

The Effect of Food Deprivation on Nociception in Formalin Test and Plasma Levels of Noradrenaline and Corticosterone in Rats

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ABSTRACT

Introduction: The concentration of noradrenalin and corticosterone as the two nociception modulators change after fasting or stress situation. The aim of present study was to investigate the effect of food deprivation on formalin-induced nociceptive behaviours and plasma levels of noradrenalin and corticosterone in rats.

Methods: Food was withdrawn 12, 24 and 48 h prior to performing the formalin test, but water continued to be available ad libitum. The formalin solution (50 µL, 2%) was injected into plantar surface of hind paw. The nociception responses of the animals during the first phase (1-7 minutes), the inter-phase (8-14), the phase 2A (15-60) and the phase 2B (61-90) was separately evaluated. The plasma concentrations of noradrenalin and corticosterone were measured using specific ELISA and IRA kits, according to manufacturer's instructions.

Results: In contrast to the increasing of 48 h food deprived animals during phase 2, the nociceptive behaviours of 12 and 24 h groups decreased through the interphase, phase 2A and phase 2B. The injection of formalin in the normal male rats significantly decreased the plasma level of noradrenalin and corticosterone. Food deprivation for 12 and 24 h increased noradrenalin level significantly in comparison with control group which has caused by fasting induced antinociceptive behaviours. There was no significant change in food deprivation for 48 h group. Food deprivation for 12, 24 and 48 h had no effect on corticosterone level in male rats.

Discussion: The present study emphasizes that the acute food deprivation diminished the nociceptive behaviours in the formalin test and show a correlation with increase in plasma noradrenalin level.

1. Introduction

Both short-term and intermittent food deprivation are well known to have antinociceptive effect, which several neuromodulatory systems such as endogenous opioid system and adrenocortical hormones are known to be involved (Bodnar, Romero, & Kramer, 1988; Hamm & Knisely, 1986). Norepinephrine participates in descending pain inhibitory system. Brainstem nuclei A1–A7 such as locus coeruleus in centrally and sym-

pathetic nerves in peripherally are the main sources of norepinephrine. Locus coeruleus in the pons has most projection to the dorsal horn of spinal cord (Proudfit, 1988; Kwiat & Basbaum, 1992) and has a key role in noradrenergic pain modulation. Locus coeruleus stimulation releases norepinephrine (Hentall, Mesigil, Pinzon, & Noga, 2003) and produces analgesia that is prevented by alpha-2-adrenoceptor antagonist's administration (Jones, 1991; Proudfit, 1988). Furthermore, norepinephrine is released by peripheral noxious stimulation (Takagi, Shiomi, Kuraishi, Fukui, & Ueda, 1979; Tyce &

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Yaksh, 1981; Yaksh & Tyce, 1981). Destruction of noradrenergic system decreased formalin-induced nociceptive behaviours in phase 2 (Martin, Gupta, Loo, Rohde, & Basbaum, 1999a). Safari et al. indicated that chemical stimulation or inactivation of lateral hypothalamus induced analgesia effect and administration of lidocaine into the LC blocked the carbachol-induced analgesia (Safari, Haghparast, & Semnani, 2009). Mustonen et al. in 2005 demonstrated that 3 and 7 days of wintertime food starvation in the male American mink decreased the plasma noradrenaline and corticosterone concentrations, respectively (Mustonen, Saarela, Pyykonen, & Nieminen, 2005a), but they observed brief increase, but not significant, in the noradrenalin concentrations after 48 h of food deprivation (Mustonen, Saarela, Pyykonen, & Nieminen, 2005b). On the other hand, formalin injection elevated the noradrenaline concentration in the locus coeruleus which might be due to the pain induced by formalin (Sajedianfard, Khatami, Semnani, Naghdi, & Jorjani, 2005b). In addition, noxious stimuli, such as foot shock as well as electrical stimulation of the locus coeruleus, accelerate noradrenaline turnover in the cerebral cortex (Singewald, Kaehler, & Philippu, 1999b). There are many evidence that showed norepinephrine is involved in pain modulation during formalin-induced nociceptive behaviors. For example, alpha-1-adrenoceptor binding was attenuated in the spinal dorsal horn during formalin-induced nociceptive behaviours in mice (Nalepa et al., 2005).

