In support of the above hypothesis, it has been reported that chronic but not acute microinjection of CaMKII inhibitors into the hippocampus is able to prevent the development of opioid tolerance (Fan, Wang, Qiu, Ma & Pei, 1999). It is thus plausible that the withdrawal syndrome, drug craving, and relapse to morphine during withdrawal may at least partly result from the potentiated synaptic strength in the hippocampus, which may be affected indirectly by CaMKIIα.

5. Conclusions

According to the obtained results in this study, there was an increase of CaMKIIα expression during morphine withdrawal being significant on day 14 of withdrawal and then subjected to , a return to a new set point thereafter. Our study suggests a possible link between the CaMKIIα expression in the hippocampus and behavioral changes associated with morphine withdrawal syndrome. Finally, a better understanding of such changes in CaMKIIα expression in the brain may hopefully provide new targets for the treatment and control of opiate addiction.

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Role of Propolis on Oxidative Stress in Fish Brain

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ABSTRACT

Introduction: Cypermethrin causes its neurotoxic effect through voltage-dependent sodium channels and integral protein ATPases in the neuronal membrane. Brain and nerve damage are often associated with low residual level of pesticides. In vitro and in vivo studies have also shown that pesticides cause free radical-mediated tissue damage in brain. Propolis has antioxidant properties. The main chemical classes found in propolis are flavonoids and phenolics. Bioflavonoids are antioxidant molecules that play important roles in scavenging free radicals, which are produced in neurodegenerative diseases and aging.

Methods: To determine the protective role of propolis, rainbow trouts were treated with cypermethrin, followed by biochemical analyses of brain tissue. Fish were divided into four groups: control, propolis-treated, cypermethrin-treated, and cypermethrin+propolis-treated.

Results: In fish brains, catalase (CAT) activity decreased ($P \leq 0.001$) and malondialdehyde (MDA) level increased ($P \leq 0.001$) in cypermethrin-treated group compared to control group. In cypermethrin + propolis-treated group CAT activity increased ($P \leq 0.001$) and MDA level decreased ($P \leq 0.001$) compared to cypermethrin group.

Discussion: The results demonstrated that the negative effects, observed as a result of cypermethrin treatment, could be reversed by adding supplementary propolis. Propolis may improve some biochemical markers associated with oxidative stress in fish brain, after exposure to cypermethrin.

1. Introduction

Cypermethrin, a synthetic pyrethroid, is widely used as a pesticide. Like other insecticides, the widespread use of cypermethrin has been associated with adverse effects on nontarget species. Consistent with its lipophilic nature, cypermethrin has been found to accumulate in body fat, skin, liver, kidneys, adrenal glands, ovaries, and brain. Cypermethrin exerts its neurotoxic effect through voltage-dependent sodium channel and integral protein ATPase in the neuronal membrane. In vitro and in vivo studies have also shown that it causes free radical-mediated tissue damage in brain, liver, and erythrocytes (Tao et al., 2008). After use, cypermethrin is released directly into the environment, enters water sources and effects the aquatic ecosystem. Large-scale population declines of many species of birds, mainly fish-eating and bird-eating species, have been attributed to exposure to insecticides through higher order food chain and upward biomagnification of residues (Muthuviveganandavel et al., 2008).

To reduce damage to environment by pesticides, biological systems have developed protective means which include antioxidant molecules. When toxic agents ex-

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haust the endogenous levels of antioxidants, exogenous antioxidative and protective compounds may be substituted to minimize adverse health outcomes (Devillers et al., 2001). The investigation of new antioxidants as potential therapeutic agents is an active field of biochemistry. A variety of organic forms of antioxidant molecules have been studied as natural therapeutic and preventive agents. Propolis, is a phenolic compound which may be capable of preventing apoptosis by decreasing oxidative stress (Kanbur et al., 2009).

Fish is an important aquatic organism. Fish products are an important source of protein for human consumption (Duran and Talas, 2009). Aquatic organisms can provide model systems for investigation of how reactive oxygen species (ROS) damage cellular compounds, how cells respond, how repair mechanisms reverse this damage, and how oxidative stress can lead to disease. Oxidative stress has become an important item for aquatic toxicology.

The most widely used assay for lipid peroxidation is malondialdehyde (MDA) formation which represents the secondary lipid peroxidation product with the thiobarbituric acid reactive substances test. MDA is the final product of lipid peroxidation. The concentration of MDA is a measure of free radical damage to lipids. Lipid peroxides could change the properties of biological membranes, resulting in eventual cell damage (Alirezaei et al., 2012).

In order to minimize such damage, cells have evolved defense systems comprising both enzymatic and nonenzymatic processes. An example of an enzymatic defense system, is the antioxidative enzyme catalase (CAT). Flavonoids comprise a nonenzymatic antioxidant molecule. Flavonoids are potent antioxidants, free radical scavengers and metal chelators. The different antioxidant properties of these compounds help to prevent the irreversible lipid peroxidation which occurs with oxidative stress (Haq et al., 2012).

In this study, rainbow trout (Oncorhynchus mykiss), one of the most popular cultural fish in Turkey, was chosen as the model organism in the human diet. We investigated the effects of the antioxidant propolis on biochemical parameters (MDA and CAT) in brain tissues of farmed rainbow trout.

2. Methods

2.1. Experimental Section

The rainbow trout (Oncorhynchus mykiss) were purchased from Camardı, Ecemis fish farm (Nigde, Turkey). Fish were fed for 15 days in a 8 x 5 x 1.5 m stock aquarium to be acclimatized. After adaptation period, 8 fish were transferred to 200 L water tank filled with natural spring water. Fish used in this study had an average weight of 245.51±5.22 g and length of 29.75 ± 3.81 cm physical and chemical properties of water are depicted in table 1.

Table 1. Some parameters of the water used in the experiment

Parameter (ppm)	Before treatment	After Treatment
Dissolved Oxygen	7.8 ± 0.2	7.6 ± 0.1
Chemical Oxygen Demand	15.1 ± 0.1	16.2 ± 0.2
Suspended Solids	36.8 ± 1.2	40.1 ± 1.7
Calcium	126.0 ± 1.5	114.1 ± 1.1
Sodium	22.4 ± 0.8	19.7 ± 0.7
Chloride	16.0±1.5	18.0±1.4
Total Nitrogen	5.8 ± 0.2	6.8 ± 0.3
Hardness (CaCO3)	174.3 ± 3.1	168.2 ± 2.8
Temperature (oC)	11.5 ± 1	12 ± 0.7
pH	7.7 ± 0.1	7.7 ± 0.1

2.2. Preparation of Propolis Extractive Solution

Method constructing propolis extraction may influence its activity, because different solvents solubilize and extract different compounds. There are three methods commonly used for extraction with ethanol, methanol and water. The chemical composition or propolis is very complex: content of more than 300 components have been identified, and its composition is directly dependent with the composition of the vegetation of the region. Moreover, propolis composition is completely variable creating a problem for the medical use and standardization. In the present work, propolis was collected from a farm at village Kocaavsar in Balikesir, Turkey. Propolis was dissolved to 30 % in ethanol, protected from light and moderately shaken for 1 day at room temperature. Afterward, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until use (Mani et al., 2006).

Biochemical parameters of rainbow trout treated propolis at various doses were investigated, and the effects of 10 ppm propolis were outlined (Talas and Gulhan, 2009).

2.3. Experimental Design

Thirty two rainbow trouts were divided into four groups, each consisting of eight animals. Each rainbow trout was weighted just before the start of the study. Rainbow trout were grown in fresh water supplemented with either, 0.0082 ppm cypermethrin (Atamanalp et al., 2002a; Atamanalp et al., 2002b), 10 ppm propolis (Talas and Gulhan, 2009), or 0.0082 ppm cypermethrin + 10 ppm propolis (Gulhan et al., 2012). Control group was grown in fresh water without any supplementation. The animals in the experimental groups were treated with 0.0082 ppm cypermethrin and/or 10 ppm propolis for 96 h. Fish were fed Excel Pond trade mark pellet feed during experiments. Fish experiments were performed in accordance with the guidelines for approved by the Committee of Animal Experiments at Cumhuriyet University, Sivas, Turkey.

2.4. Preparation of Tissues for Biochemical Analyses

After exposures, 2 mL of blood was obtained from caudal vein of rainbow trout. Brain tissue of fish were removed and frozen in liquid nitrogen. Tissues were stored at −80°C until used. The tissues were separated into two parts for determination of CAT activity or lipid peroxidation. Tissues were weighed and then homogenized in 100 mL of 2 mM phosphate buffer, pH 7.4 using PCV Kinematica Status Homogenizer. Homogenized samples were then sonicated for 1.5 min (30 s sonications interrupted with 30 s pause on ice). Samples were then centrifuged at 12,000g for 15 min at 4°C and supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at −80°C. Supernatants were used for determination of total protein and measurement of CAT activity. The second part of tissues homogenate was used for lipid peroxidation analysis. Tissues were washed three times with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The homogenates were assayed for MDA, the product of lipid peroxidation.

2.5. Protein Assay

Supernatants of tissues were used for determination of total protein. Total protein was quantified by the colorimetric method of Lowry et al. using BSA as the standard (Lowry et al., 1951).

2.6. Analysis of CAT Activity

The CAT activity in the tissues was determined by measuring the decompositon of hydrogen peroxide at 240 nm, according to the method of Aebi. It was expressed as kU/g protein, where k is the first-order rate constant (Aebi, 1984).

2.7. Measurement of MDA Levels

Tissue MDA levels were determined spectrophotometrically using thiobarbituric acid (TBA) solution (Yagi, 1984). The reaction mixture containing tissue homogenate, phosphoric acid, TBA and sulfuric acid was heated 60 minutes in a boiling water bath. After cooling, n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and absorbance was measured at 532 nm. For quantification an external standart curve was prepared using 1, 1, 3, 3 tetraethoxypropane. Values were expressed as nmol/g tissue.

2.8. Statistical Analysis

Biochemical data were analyzed with SPSS 9.0 for Windows using one-way analyses of variance (ANOVA). Differences between means were determined using Duncan's multiple range test in which the significance level was defined as ($P \leq 0.001$).

3. Results

Effects of cypermethrin and propolis on CAT activities and MDA levels on rainbow trout brain is shown

in table 2. CAT activity decreased in the brain tissue of cypermethrin treated fish compare with control group (P≤0.001) (Table 2). There was no statistically significant change (P≥0.001) in CAT activity of propolis treated group compared with control group in the brain tissue of fish (Table 2). There was statistically significant increase in CAT activity of L- cypermethrin+propolis group compared with cypermethrin exposed group (P≤0.001) in the brain tissue of rainbow trout (Table 2).

There was a statistically significant increase (P≤0.001) in MDA after cypermethrin exposure compared with control group. In propolis treated group there was a significant reduction (P≤0.001) of MDA levels compared with control and cypermethrin groups (Table 2). Propolis plus cypermethrin exposure caused a statistically significant decrease (P≤0.001) in MDA levels compared to cypermethrin treated and control groups in the rainbow trout brain tissues (Table 2).

Table 2. Changes on the MDA levels and CAT activities in rainbow trout brain tissues

Groups	MDA (nmol/g tissue ± SEM)	CAT (kU/g protein ± SEM)
Control	17,66±0,72c	0,0125±0,0026a
Cypermethrin	22,86±1,48b	0,0056±0,0003b
Propolis	10,38±0,54a	0,0110±0,0021a
Cypermethrin+propolis	11,78±0,38b	0,0129±0,0015a

All data points are the average of n=8 with ±STDEV.

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abcstatistically significant (P≤0.001)

4. Discussion

Toxicants can have a important role in the development and progression of many disease processes. Exposure to contaminants will depend on the particular dietary and ecological lifestyles of the aquatic organisms. Problems of mutagenicity, teratogenicity, or carcinogenicity with pyrethroids are rare, as they do not appear to pose serious residue problems in food and food products. Membrane phospholipids of aerobic organisms are permanently subjected to oxidant challenges from endogenous and exogenous sources. Peroxidized membranes and lipid peroxidation products represent constant threats to aerobic cells. The most widely used assay for lipid peroxidation is MDA formation as a secondary lipid peroxidation product, with the thiobarbituric acid reactive substances test. The concentration of MDA is the direct evidence of lipid damage caused by free radicals (Orun et al., 2011; Talas and Duran, 2012).

The first line of defense mechanism against damaging effects of ROS are antioxidant enzymes such as CAT which directly scavenges the superoxide radicals and hydrogen peroxide, converting them to less reactive species. Superoxide dismutase catalyzes the dismutation of $O2^{\cdot}-$ to H2O2, and CAT reduces H2O2 to 2 H2O (Atli et al., 2006). Increased level of MDA and a reduction in the activity of CAT were observed in brain tissue of cypermethrin exposed fish. Thisis in agreement with previous reports (Ates et al., 2008; Orun et al., 2008; Ventura-Lima et al., 2009; Orun et al., 2011; Gogebakan et al., 2012; Oliva et al., 2012).

Fish exposed to cypermethrin show a tendency toward decreased antioxidant enzyme activity. In the present study, CAT activity in fish brain was decreased by pesticide exposure. Similar findings have been reported by several researchers (Orun et al., 2008; Ventura-Lima et al., 2009; Orun et al., 2011; Oliva et al., 2012).

Negative correlations were found between CAT activities and MDA levels. Lipid peroxidation, considered a complex self propagating process producing high levels of cell degradation, increases the rigidity (decreases the fluidity) of cellular membranes (Ates et al. 2008; Talas et al. 2008).

Antioxidative effects have been noted with various food and natural products. Most recent studies have shown that natural protective compounds have gained popularity as some of the widely used synthetic pharmaceuticals and therapeutics might have some unexpected adverse effects. Propolis may be a potential natural therapeutic and preventive agent against pesticide damage. propolis. Natural antioxidants are essential for homeostasis in many biological systems. Due to antioxidant and preservative properties of propolis, it may both prolong the physiological functions of some aquatic organisms and contribute to the health benefit of consumers that consume aquatic animals. Increased CAT activity

after propolis treatment in fish exposed to cypermethrin may be related to antioxidant effect of propolis. Our results are in accordance with previous reported results (Ramanathan et al., 2003; Nandi et al., 2008).

Propolis has flavonoids and phenolic compounds. Flavonoids are potent antioxidants, free radical scavengers, and they inhibit lipid peroxidation and exhibit various physiological activities, including antihypertensive and vasodilating activities. In this study, a protective role for propolis was demonstrated.

Propolis is a therapeutical natural substance. It supports the immune system and has antioxidative properties. There are many studies concerning the therapeutic effects of propolis on biochemical and physiological changes in organisms (Gulhan et al., 2012; Talas et al., 2012). Propolis extracts, prepared with ethanol were shown to have positive effects in both animals and patients (Talas and Gulhan, 2009; Gogebakan et al., 2012). We demonstrated that the negative effects, observed as a result of cypermethrin exposure, could be reversed by adding supplementary propolis.

Many reports indicate that propolis and its constituents protect against neuronal death at least partly by the mediation of their antioxidant activity. Numerous studies have led in recent years to the idea that different propolis samples could be completely different in their chemistry and biological activity. Kasai et al. (2011) reported that Chinese propolis contains many biologically active constituents expected to be useful for improvement of the neuropathological conditions in the injured spinal cord. For example, caffeic acid phenethyl ester (CAPE), biologically active constituent in propolis, is a constituent identified to exert antiinflammatory activity and to protect the brain from ischemia-reperfusion injury (Kasai et al., 2011). The flavonoids in propolis, potentially represents antioxidative capacity in neuronal cell death. With regard to the effects of propolis on the nervous system, evidence showing that propolis and/or its components are neuroprotective against various brain insults in vivo or neuronal damage in vitro has been rapidly accumulating (references are needed). For instance, CAPE and water soluble components of propolis are neuroprotective against excitotoxic insults in ischemia/reperfusion injury. Chinese propolis may become a promising tool for wide use in the nervous system for reducing the secondary neuronal damage following primary physical injury (Bankova, 2005; Izuta et al., 2008; Kasai et al., 2011). In agreement with these results we demonstrated the direct effect of propolis on brain tissue.

Recent interests are focusing on the use of nonenzymatic antioxidants such as flavonoids in reducing the toxicity associated with pesticide-induced oxidative stress in experimental models (Kanbur et al., 2009; Alirezaei et al., 2012; Gulhan et al., 2012). Plant-based pharmaceuticals including flavonoids have been employed in the management of various diseases. They are an essential part of human diet and are present in plants that have been used for centuries in medicine. Antioxidant properties, ROS scavenging, and cell function modulation of flavonoids could account for the large part of their pharmacological activity (Narenjkar et al., 2011).

In the present study, it was determined that cypermethrin increased oxidative stress parameters in brain tissue of rainbow trout. The experimental results indicate that propolis may be beneficial in reversing adverse effects in the brain of rainbow trout exposed to cypermethrin. Chemicals used in agriculture may have negative effects on aquatic organisms and environmental monitoring reports need to be done periodically in order to limit and prevent adverse environmental contaminations.

Our results indicate that propolis has antioxidant and neuroprotective effect and can prevent brain dysfunction in fish.

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Functional Interaction between the Shell Sub-Region of the Nucleus Accumbens and the Ventral Tegmental Area in Response to Morphine: an Electrophysiological Study

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ABSTRACT

This study has examined the functional importance of nucleus accumbens (NAc)-ventral tegmental area (VTA) interactions. As it is known, this interaction is important in associative reward processes. Under urethane anesthesia, extracellular single unit recordings of the shell sub-region of the nucleus accumbens (NAcSh) neurons were employed to determine the functional contributions of the VTA to neuronal activity across NAcSh in rats. The baseline firing rate of NAcSh neurons varied between 0.42 and 11.44 spikes/sec and the average frequency of spontaneous activity over 45-minute period was 3.21±0.6 spikes/sec. The majority of NAcSh neurons responded excitatory in the first and second 15-min time blocks subsequent to the inactivation of VTA. In the next set of experiments, eight experimental rats received morphine (5 mg/kg; sc). Three patterns of neuronal activity were found. Among the recorded neurons only three had an increase followed by morphine administration. Whereas the other three neurons were attenuated following morphine administration; and there were no changes in the firing rates of the two neurons left. Finally, unilateral reversible inactivation of VTA attenuated the firing activity of the majority of ipsilateral NAcSh neuron in response to morphine, except for a single cell. These results suggest that transient inactivation of VTA reduces the ability of neurons in the NAcsh to respond to systemic morphine, and that NAcSh neuron activity depends on basal firing rate of VTA inputs.

1. Introduction

The mesolimbic dopaminergic system projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) is critical for the initiation of opioid reinforcement and reward-related behaviors. Related anatomical and physiological evidences implicate the NAc and its afferents in mediating behavioral effects of drugs abuse such as ethanol (Bunney, Appel, & Brodie, 2001), cocaine (Navailles, Moison, Cunningham, & Spampinato, 2007), nicotine (Mansvelder & McGehee, 2000) and morphine (Bunney et al., 2001; Olmstead & Franklin, 1997; Rezayof, Nazari-Serenjeh, Zarrindast, Sepehri, & Delphi, 2007). Based on histochemical (Jongen-Relo, Groenewegen, & Voorn, 1993) and connective (He, Wang, & Wei, 2007) differences, the NAc has been divided into core and shell regions, besides several reports indicating that both parts are involved in reward related behaviors (Ikemoto, Glazier, Murphy, & McBride, 1997; Mansvelder & McGehee, 2000; Moaddab, Haghparast, & Hassanpour-Ezatti, 2009; Pennartz, Groenewegen, & Da Silva, 1994). Various biochemical and physiological adaptations in the VTA neurons (both dopaminergic and GABAergic neurons) following chronic exposure to morphine have been reported, and neuroplastic changes within the VTA neurons are believed to contribute to drug addiction

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(Chu et al., 2008). Dopaminergic neurons in the VTA are the origins of mesolimbic/ mesocortical dopamine pathway and they also provide dopaminergic innervation to the NAc (Bunney et al., 2001; Moaddab et al., 2009; Oades & Halliday, 1987). The neural activity of NAc neurons is strongly modulated by various cortical and subcortical limbic inputs, and thus may represent a site in which the motivational component of behavior gains access the motor system (Esmaeili, Kermani, Parvishan, & Haghparast, 2012; He et al., 2007; Olmstead & Franklin, 1997; Winder, Egli, Schramm, & Matthews, 2002). These findings suggest that the NAc is well positioned to integrate a wide range of limbic and motor information (Esmaeili et al., 2012; Lisman & Grace, 2005; Meredith et al., 1999). This converging connectivity suggests that emotional motivational influences gain access to behavior through the limbic-motor interface of the NAc (Martin, 1991). Also, it has been shown that systemically administered opiates inhibit or excite spontaneously active single units of the nucleus accumbens septi (NAS) (Hakan, Eyl, & Henriksen, 1994). Moreover, in another research, single unit recordings of NAS neurons in halothane-anesthetized rats revealed that microinfusions of morphine into the VTA primarily inhibited spontaneously active NAS units. These inhibitory effects were reversed by subcutaneously administration of alpha-flupenthixol, a DA receptor antagonist, suggesting a role for dopamine (DA) in the observed opiate-induced effect. VTA opiate microinfusions also inhibited the evoked (driven) responses of silent cells (spontaneously inactive) in the NAS elicited by stimulation of hippocampal afferents to the NAS. In addition, this inhibition of driven response was reversed by naloxone but not by alpha-flupenthixol, implying a VTA-mediated non-DA mechanism (Hakan & Henriksen, 1989).