Administration of noradrenaline into the central grey before and at the end of short term food deprivation, couldn't change pain threshold (Bhunja, Bharambe, Singh, Premendran, & Pande, 2000). In men, long-term food deprivation increased release of adrenaline (Uvnas-Wallenstein & Palmblad, 1980). Based on the above literatures, indicating that fasting affects formalin-induced nociceptive behaviors and noradrenaline concentration, it can be hypothesized that food deprivation in a time-dependent manner modulates formalin induced nociceptive behaviors and it might be correlated to pain modulation of noradrenalin and corticosterone after food deprivation. To test this hypothesis, formalin tests were performed following 12, 24 and 48hr food deprivation and noradrenalin and corticosterone levels were measured in male rats.

2. Methods

2.1. Subjects

All experiments were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23,

revised 1996) and were approved by the Research and Ethics Committee of Qazvin University of Medical Sciences, Qazvin, Iran. Efforts were made throughout the experiments to minimize the animal discomfort and to reduce the number of animals used. Adult male and female Wistar rats (220–300 g) were purchased from Razi Institute (Hesarak Karj, Iran), and were housed in groups of three in a temperature controlled room, under a 12 h light–dark cycle with lights on at 7:00 to 19:00. Food and water were provided ad libitum. During the experiments, attention was strictly paid to the regulations of local authorities for handling laboratory animals.

2.2. Food Deprivation

Food was withdrawn 12, 24 and 48 h prior to performing the formalin test, but water continued to be available ad libitum. Control rats had free access to both food and water.

2.3. Formalin Induced Nociceptive Behaviour

Formalin-induced nociceptive behaviour is a widely used animal model of persistent pain (Abbott, Franklin, & Westbrook, 1995; Dubuisson & Dennis, 1977). Rats (8-9 per groups) were moved to the test room at least 1 h before the commencement of the experiment. In the present study, the rats were first acclimatized for 30 minutes in an acrylic observation chamber (30 cm in diameter and in height) and then 50 μ L of 2% formalin was injected subcutaneously into the plantar surface of the right hind paw with a 30 gauge needle. Each rat was then immediately returned to the observation box, and the behavioural recording commenced. A mirror, placed at a 45° angle beneath the box, permitted the observation of behaviours without moving the box. Pain behaviour was scored as follows: 0, the injected paw was not favoured; 1, the injected paw had little or no weight placed on; 2, the injected paw was elevated and not in contact with any surface; and 3, the injected paw was licked or bit. Scores were continuously observed for the duration of the experiment (90 minutes). The nociceptive behaviour score for each 3-minutes interval was calculated as the weighted average of the number of seconds spent in each nociceptive behavioural condition, from the start of the experiment. The scores were recorded in normal rats as well as in those who received 12, 24 and 48hr food deprivation. In each group, the behavioural responses of each rat during the first phase (1-7 minutes), the inter-phase (8-14), the phase 2A (15-60) and the phase 2B (61-90) were separately evaluated (Azhdari Zarmehri H. et al., 2011; Azhdari Zarmehri, Semnani, & Fathollahi, 2008).

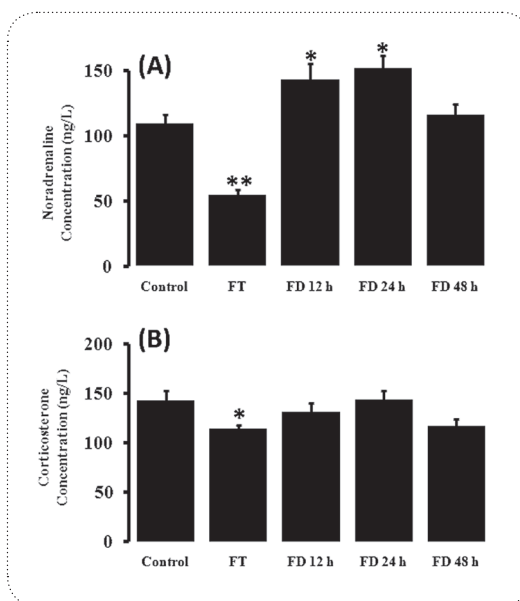
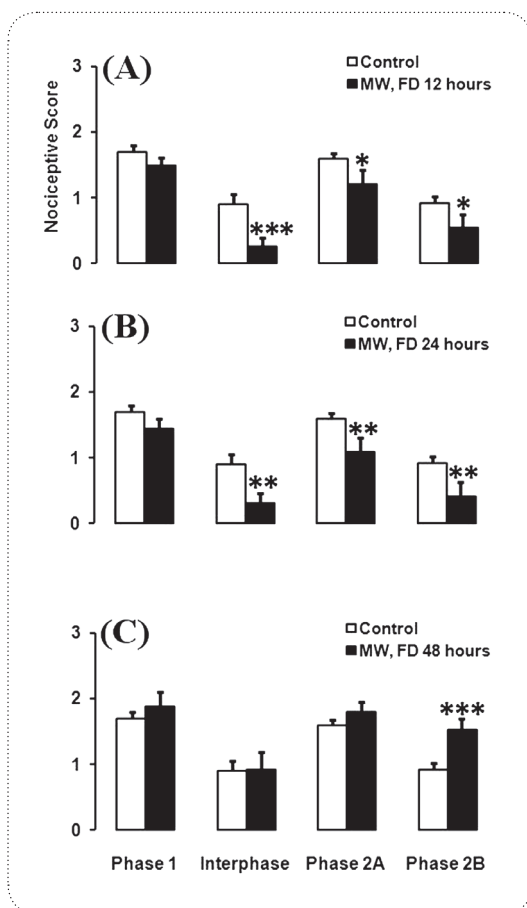


Figure 1. Effect of 12 (A), 24 (B) and 48 h (C) food deprivation on formalin test. Subcutaneous formalin (2%, 50 μ L) injection into the right hind-paw of rats on the time course of the nociceptive behaviours score measured every 3 minutes for 90 minutes. The columns represent the mean of nociceptive score in each phase: phase 1 (1–7 minutes), inter-phase (8–14 minutes), phase 2A (15–60 minutes) and phase 2B (61–90 minutes).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ food deprivation group in comparison with control one in male rats.

2.4. Blood Sampling and Noradrenalin and Corticosterone Measurements

Under deep anaesthesia, blood was collected from the heart of rats ($n=6$ for each group). First from the control group and then from food deprived groups. Blood was allowed to clot and sera were separated using centrifugation at 5000 rpm for 5 min and stored at -80°C until use. Total serum level of noradrenalin was measured using ELISA kit (Glory Science Co. USA) and total serum level of corticosterone was measured by radioimmunoassay kits (Immunotech, France). Test principle of ELISA kits was based on a double-antibody sandwich ELISA to assay noradrenalin level. In radioimmunoassay kits there is a competition between analysis in samples and ^{125}I -labeled reagent in antibody-coated tubes.

2.5. Data Analysis

Data are presented as mean \pm S.E.M. and analysed by one-way analysis of variance and t-test between groups. The mean nociceptive score in each phase (phase 1, interphase, phase 2) of the formalin test was analysed using one-way analysis of variance (ANOVA) followed by

Dunnett's post hoc test. Phase 1 (1–7 minutes), the inter-phase (8–14 minutes) and the phase 2 (2A: 15–60 and 2B: 61–90 minutes) of the formalin test were analysed separately. The defined level for statistical significance was $P < 0.05$.