Morphine applied iontophoretically to cells within the NAS inhibited spontaneous activity but not fimbria-driven cellular activity, suggesting that the systemic effects of opiates on NAS activity can be mediated directly in the NAS as well as through VTA afferents. Moreover, since VTA-induced inhibition of fimbria-driven activity was reversed by systemic opiates, opiates also can exert effects through other, as yet unidentified NAS afferent systems (Gysling & Wang, 1983). Therefore, in this study, we tried to investigate the effects of reversible inactivation of the ventral tegmental area on the alteration in neural firing of shell sub-region of NAc neurons and the effects of morphine on this alteration in the rats.

2. Methods

2.1. Animals

Twenty nine adult male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 240-300 g were used in these experiments. Animals were housed in groups of three per cage in a 12/12 h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.) with free access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for 1 week before the experimental procedure started. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

2.2. Drugs

Morphine sulfate (Temad, Iran) and lidocaine, which was dissolved in sterile saline (0.9%) as a vehicle, were used in this study. Control animals received saline.

2.3. Experimental Procedures

Testing sessions were carried out in a quiet room, with the room temperature kept at 25ºC. There were three groups as follows:

2.3.1. Effects of Intra-VTA Lidocaine Administration on the Firing Rate of NAcSh Neurons

In this set of experiments, after stabilization period (15 min) and baseline recording (30 min), lidocaine 2% (0.5 µl/rat) was administrated into the VTA, and 15 min later subcutaneous (sc) saline (1 ml/kg) was applied. Control animals received saline (0.5 µl/rat) instead of lidocaine 2%.

2.3.2. Effects of Systemic Administration of Morphine on the Firing Rate of NAcSh Neurons

In the next set of experiments, the effects of morphine on a total of eight single neurons recorded in the NAcSh were determined. After stabilization period (15 min) and baseline recording (30 min), saline (0.5 µl/rat) was applied into the VTA as a vehicle instead of lidocaine. 15 min later the administration of morphine (5 mg/kg; sc) was done. In control animals, saline (1 ml/kg) was administered instead of morphine.

2.3.3. Effects of Reversible Inactivation of VTA on NAcSh Neural Response After Morphine Administration.

In this set of experiments, after stabilization period (15 min) and baseline recording (30 min), the animals received lidocaine 2% (0.5µl/rat) and after 15 min morphine (5 mg/kg; sc) was administrated. Control animals received saline (0.5 µl/rat) instead of lidocaine 2%.

In all aforementioned experimental groups, after the second injection, single unit recording was followed by 30 min period without any intervention. In this study, a total of 29 single NAcSh neurons were analyzed.

2.4. Electrical Recording and Data Acquisition

Animals were initially anesthetized with urethane intraperitoneally (1.2 g/kg) with additional doses (0.1 g/kg) administered every 1 h as needed for maintaining a deep and constant level of anesthesia as determined by lack of movement in response to a strong tail pinch. Then tracheotomy was done in order to prevent suffocation and the animals were placed in a stereotaxic instrument (Stoelting; USA). Body temperature was maintained at 36-36.7ºC with a thermistor-controlled heating pad. Two 2-mm diameter holes were drilled in the skull; one above the NAcSh (1.2 mm rostral to bregma, 0.8 mm lateral to the sagittal suture) and the other on top of the VTA (4.75- 5 mm caudal to bregma, and 0.8- 0.9 mm lateral to the sagittal suture) based on the rat brain atlas (Paxinos & Watson, 2007). The Dura was removed and the hole was covered by a drop of mineral oil, for facilitating the microelectrode entrance. The injector cannula was stereotaxically aimed to the VTA (8.2- 8.4 mm ventral to skull surface).

Extracellular recording from individual neurons was obtained with tungsten microelectrode (Harvad Apparatus, USA; Parylene Coated; 127-µm diameter shaft with extra fine tip; 5 MΩ impedance tip). Microelectrode was stereotaxically advanced into the NAcSh (7 mm ventral to skull surface). Spike signals which were received from neurons by a preamplifier, were amplified by a differential amplifier (DAM-80, WPI, USA; ×1000 gain; 300 Hz and 10 KHz for low and high filters, respectively) and were modified from analog to digital form by data acquisition, as signals. Then the signals were displayed continuously on the computer via a homemade software and were auditory monitored (Haghparast A, 2010; Haghparast A 2012). Only the single cells having a consistent spike amplitude and waveform during the experimental procedure were studied. The action potentials were categorized by the initial direction of the voltage deflection (positive or negative); amplitude (peak-to-peak), and duration were also determined. Action potentials were isolated from background activity with two windows which generated output pulses for signal units based on spike height (amplitude), and which counted the number of spikes per unit time (bin widths were 1000 ms). Sampling of extracellular recordings was done using an electrophysiological data acquisition (D3109; WSI; Iran) on an IBM Pentium computer for on-line data collection (Haghparast A, 2010; Haghparast A 2012). In this manner, the computer saves the number of output signals as spikes in time unit that is set manually (0.1-12000 sec). In these experiments, time setting for data collection was 4800 sec with 1000 ms bin size as a file which was saved continuously during experiment in hard disk, and unit activity was calculated by computer as an average frequency (spikes/sec). In the present study, the signal to noise ratio was considered at least 3 to 1. For data presentation, unit activity is shown at 1- and/or 5-min intervals.

2.5. Data Analysis

In this study, discharges of each neuron were counted in 60 s time bins using a data acquisition interface program to construct peri-stimulus time histograms (PSTHs), with a time range of 15 min (stability period) and 30 min (baseline recording) to 35 min after the injections of saline/lidocaine (intra-VTA microinjection) and saline/morphine (sc). The data were later analyzed off-line with the homemade analysis software for windows. In order to detect the neuronal response patterns to lidocaine and morphine administration over time, the whole period of observation was sectioned into 1-min time bins. An increase or decrease in firing rates over two-fold of the standard deviation of the baseline for at least 3 consecutive bins (i.e., 3 min) was considered as an excitatory or inhibitory response, respectively. A clustering analysis (K-means, SPSS) was performed to classify neuronal responses depending on the similarities in patterns of excitation or inhibition (both latency and duration of response) induced by injection of drugs (Haghparast et al. 2010, 2012). The number of neurons exhibiting excitatory or inhibitory response to lidocaine and morphine injections at each time bin was counted. To calculate the significance, the mean ± SEM values of the 1 min time blocks, representing the lidocaine and morphine responses of each of the groups, were compared with the control group. Statistical analysis of the data was done using one-way ANOVA and followed by Student-Newman-Keuls test for multiple comparisons. P-value < 0.05 was statistically considered significant.

2.6. Histological Verification

After completion of the recordings, subjects were overdosed with urethane and the electrode position was confirmed by electrolytical markings (50 µA of negative current for 10-15 sec) with signs of electrode penetration to confirm microelectrode placement within the NAcSh. Under deep anesthesia, the animals were perfused transcardially with 0.9% saline followed by 10% formalin. The brains were removed and placed in a 10% formalin solution for at least three days. The recording site was subsequently examined in coronal sections (150 µm) by light microscopy by an observer unfamiliar with the electrophysiological data. Recording site was histologically verified and plotted on standardized sections derived from the atlas of Paxinos and Watson (2007) and only those data that were histologically verified to be located in NAcSh were included in the data analysis.