3. Results

3.1. Effect of 12, 24 and 48hr Food Deprivation on Formalin-Induced Nociceptive Behaviours

Food-deprived male rats were compared with non-food deprived controls to determine if the 12 h food deprivation causes the induction of significant nociceptive behaviour with formalin in rats. Following 12 and 24hr food deprivation male rats exhibited decrease in formalin induced nociceptive behaviour. There was no significant difference in behavioural response between 12hr food deprived ($n=11$) and control ($n=11$) ones during phase 1 [$T(1,20)=1.688$; $P=0.107$], while there were significant differences in the interphase [$T(1,20)=3.922$; $P=0.001$], phase 2A [$T(1,20)=2.453$; $P=0.023$] and phase 2B [$T(1,20)=2.202$; $P=0.04$]. Although 24hr food deprivation decreased nociceptive behaviour in phase 1,

no significant difference was observed in behavioural response between 24hr food deprived (n=11) and control (n=11) ones during phase 1 [T(1,20)=1.949; P=0.065], while there were significant differences in the interphase [T(1,20)=3.572; P=0.002], phase 2A [T(1,20)=3.231; P=0.004] and phase 2B [T(1,20)=2.994; P=0.007]. Following 48hr food deprivation male rats exhibited increase in formalin induced nociceptive behaviour during phase 2. There was no significant difference in behavioural response between 48hr food deprived (n=11) and control (n=8) ones during phase 1 [T(1,16)=0.404; P=0.691], interphase [T(1,16)=0.372; P=0.714] and phase 2A [T(1,16)=1.667; P=0.114], while there were significant differences in phase 2B [T(1,16)=4.083; P=0.001; (Fig. 1.)].

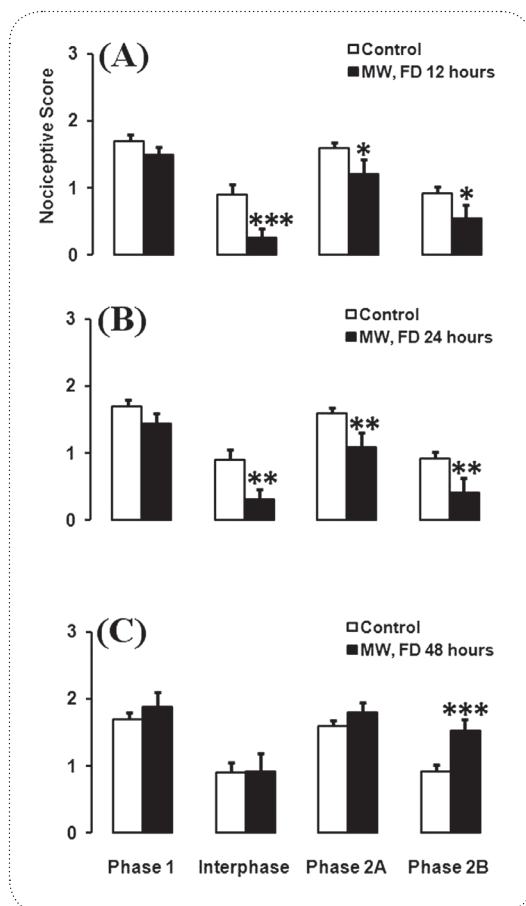
3.2. Effect of Food Deprivation on Plasma Noradrenalin and Corticosterone Concentrations

Under deep anaesthesia, blood was collected from the heart of rats in order of control, formalin test (after finishing formalin test procedure), and food deprived groups (12, 24 and 48hr). The effect of food deprivation and formalin test on plasma noradrenalin level (mean \pm SEM) is shown in Fig. 2. The injection of formalin in the male rats significantly decreased the concentration of plasma noradrenalin [T (1, 14) = 12.431, (p=0.003)]. Food deprivation for 12 and 24hr increased noradrenalin level in male rats, as compared with control [for 12 hours; T (1, 18)=7.990, (p=0.011), for 24 hours; T (1, 18)=3.734, (p=0.042)] and food deprivation for 48hr had no significant effect, as compared with control [T (1, 18)=0.498, (p=0.489)]. The injection of formalin in the male rats significantly decreased the concentration of plasma corticosterone [T (1, 14) = 5.430, (p=0.037)]. Food deprivation for 12, 24 and 48hr had no effect on corticosterone level in male rats, as compared with control [for 12 hours; T (1, 18)=0.276, (p=0.606), for 24 hours; T (1, 18)=0.263, (p=0.614), for 48 hours; T (1, 18)=1.372, (p=0.258)].

4. Discussion

Although 12 and 24hr food deprivation decreases nociceptive behaviour in the formalin test, 48hr food deprivation exhibited increase in formalin induced nociceptive behaviour. The injection of formalin in the male rats significantly decreased the concentration of plasma noradrenalin and corticosterone. This result is in consistent with Sajedianfard et al. (2005) who showed that after the injection of formalin, the noradrenaline concentration in the locus coeruleus increased during the first phase of the formalin test but not during the second phase. They

suggested that part of the increase in noradrenaline concentration in the locus coeruleus is certainly due to the pain induced by formalin injection (Sajedianfard, Khatami, Semnani, Naghdi, & Jorjani, 2005a). In addition, noxious stimuli, such as foot shock as well as electrical stimulation of the locus coeruleus, accelerated noradrenaline turnover in the cerebral cortex (Singewald, Kaehler, & Philippu, 1999a). Food deprivation for 12 and 24hr increased noradrenalin level in male rats which have correlated fasting induced antinociceptive behaviours and food deprivation for 48hr had no significant effect, as compared with control. Food deprivation for 12, 24 and 48hr had no effect on corticosterone level in male rats. Food deprivation and nociception are physiological conditions that are associated with homeostatic functioning and have bidirectional effect. In consistent with our result, it has been shown that food deprivation induces analgesia (McGivern, Berka, Berntson, Walker, & Sandman, 1979; Hamm, Knisely, Watson, Lyeth, & Bossut, 1985; Davidson, McKenzie, Tujo, & Bish, 1992). We demonstrated that 12 and 24hr food deprivation induced antinociceptive effect and increased noradrenaline level which might cause the analgesic effect of acute fasting. On the other hand, McGivern & Berntson in 1980 showed that this fasting induced analgesia is decreased by naloxone, suggesting that endogenous opioid systems may be involved (McGivern & Berntson, 1980). Food and/or water-deprivation induces analgesia or hyperalgesia and the magnitude of the increase in pain threshold depends on the duration of deprivation (Konecka, Sroczyńska, & Przewlocki, 1985). In consistent with our study, Khasar et al. demonstrated the fasting induced pronociceptive effect (Khasar, Reichling, Green, Isenberg, & Levine, 2003). Although in both studies, formalin test was used as tonic pain model, some conditions are different between the two studies; Khasar et al. observed formalin induced nociceptive behaviour for 60 min, but in this study observation of nociceptive behaviour was followed for 90 minutes and showed pronociceptive effect during phase 2B in male rats. Other methodological differences should also be considered (e.g. fasting condition, lighting, noise, odours, handling stress or anaesthesia prior to formalin injection all known to influence the formalin test). The duration or intensity of fasting has no correlation with the level of corticosterone that is in the circulation. Although 48hr food deprivation had pronociceptive effect during the second part of phase 2, it had no significant effect on phase 1 or first part of phase 2. The difference between nociceptive behaviours in the two phases might be due to several reasons. It is thought that phase 1 may be caused by increased activity in primary afferent nociceptors due to their direct activation by formalin (Hunnskaar, Berge, & Hole, 1986). Although



interphase was previously considered as an inactive phase, Henry et al (1999) showed that active inhibitory mechanisms are involved in this period (Franklin & Abbott, 1993a; Henry, Yashpal, Pitcher, & Coderre, 1999b), and it seems that sex hormones might also play a role in modulation of pain during this period (Aloisi, Albonetti, & Carli, 1994; Aloisi & Ceccarelli, 2000; Gaumond, Arsenault, & Marchand, 2002; Gaumond, Arsenault, & Marchand, 2005; Gaumond, Spooner, & Marchand, 2007). Phase 2, in addition to increased activity in sensitized primary afferent neurons, may also involve sensitization of nociceptive network in the spinal and supraspinal systems (Dickenson & Sullivan, 1987; Coderre, Vaccarino, & Melzack, 1990). Several studies suggest that the interphase of the formalin test is the result of endogenous pain-suppressing mechanisms (Franklin & Abbott, 1993b; Henry, Yashpal, Pitcher, & Coderre, 1999a). Martin et al., in 1999 showed that neurotoxic destruction of descending noradrenergic pathways had no effect on nociceptive responses in the hot-plate, tail-flick and formalin test one week post-toxin. However, two weeks post-injection, they observed a decrease in formalin-induced nociceptive behaviours in phase2 and also a reduction in formalin-evoked fos expression