3. Results

3.1. Electrophysiologic Profile of NAcSh Recording

Histological evaluations revealed that electrophysiological recordings were obtained from 29 neurons located throughout the shell part of the nucleus accumbens. After isolating a unit and determining the stability of its firing rate (30–40 min), background activity

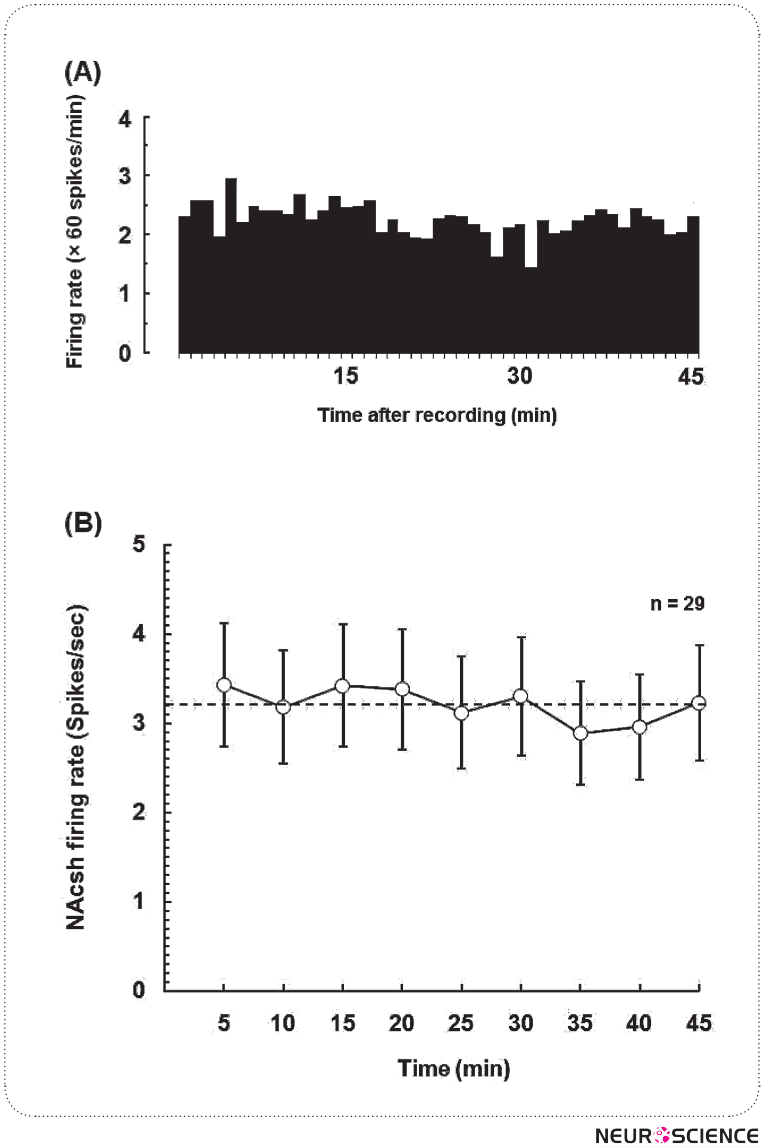

Figure 1. (A) An example of spontaneous activity of neuron (2.16 ± 0.27 spikes/sec) recorded from the NAcSh in urethane-anesthetized rat. (B) Average firing rate of the NAcSh neurons in control (open circles), anesthetized rats (n = 18 to 29 neurons at each time point) at 5-min set intervals for the 45-min recording time period. Dash line shows the mean baseline activity (3.21±0.6 spikes/sec) in the NAcSh.

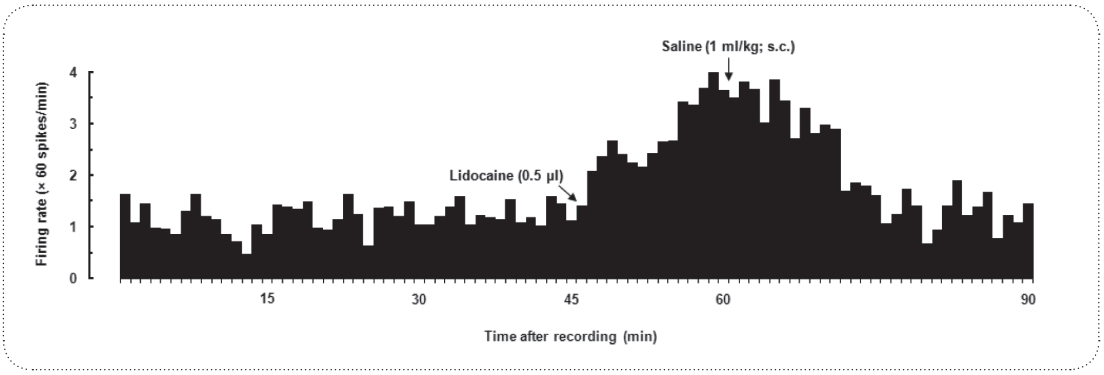

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Figure 2. A typical effect of administration of 2%lidocaine (0.5µl) alone into the VTA on spontaneous activity of neurons in the NAcSh followed by saline (1 ml/kg; sc) injection. The firing rate of neuron continually recorded 90 min following injection of lidocaine at 45th-min of recording period.

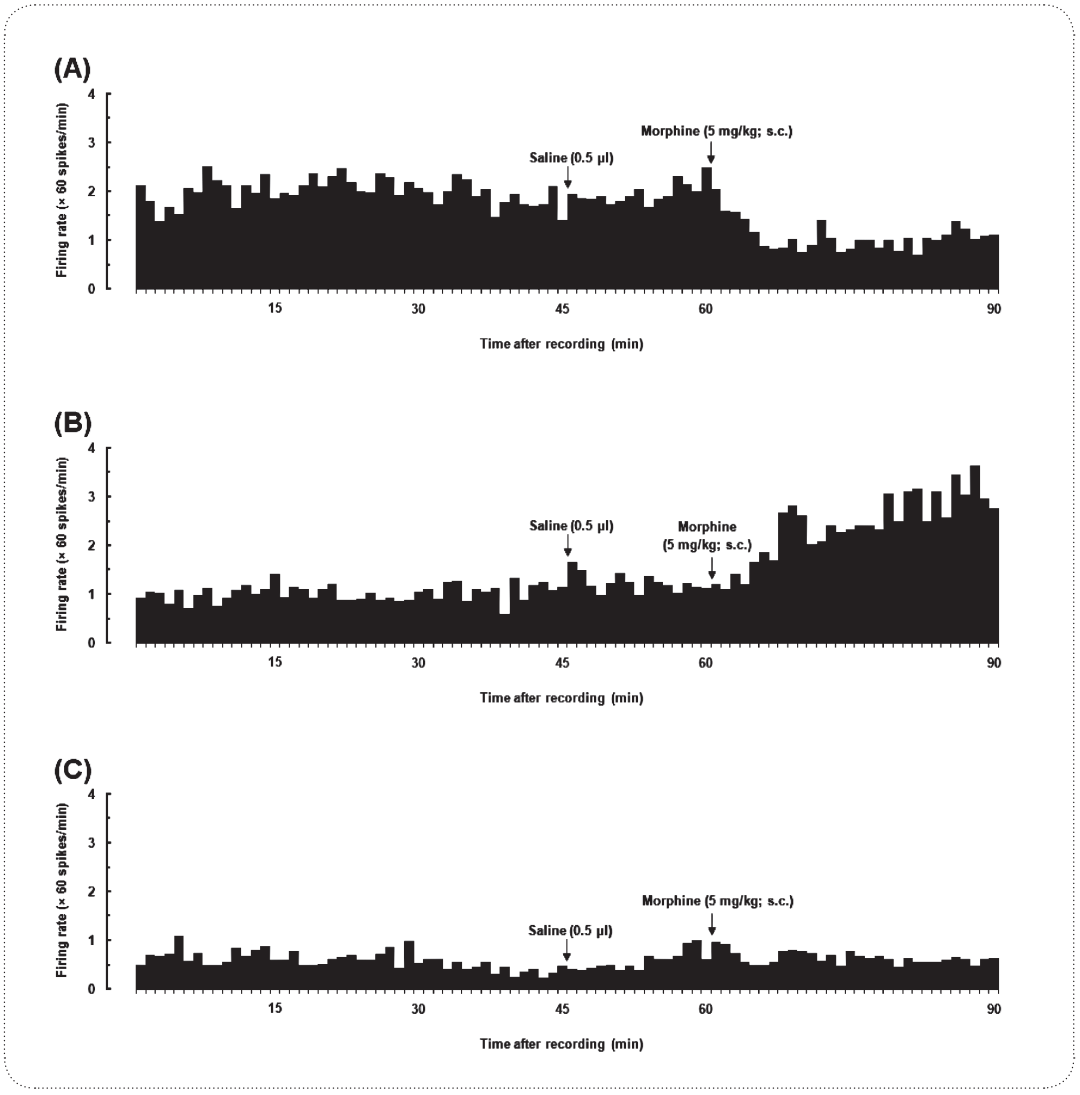

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Figure 3. Examples of the effect produced by morphine on NAc neurons recorded from anesthetized rats. The panel depicts the (A) decreasing firing rate and (B) increasing after morphine administration. In the bottom graph (c) neural firing rate didn't have any alteration.

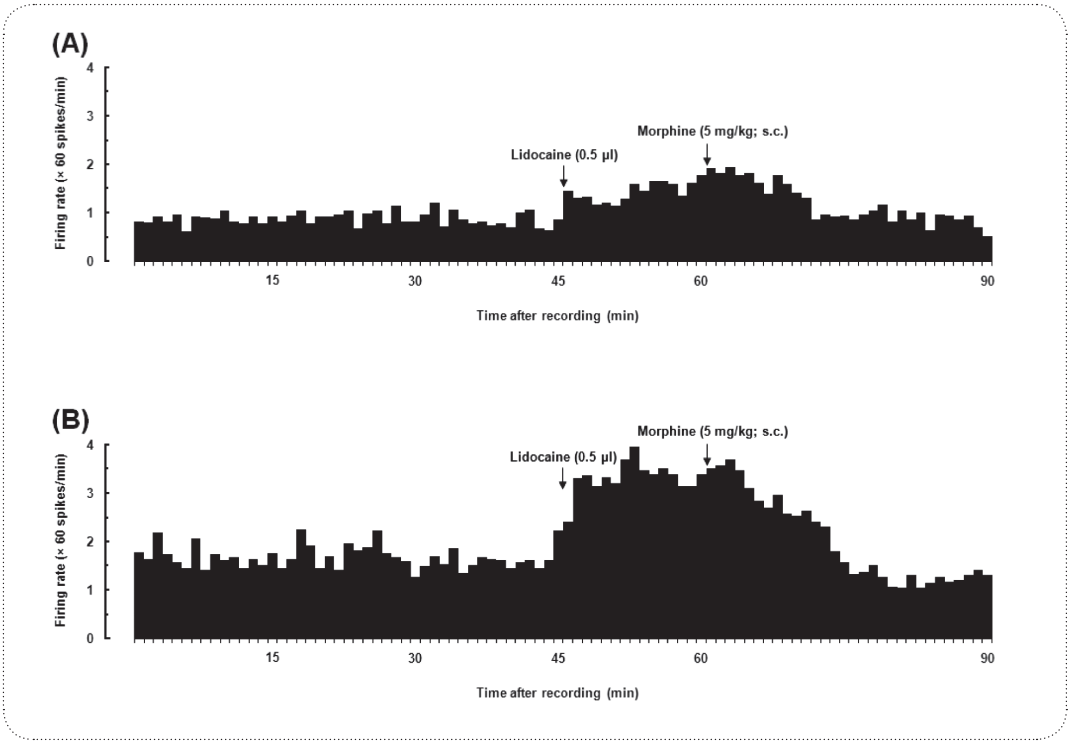

Figure 4. Typical effects of intra-VTA administration of lidocaine followed by systemic injection of morphine. The upper figure (A) depicts neither lidocaine nor morphine didn't changed neural firing rate in NAsch. In the lower figure (B), lidocaine increased the NAcSh neural activity and firing rate decreased after morphine administration.

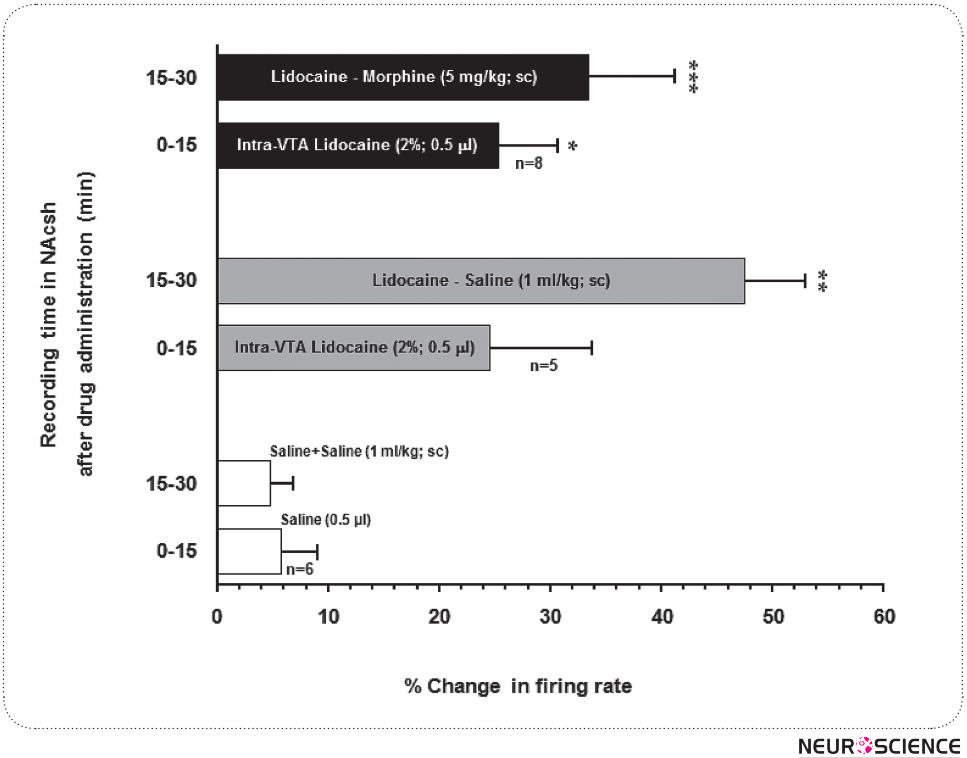

Figure 5. The histogram presents the average changes in percentage of firing rate of neurons in baseline recording and after intra-VTA injection of 2%lidocaine and lidocaine + morphine. Values expressed as mean ± SEM.

*P < 0.05, **P < 0.01, ***P < 0.001 compared to saline respective group.

is tested merely for determining spontaneous firing rate of NAcSh neurons during 45 min (Fig. 1A). The saline experiments served as control for the effect of the injection procedure and the volume administered on neural activity over the recording period. Data was subjected to one-way ANOVA and showed that there were no significant differences in any 5-min points of baseline firing rate of neurons in the NAcSh during 45-min recording time after the stabilization period [$F(8,223) = 0.6833$, $P = 0.8711$; Fig 1B]. Their baseline firing rate varied between 0.42 and 11.44 spikes/sec and the average frequency of spontaneous activity over the 45-min time period was 3.21 ± 0.6 spikes/sec as shown in Fig 1B. Neurons in the NAcSh exhibited mostly action potentials with biphasic waveforms (~ 90%; 26 cells), a width of 1.3 to 2.8 ms in duration and an inflection in the initial positive component with different amplitudes (130-240 µV).

3.2. Response of NAcSh Neurons to VTA Lidocaine Administration

After stabilization period (15 min) and baseline recording (30 min), lidocaine 2% (0.5 µl/rat) was administrated into the VTA, and 15 min later, saline (1 ml/kg; sc) was applied. In 5 out of 6 neurons, 1-7 min after injection of lidocaine, neural firing rate was increased in the NAcSh and it continued for 19-33 min (Fig. 2). The spontaneous activity of NAcSh was variable, between 0.58 and 8.92 spikes/sec and the average of firing rate was 3.27 ± 1.01 spikes/sec. The maximal percentage of lidocaine-induced activation of neural activity in the first 15-min time period (0-15) after lidocaine injection was 24.54 ± 9.16% and in the second 15-min (15-30) which was coincidence with the injection of saline (1 mg/kg; sc) was 47.57 ± 15.44%. In addition, in the case of the one remained neuron, lidocaine increased the neural activity only 8.1 ± 0.27%, hence it was considered as an ineffective neuron. Student's paired t-test ($t5 = 2.88$, $P<0.05$) revealed that the average unit activities of NAcSh neurons after lidocaine administration into the VTA were significantly different from those of pre-injection recording time.

3.3. Responses of NAcSh Neurons to Systemic Administration of Morphine

In the next set of experiments, the effects of morphine on a total of 8 single neurons recorded in the NAcSh were determined. After stabilization period (15 min) and baseline recording (30 min), saline (0.5 µl; one side) as a vehicle was applied into the VTA; and 15 min later the sc administration of morphine (5 mg/kg) was done. As it can be seen in Fig. 3 after morphine injection, the following neural activity profiles were seen in the NAcSh: in 3 neurons, 1-3 min after morphine injection, the neural activity significantly decreased and it continued till the 90th minute (Fig. 3A). Their baseline firing rate varied between 1.05 - 8.4 spikes/sec and the average frequency of spontaneous activity over the 90 min time period was 3.1 ± 1.15 spikes/sec while the mean percentage of the inhibition of these neurons after morphine injection was 49.43 ± 10.22%. In 3 out of 8 neurons, 3-7 min after morphine injection (5 mg/kg; sc) the neural activity of NAcSh neurons were significantly augmented which lasted until the 90th minute (Fig. 3B). The baseline firing rate was varied between 0.71- 8.02 spikes/sec and the average frequency of spontaneous activity over the 90-minute time period was 3.19 ± 2.22 spikes/sec. Moreover, the mean percentage of potentiation after morphine injection was 71.3 ± 7.61% in these neurons. Finally, in 2 out of 8 neurons, no effect was observed after morphine injection (Fig. 3C). Here, the baseline firing rate and the average frequency of spontaneous activity over the 90-minute time period was 1.11-5.43 spikes/sec and 2.96 ± 1.17 spikes/sec, respectively.

3.4. Effect of VTA inactivation by lidocaine on NAcSh neural activity alteration caused by morphine administration

After stabilization period (15 min) and baseline recording (30 min), animals received 2% lidocaine (0.5 µl/rat in VTA), 15 min later morphine (5 mg/kg; sc) was administrated. Analysis of data in this experiment showed that there are two neural activity profiles for NAcSh neurons after morphine administration, as shown in Fig. 4A, B. In 8 out of 9 neurons, the mean activation of NAcSh neurons in 15-min after lidocaine administration was 25.4 ±5.23% while in the next 15-min (15-30), concurrent with morphine administration, was 13.53 ±17.69%. Meanwhile, the average frequency of spontaneous activity over this time was 2.57 ± 0.74 spikes/sec. In other words, student t-test revealed that the administration of lidocaine into the VTA suppressed the neural alteration followed by systemic administration of morphine (Fig. 5).

4. Discussion

The purpose of this study is to provide a conceptual framework for understanding the way the mesolimbic neurotransmission systems work in morphine-rewarding behaviors. To our knowledge, this is the first re-

port of electrophysiological effects of VTA inhibition on provoked NAcSh neural activity through systemic morphine administration. A body of work indicates that impermanent inactivation of VTA reduced the effect of systemic administration of morphine on NAcSh. These finding is concurrent with our previous finding that support the necessity of projection from VTA to NAcSh for the formation of reward-related effects of abused drugs (Moaddab et al., 2009). It is well documented that the mesolimbic dopaminergic pathway that is projected from the VTA to the NAc is critical for the reinforcing effects of opioids and other abused drugs (Esmaeili et al., 2012; Ikemoto et al., 1997; Moaddab et al., 2009). These dopamine neurons are activated by systemic morphine injection or natural rewarding stimuli such as food or sex, resulting in increased dopamine release in the targeted brain regions particularly VTA and NAc (Leone, Pocock, & Wise, 1991). With this regard, previous studies revealed that opiates administered systemically increase the firing of VTA-dopamine neurons recorded in vivo (Matthews & German, 1984), moreover, microiontophoretic administration of morphine or enkephalin analogues significantly increases the spontaneous activity of the VTA and substantia nigra pars compacta (SNC) cells. Also, these effects were not reversed by neither iontophoretic nor intravenous naloxone, proposing that morphine- induced activation of the VTA dopamine cells could be indirectly mediated by non-dopaminergic cells (R. Hakan & Henriksen, 1989). The direct action of opioids on neurons elsewhere in the nervous system, including other catecholamine-containing cells, is inhibitory (Mihara & North, 1986; North & Tonini, 1977). This finding raises the possibility that the excitatory effect of the opioids on the principal, dopamine-containing cells, occurs indirectly; that is, opioids may inhibit non-dopamine neurons in the VTA (secondary neurons), specifically GABA-containing neurons that provide inhibitory synaptic input to the dopamine cells (Gysling & Wang, 1983). Our results in the second part shows that, approving those of similar works done before, transient inactivation of VTA by 2% lidocaine provokes neural firing rate in the NAcSh. It seems that the observed increase in neural activity followed by lidocaine injection was due to inhibition of dopaminergic and GABAergic projections.

The nucleus accumbens as a central part in the neural circuitry involved in drug addiction exhibits spontaneous neuronal activity as well as evoked (driven) neuronal responses to ipsilateral fimbria stimulation (Yang and Mogenson, 1984). Spontaneous active NAc single units are predominantly inhibited (but also excited or unaffected) by systemically administered opiate drugs (R. L. Hakan & Henriksen, 1987; R Yang & J Mogenson, 1984). Along with mentioned studies, we found out that the subcutaneous administration of morphine changed the NAcSh neural activity in opposite directions. Based on this phenomenon, it seems that the observed accretion in NAcSh neural activity following morphine injection was due to alterations in the neural substrates which is leading to the activation of dopaminergic neurons. In contrast, it can be suggested that GABA interneuron activation, following morphine administration caused inhibition in NAcSh neural activity. The shell portion of the accumbens appears to be more important than the core for drug reward. Ikemoto et al. showed that rats learn to self-administer psychomotor stimulants such as amphetamine or cocaine or dopamine receptor agonists into the accumbens shell, but not into the core (Ikemoto et al., 1997; Ikemoto, Qin, & Liu, 2005). To function, NAc is dependent on neurotransmitergic inputs from other brain areas involved in the reward circuitry specially the VTA.

Dopaminergic and GABAergic inputs from VTA typically converge on the NAc and there is considerable evidence suggesting that the NAc may have a pivotal role in the integration of limbic inputs relevant to motivated behaviors (Everitt & Wolf, 2002). Previous studies indicated that the lesion of dopaminergic projection from the VTA to the NAc or the blocking of dopaminergic transmission reduces the reinforcing effects of drugs in several experimental paradigms including conditioned place preference (Gholami, Zarrindast, Sahraei, & Haerri-Rohani, 2003; Moaddab et al., 2009). Our previous study revealed that reversible inactivation of VTA significantly decreased the acquisition and expression of morphine-induced CPP (Moaddab et al., 2009). These findings are of particular importance in light of the present report, showing that intraperitoneal administration of morphine was not able to change the firing of NAc neurons while VTA was provisionally inactivated. It can be concluded that observed alterations in neural activity followed by systemic morphine administration fit within the dopaminergic hypothesis of reward, and inhibition of neural activity of the VTA leads to the increase in neural activity of the NAc. Meanwhile, based on the two entrances of GABA and dopamine from the VTA to NAc, the augmentation of NAcSh neural activity could be due to the blockade of these neurotransmitters pathway. Finally, it could be concluded that the activated neuronal pathways by morphine, have to pass the VTA in order to get to the NAc, and ability of neurons in the NAcsh for responding to morphine depends on basal firing rate of VTA-NAc dopaminergic inputs.

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Rapidly Changing Tachyarrhythmia in Acute Stroke

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A B S T R A C T

Introduction: we report a 56-year-olds female with supraventricular arrhythmia due acute ischemic stroke without structural heart disease.

Case Description: A patient presented with sudden onset of lethargy, right hemiplegia, and global aphasia. There was previous history of stroke 1 year ago presented with left hemiplegia that recovered completely during 10 days. There was no history of comorbid illness. The brain CT revealed extensive hypodensity in left temporoparietal region suggestive of infarct without midline shift. General examination revealed hypotension and bradycardia that treated with dopamine that gradually recovered during 5 days thus infusion of dopamine discontinued, and muscular power in paretic limbs and aphasia was recovered. In 6th day of admission electrocardiographic monitoring of patient showed a rapidly changing tachyarrhythmia including sinus tachycardia, atrial fibrillation, and atrial flutter that quickly interchanged to another, without hemodynamic instability and alteration in mental status. Laboratory tests and TEE study were normal. During 48 hour arrhythmia relived spontaneously.

Discussion: Stroke can cause any type of cardiac arrhythmias that may not be constant.

1. Introduction

Approximately 75% to 92% of patients with intracranial bleeding or ischemic stroke develop new ECG abnormalities (Kevin A.B. et al., 2008). These may include, cardiac arrhythmias (CA), such as ventricular ectopic beats (VEB) or supraventricular ectopic beats (SVEB); ventricular arrhythmias (VA), especially ventricular tachycardia (VT); atrial flutter/fibrillation (AF); and repolarization abnormalities (QT interval prolongation, ST segment changes, large upright or inverted T-waves, and septal U waves) (Ornella D. et al., 2002). The Modern neuroimaging data, including positron emission tomography and functional magnetic resonance imaging, have revealed that a network consisting of the insular cortex, anterior cingulate gyrus, and amygdale play a crucial role in the regulation of central autonomic nervous system (Michiaki N. et al., 2010; Furio C. et al., 2004; Fred R. et al., 2008; Kevin AB et al., 2008). These data strongly indicate that the brain has a major influence on cardiac structure and function and that this is likely mediated through alterations in patterning of sympathovagal relationships (Fang L. et al., 2006).

2. Case Report

We are a 56-year-old female with sudden onset of lethargy, hemiplegia of right upper and lower limbs, deviation of corner of mouth to the left side and global aphasia within last 8 hours. There was previous history of stroke 1 year ago, presented with hemiplegia of left upper and lower limbs and aphasia that recovered completely during 10 days. There was no history of Diabetes, Congestive heart failure, High blood pressure, Ischemic heart disease (coronary artery disease) or other comorbid illnesses. General examination revealed

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a pulse rate of 35-40/ min and blood pressure of 90/50 mmHg, that was treated with infusion of dopamine (10-20μ/kg/min). The central nervous system examination showed lethargia, right upper motor neuron facial nerve palsy, and muscle power of upper and lower limbs were1/5, with right extensor plantar response. Examination of other systems was normal. Daily laboratory tests revealed normal levels of serum sodium, potassium, Calcium, magnesium, urea and creatinin. The initial serum troponin T and creatine phosphokinase levels were normal. The brain CT revealed extensive hypodensity in left temporoparietal region, suggestive of infarct without midline shift (Figure 1). Doppler sonography of carotid and vertebrobasilar artery revealed a floating thrombosis in right brachiocephalic artery after the origin of common carotid artery without remarkable stenosis. Gradually bradycardia and hypotension recovered during 5 days, thus infusion of dopamine was discontinued, and muscular power in paretic limbs and aphasia were recovered.

In 6th day of admission electrocardiographic monitoring of patient showed a rapidly changing tachyarrhythmia including sinus tachycardia, atrial fibrillation, and atrial flutter that quickly interchanged to another without hemodynamic instability and alteration in mental status (Figure 2). Laboratory tests rechecked, and levels of serum sodium, potassium, Calcium, magnesium, urea, creatinin, thyroid function tests and troponin I and creatine phosphokinase were normal. Transesophagial echocardiography (TEE) revealed normal LV and RV systolic and diastolic function, no LA and RA clot, with no regional wall motion abnormality (RWMA). During 48h without any specific antiarrhythmic treatment,

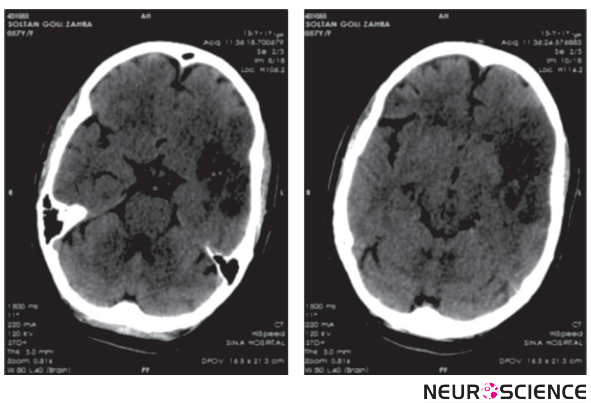

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Figure 1. Brain CT showed extensive hypodensity in left temporoparietal region suggestive of infarct. No midline shift and mass effect.

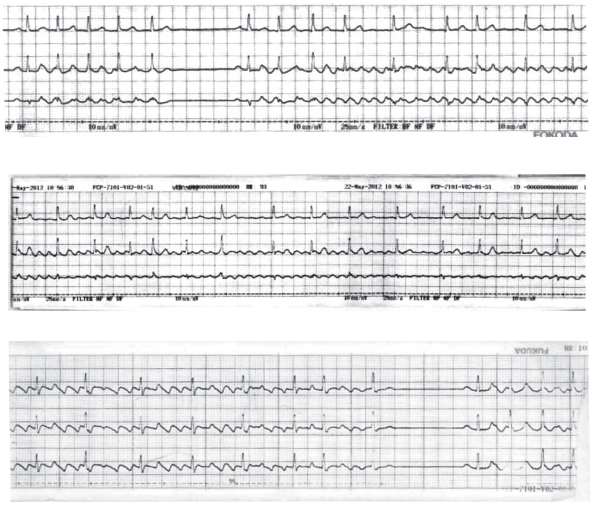

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Figure 2. Electrocardiogram showed a rapidly changing tachyarrhythmia including sinus tachycardia, atrial fibrillation, and atrial flutter that quickly interchanged to another.

arrhythmias relieved and normal sinus rhythm established. 5 days later, patient was discharged without any arrhythmia in electrocardiogram.

3. Discussion

We described a case of ischemic stroke with involvement of left temporoparietal region that reveals rapidly changing tachyarrhythmia. Cardiac abnormalities in patients with acute stroke were first reported in 4 patients in 1947 (Stephen O. et al., 1992). In humans, stroke in both hemispheres has been shown to produce changes in autonomic mechanisms, which leads to myocardial necrosis, arrhythmias, and even sudden death through related mechanisms. However, the localization of stroke may have differential effects (Sadberk L.T. et al., 1999). Lane et al have shown that right hemisphere infarction is associated with a greater number of supraventricular tachycardia, and they speculated that a decrease in cardiac parasympathetic activity in right sided infarction may cause the probable reciprocal rise in the sympathetic tone (Lane R.D. et al., 1992). It has been shown in humans that, lesions ablating part or all of the left anterior insula and its efferent connections and ablation of inhibitory circuminsular efferent's to the right insular cortex are of consequence with regard to determining cardiovascular outcomes after neurological damage (Stephen O. et al., 2006). There is evidence supporting long-term activation of the autonomic nervous system after stroke with increased levels of norepinephrine and pathologic nighttime blood pressure increases, a combination that represents an independent risk for future

cardiovascular and cerebrovascular events (Fred R. et al., 2008). Serious arrhythmic tachycardia (ventricular or supraventricular >130 beats/min) was more frequent than bradycardic arrhythmia (sinus-node dysfunction, bradyarrhythmia, or atrioventricular block °II and °III).12 Atrial fibrillation is the most common arrhythmia reported, occurring with a frequency of 9% (Giuseppe M. et al., 2008).

Although the exact mechanism for arrhythmia during stroke has not yet to be known, we propose some probable causes of tachyarrhythmia in this patient.

In the present case, there are two interesting findings that distinguish it from other patients, which have been reported so far. The first note was beginning time of arrhythmia based on literature, the common time period for occurrence of arrhythmia is in the first 48 hours, but in our case, arrhythmia occurred 6 days after admission.

The previous studies have analyzed patients with first over ischemic stroke, but our patient had history of right side stroke in one year ago, which could be the cause of this difference and late occurrence of tachyarrhythmia. Also other probable cause of late tachyarrhythmia may be presence of floating thrombus in right brachiocephalic artery that could be cause recurrent microemboli to right carotid artery which cause right hemispheric ischemia that indistinguishable in brain CT studies. This kind of microemboli can cause transient right insular ischemia with parasympathetic blocked, which increase brain sympathic tone and produce tachyarrhythmia in our patient.

Second, type of arrhythmia in this patient differed from similar cases reported previously. The most common reported type is atrial fibrillation (AF), but interestingly we detected a variable form of tachyarrhythmia which quickly interchanged to another. This variability in the type of arrhythmia may be due to the local stimulatory effects of existing floating thrombosis in great vessels (brachiocephalic artery) on the endothelium, or its dynamic retrograde effects on the left and consequently right heart.

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Cell Therapy in Spinal Cord Injury: a Mini- Reivew

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ABSTRACT

Spinal cord injury (SCI) is a debilitating disease which leads to progressive functional damages. Because of limited axonal regeneration in the central nervous system, there is no or little recovery expected in the patients. Different cellular and molecular approaches were investigated in SCI animal models. Cellular transplantation of stem cells can potentially replace damaged tissue and provide a suitable microenvironment for axons to regenerate. Here, we reviewed the last approaches applied by our colleagues and others in order to improve axonal regeneration following SCI. We used different types of stem cells via different methods. First, fetal olfactory mucosa, schwann, and bone marrow stromal cells were transplanted into the injury sites in SCI models. In later studies, was applied simultaneous transplantation of stem cells with chondroitinase ABC in SCI models with the aid of nanoparticles. Using these approaches, considerable functional recovery was observed. However, considering some challenges in stem cell therapy such as rejection, infection, and development of a new cancer, our more recent strategy was application of cytokines. We observed a significant improvement in motor function of rats when stromal derived factor-1 was used to attract innate stem cells to the injury site. In conclusion, it seems that co-transplantation of different cells accompanies with other factors like enzymes and growth factors via new delivery systems may yield better results in SCI.

1. Introduction

The trend in the major causes of death has changed over the past years from infectious diseases to cardiovascular diseases, cancers and road traffic accidents. Road traffic accident is one of the main causes of Spinal Cord Injury (SCI), a debilitating disease, which leads to progressive functional damages. It is estimated that the annual incidence of spinal cord injury is approximately 50 per 1 million people (Ackery, Tator, & Krassioukov, 2004). Prevalence of traumatic SCI in Tehran ranged from 1.2 to 11.4 per 10,000 people (Rahimi-Movaghar et al., 2009).There are about 89000 patients suffering from this problem in Iran, 66% of them caused by road traffic accidents (Joghataei, 2009). SCI represents an injury with catastrophic outcomes, both for the individual on a personal level, and for society with respect to the extent of burden on the health care and living expenses (Kwon, Sekhon, & Fehlings, 2010). Although, considerable research efforts were undertaken, only limited rehabilitating therapies are available in human patients. Unlike the peripheral nervous system (PNS), the adult mammalian the CNS have limited capacity to spontaneously regeneration following an injury. The Edwin Smith surgical papyrus, which dates back to 1550 BC, states that "If you examine a man with a neck injury … and find he is without sensation in

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both arms and both legs, and unable to move them, and he is incontinent of urine …it is due to the breaking of the spinal cord caused by dislocation of a cervical vertebra. This is a condition which cannot be treated (Filbin, 2003). Many approaches were used by investigators in order to facilitate axonal regeneration and consequent functional recovery following SCI. Here, we review briefly different strategies applied by researchers and our colleagues in animal models of SCI.

2. Inhibitors of Axonal Regeneration in the Adult CNS

Injury to the CNS induces tissue damage, which creates barriers to regeneration. One of the main barriers is the glial scar, in which astrocytes and some other cells establish a dense cellular response surrounding the lesion site (Dawson, Levine, & Reynolds, 2000; P. Lu, Jones, & Tuszynski, 2007; Silver & Miller, 2004). These cells express several inhibitory molecules including chondroitin sulfate proteoglycans (CSPGs) and keratan sulfate proteoglycans (KSPGs) which fill the extracellular matrix (ECM) surrounding the lesion site (Jones & Tuszynski, 2002; Tang, Davies, & Davies, 2003). The astrocyte response to injury is referred to as reactive gliosis (Silver & Miller, 2004). As axons cannot regenerate beyond the glial scar, this extracellular glial scar is thought to be a major limiting factor following CNS injury (Grimpe & Silver, 2002; Silver & Miller, 2004); However, increasing evidences suggests a beneficial role of this scar tissue in reestablishing the physical and chemical integrity of the CNS. Another event which makes more complex the regeneration process is progressive cavitation in which, after days to weeks, a CNS injury can expand in size; leading to a scar-encapsulated cavity more expanded the size of initial lesion (Balentine, 1978; Fitch, Doller, Combs, Landreth, & Silver, 1999).

3. New Approaches to Spinal Cord Injury Treatment

Recently, basic scientists have being applied several strategies aimed to provide new treatments (Barnett & Riddell, 2004). These include (a) promoting the survival and growth of damaged axons using different neurotrophins; (b) neutralizing inhibitory molecules associated with the failure of axonal regeneration, (c) providing a permissive growth environment by transplanting neural cells, and (d) gene therapy (Bamber et al., 2001; Bradbury et al., 2002; Qiu et al., 2002); (Harrop, Poulsen, Xiao, Freese, & During, 2004; Poulsen, Harrop, & During, 2002) .

4. Challenges of Cell Therapy for Spinal Cord Injury

Stem cells can be classified into two major categories, (i) Embryonic stem cells (ES) are pluripotent stem cells capable of differentiate to most tissues of the organism. Human ESCs are derived from discarded, non-transferred human embryos, from the inner

cell mass of a blastocyst using an immunosurgical technique. (ii) Adult stem cells are undifferentiated cells found throughout the body that divide to replenish

dying cells and regenerate damaged tissues. Adult stem cells can be isolated from a tissue sample obtained from an adult organ. Compared to adult stem cells ES cells are clinically more effective for neurological disorders. (Paspala et al 2009)

4.1. Olfactory Ensheathing Cell Transplantation

A range of cells have been investigated for use in transplantation; These include neural stem cells and glial cells such as olfactory ensheathing cells, Schwann cells and oligodendrocyte precursor cells (Barnett & Riddell, 2004). One of the most favorable candidates for cellular transplant-mediated repair of CNS lesions is the olfactory ensheathing cells (OEC). OECs reside in the olfactory system, which supports neurogenesis throughout the life (Farbman, 1990).The olfactory system originates from the olfactory placode and is made up of olfactory epithelium (PNS tissue) and olfactory bulb (CNS tissue) (Barnett & Riddell, 2004). After injury or during normal cell turnover, new olfactory receptor neurons (ORNs) are generated from the basal stem cells in the olfactory epithelium, which extend axons through the cribriform plate and re-enter the olfactory bulb, re-synapsing with second-order neurons in the glomerular layer (Schwob, 2002). This is one of the rare situations in which peripheral axons are able to enter the adult CNS environment and form synapses, and it is thought that this unusual ability may be due in part to unique specialized properties of OECs (Farbman, 1990). In a study by Raisman et al, the upper cervical corticospinal tract was transected on one side in adult rats; then a suspension of ensheathing cells cultured from adult rat olfactory bulb was injected into the lesion site; this induced unbranched, elongative growth of the transected corticospinal axons. The axons grew through the transplant and continued to regenerate into the denervated caudal host tract. Rats with complete transections and no transplanted cells did not use the forepaw on the lesioned side for directed reaching; while, Rats in which

the transplanted cells had formed a continuous bridge across the lesion exhibited directed forepaw reaching on the lesioned side (Li, Field, & Raisman, 1997).

In most previous studies, transplantation was carried out at the same time the lesions were made. The potential benefit of such an approach to human spinal cord injuries in clinical situation should take in account the unavoidable time delay between the time of injury and the time of transplantation. This issue leaded the researchers to work on delayed transplantation. For example, Lu et al, and Plant et al, reported an improvement of locomotor function following delayed transplantation of OECs into complete or incomplete thoracic spinal lesions (J. Lu, Feron, Mackay-Sim, & Waite, 2002; Plant, Christensen, Oudega, & Bunge, 2003). In a study by Naghmeh Keyvan-Fouladi et al, destruction of the dorsal corticospinal tract on one side at the level of the first cervical segment abolished the use of the ipsilateral forepaw for retrieval for at least 6 months after operation; in lesioned rats that had shown no retrieval for 8 weeks after operation, a suspension of olfactory ensheathing cells was injected into the lesion site; starting between 1 and 3 weeks after transplantation, all rats with transplants bridging the lesion site resumed retrieval by the ipsilateral forepaw; furthermore, by biotin dextran anterograde tracing they also showed regenerating corticospinal axons crossing the bridge, traveling caudally for about 10mm in the distal part of the corticospinal tract and forming terminal arborizations in the spinal gray matter. They provide an assay for determining the effectiveness of different methods of cell preparation or different cell types (Keyvan-Fouladi, Raisman, & Li, 2003). In another study by same group, it was reported that transplantation of cultured adult peripheral nerve schwann cells also restores function, but the effect is delayed until around 30 days after transplantation and reaches only around 5-10% of normal (Keyvan-Fouladi, Raisman, & Li, 2005)

4.2. Fetal Olfactory Mucosa Transplantation

Unlike rats, the olfactory bulb in humans is relatively small and inaccessible (Franklin, 2002). Since transplantation of fetal olfactory mucosa (FOM) is more safe and feasible, in one study we investigated the possible beneficial effects of FOM transplantation on the recovery of locomotor function and also spinal tissue sparing following spinal cord hemisection (Delaviz et al., 2008). Adult female rats were spinally hemisected at the L1 level and were randomized into the three groups. The first group was immunosuppressed injured animals received Cyclosporine A (CsA) and FOM graft. The second group received CsA and fetal respiratory mucosa (FRM) graft, and the control group, non-immunosuppressed rats, received saline and gel foam. From weeks 6-8, the functional recovery of the FOM rats significantly increased in comparison to the FRM, although a significant difference in tissue sparing was not apparent. From weeks 2-8, the functional recovery of the FOM and FRM groups as well as tissue sparing of the FOM group increased significantly compared to the control group .This study showed that transplantation of fetal olfactory mucosa with its lamina prafopria and olfactory neuroepithelium results in promotion of tissue sparing and functional recovery in mammals with partial spinal cord injury.

4.3. Bone Marrow Stromal Cells and Schwann Cell Transplantation

Different studies indicate that bone marrow stromal cells (BMSCs), can differentiate into adipocytes; chondrocytes; and osteocytes after transplantation in mice and rats (Pittenger et al., 1999). Oligodendrocytes, are less in number compare to neurons, and are able to express the markers of these cells (Steidl et al., 2002). They can promote injured tissue repair by reducing cavity formations caused by tissue necrosis in the site of lesions (Wu et al., 2003). BMSCs create a suitable axonal growth environment through the expression of growth factors such as neurotrophins (Chen et al., 2002; Mahmood, Lu, Wang, & Chopp, 2002). They can also improve vascularization leading to repair of damaged tissue (Mahmood et al., 2002).

Another cell type that has been used for repair of injured spinal cord is the schwann cell (SC). SCs can myelinate and ensheath axons and provide physical support for axonal growth when they are injected into the area of spinal cord lesion (Bunge, 2006; Pearse & Barakat, 2006) . SCs produce neurotrophic factors and cell adhesion molecules leading to promotion of axonal growth (Oudega & Xu, 2006). They also suppress the cavity formation when transplanted into the injured spinal cords (Pearse et al., 2007; Takami et al., 2002). Studies have shown that SC transplantation significantly improves tissue sparing and results in an increase in the number of myelinated axons in implantation areas (Takami et al., 2002). It has been reported that SC injection into the contused spinal cord promotes myelination and regeneration of supraspinal axons and therefore improves locomotor recovery (Takami et al., 2002). The advantage of the SC compared to other commonly used cells in the cell therapy is the superior ability of this cell to myelinate the demyelinated axon (Oudega, Moon, & de Almeida Leme, 2005).

Several studies have reported that transplantation of BMSCs in the contused spinal cord of rats improves locomotor recovery, and intraspinal administration of SCs facilitates axonal regeneration after SCI. Although the efficacy of these methods has been proven, when used individually, they result in a small number of regenerated axons, and a modest locomotor recovery (Takami et al., 2002) .Since a combination-therapy strategy seems more promising, we investigated whether co-transplantation of these cells can improve treatment outcome (Joghataei Mohammad Taghi., 2010) . In our study, contusion model of SCI was created at the T8-9 level in adult male rats. BMSCs and SCs were cultured and prelabeled with BrdU and 1,1' dioctadecyl 3,3,3',3' tetramethylindocarbocyanin perchlorate respectively. The rats were divided into seven groups. These groups included: a control group, three experimental groups and three sham groups. In the control group only laminectomy was performed. The three experiment groups were the BMSC, SC and co-transplant groups, and 7 days after injury, they received intraspinal BMSCs, SCs, or combination of BMSCs and SCs, respectively. The sham groups received serum in the same manner. Four weeks after the contusion injury, observation of sections stained with cresyl violet revealed the formation of several differently sized vacuoles and cystic cavities at the site of injury. The cyst formation is due to the death of neurons; interneurons; and glial cells after SCI. It was showed that BrdU-positive BMSCs, transplanted in the site of injury, survived and reorganized around the cavity center. Fluorescence microscopy also shows the presence of S-100-positive cells at the site of injury, confirmed that intra-spinaly transplanted SCs survived for a long time. Immunohistochemistry findings confirmed the presence and viability of transplanted cells at the area of lesion. Eight weeks after transplantation, the immunohistochemistry results showed that transplants of BMSCs and SCs at the site of injury survived. In functional assessment, statistical analysis revealed significant differences between the experimental and control groups, between the experimental and sham groups and between the co-transplant and SC and BMSC groups; in contrast, the statistical difference between SC and BMSC groups was not significant.

5. Intra-Thecal Delivery of Chemokines

Considering potential complications of cell transplantation such as rejection; infection; and development of new cancer our recent approach was using chemokines like stromal-derived factor-1 (SDF-1) , cytokines like granulocyte colony-stimulating factor (G-CSF) and other factors for attracting and mobilizing innate stem cells toward the injury site. In a study by Kawada et al, it has been shown that G-CSF mobilizes bone marrow-derived stem cells to repair the ischemic myocardium (Kawada et al., 2004). In an experiment by Koda et al, they showed that G-CSF mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice (Koda et al., 2007); and recently we tested the effect of SDF releasing pump on functional recovery in experimental model of spinal cord injury in rats. In this study, Brdu immunohistochemistry, and BBB tests showed a significant improvement in treated rats (Zendedel et al., 2012)

6. Self-Assembling Peptides

One of the effective strategies for spinal cord regeneration is the transplantation of scaffold contained stem cells to the lesion site; because implanted cells accompanied by scaffold may act as a bridge and also as secretors of pre-regenerative molecules such as growth factors. Different members of a self-assembling peptide hydrogel family have been evaluated as three-dimensional (3D) culture systems for carrying different cells. BD PuraMatrix peptide hydrogel, a three-dimensional cell culture model of nanofiber scaffold derived from the self-assembling peptide RADA16, can be applied to regenerative tissue repair in order to develop novel nanomedicine systems. In another study by our group, self-assembling nano-fiber scaffold (SAPNS) and schwann cells isolated from human fetal sciatic nerves transplanted into the spinal cord after injury in rats. Immunohistochemical analysis of grafted lumber segments at 8 weeks after grafting revealed reduced asterogliosis and considerably increased infiltration of endogenous S-100 positive cells into the injury site; suggesting that PuraMatrix plays an important role in the repair observed following transplantation of SAPNS with human fetal SC (Moradi et al, 2012)

7. Conclusion

In conclusion, cell therapy is a promising strategy in the field of SCI treatment. However, the approaches of cell therapy are an ever changing field. It seems that co-transplantation of different cells accompanies with other factors like enzymes and growth factors via new delivery systems may yield better results in SCI.

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درباره نشریه

فصلنامه علوم اعصاب پایه و بالینی، یک فصلنامه ی علمی، بین المللی و میان رشته است که به چاپ و انتشار مقالات پژوهشی، مروری، مقالات کوتاه، نقدها، یادداشت های روش شناختی، گزارشات، و چشم اندازها به زبان انگلیسی می پردازد و در حوزه های گوناگون علوم اعصاب بالینی، شناختی، رفتاری، سیستمی، سلولی، و ملکولی متمرکز شده است. در این میان، نشریه علوم اعصاب پایه و بالینی بر آن اســت تا با شکل دهی شــبکه متخصصین علوم اعصاب منطقه خاورمیانه، امکان گسترش دانش علوم اعصاب و همچنین شناخت هرچه بیشتر روش های درمانی بیماری های مرتبط با مغز و اعصاب فراهم آید.

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آثار و مقالاتی در فصلنامه علوم اعصاب پایه و بالینی پذیرفته می‌شوند که خود حاصل پژوهش های نوین و دسته اولی بوده که از طریق آزمایشات علمی و بر اساس اهداف و فرضیه‌های کاملاً مشخص ارائه گردند. این نشریه در نظر دارد تا زمینه‌ای را جهت ایجاد ارتباطات میان رشته‌ای و ارتقای داوری موثر مقالات برقرار نماید و رسانه‌ای علمی برای مخاطبین ملی، منطقه‌ای و بین‌المللی قرار گیرد. بدیهی است که این مجله فارغ از منافع غیرعلمی می‌باشد. فصلنامه علوم اعصاب پایه و بالینی سعی دارد تا شبکه همکاری های ملی، منطقه‌ای و بین‌المللی را در زمینه علوم اعصاب در ایران، خاورمیانه، آســیای میانه و شــمال آفریقا ســهولت بخشد و رسانه‌ی جامعه علوم اعصاب ایرانی و منطقه‌ای در جهان گردد. بنابراین، سرمقالات، مقالات مروری، مقالات علمی، مقالات کوتاه، و یادداشت های روش شناختی در راستای اهداف مذکور تعریف، و پذیرفته، و منتشر می‌گردند.

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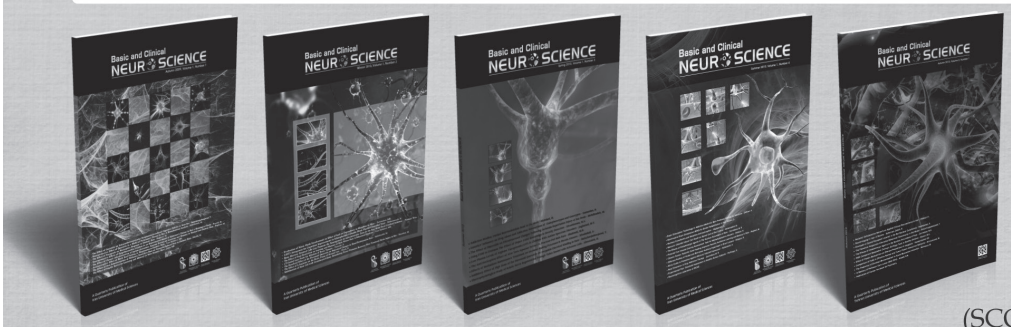

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