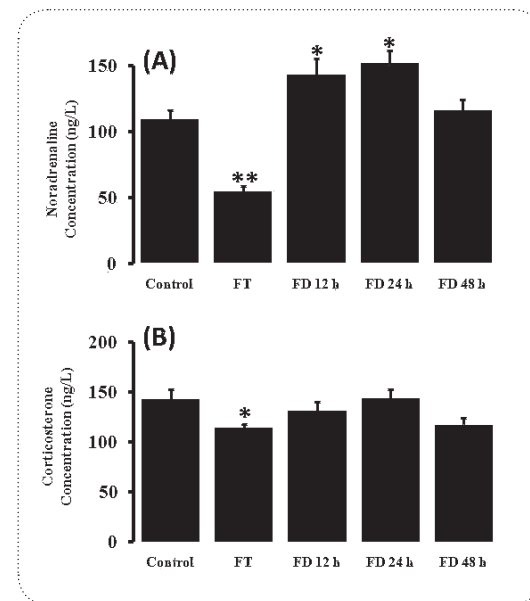


Figure 2. Effect of food deprivation on plasma noradrenaline (A) and corticosterone concentration of male rats that had ad libitum access to their diets or were food deprived for 12, 24 and 48hr.

** P<0.01 and ***P<0.01 compared to control group

in the dorsal horn of spinal cord (Martin, Gupta, Loo, Rohde, & Basbaum, 1999b). They proposed that acute and persistent nociception are differentially regulated by descending noradrenergic pathways. Another study showed that electrical stimulation of neurons in the locus coeruleus induced different analgesic effect in Sprague-Dawley rats obtained from two different vendors (Harlan and Sasco rats). The analgesic effect in Harlan rats was blocked by a selective α_2 -adrenoceptor antagonist, yohimbine or by phentolamine, a non-selective α_2 -adrenoceptor antagonist, but not in Sasco rats. These observations indicate that coeruleospinal noradrenergic neurons in Harlan and Sasco Sprague-Dawley rats have different physiological functions (West, Yeomans, & Proudfit, 1993). Holden and Naleway (2001) demonstrated that neurons in the lateral hypothalamus activate spinally projecting methionine enkephalin neurons, as well as two populations of A7 noradrenergic neurons that exert a bidirectional effect on nociception (Holden & Naleway, 2001). Noradrenergic pain modulation system is affected by norepinephrine and noradrenergic receptors, supraspinal site, spinal segmental, receptor type and pain model (Pertovaara, 2006).

We suggest that extended fasting time might produce tolerance in endogenous inhibitory system that is involved in decreasing nociceptive behaviours or finishing phase 2 of formalin test. Consistent with these results, humans subjected to food deprivation without electrolyte substitute for 72hr showed an increase in plasma cortisol, beta-endorphin, noradrenalin and dopamine which were much greater on the first morning of the fasting state (Beer et al., 1989), on the contrary, the plasma noradrenalin level of the minks diminished after 3–5 days of food deprivation (Mustonen, Saarela, Pyykonen, & Nieminen, 2005c). It seems that acute fasting could increase the concentration of plasma noradrenalin which produces analgesia in formalin test. Munro in 2007 demonstrated that 5-HT and noradrenaline reuptake inhibitor decrease formalin-induced nociceptive behaviours and also showed that systemically injected dopamine receptor agonists decrease formalin-induced nociceptive behaviours in rats (Munro, 2007). Furthermore, bicifadine, a reuptake inhibitor of 5-HT, noradrenaline and dopamine has been shown to decrease nociceptive behaviours in animal pain models (Basile et al., 2007). The increase in noradrenaline after acute fasting which was observed in our study, can cause analgesic effects mediated by adrenoceptors in the dorsal horn of the spinal cord via the descending pain inhibitory pathway (Millan, 2002). For example, noradrenaline injected to the spinal cord inhibits the response of dorsal horn neurons (Millan, 2002). Inconsistent with this study, they observed brief increase in the noradrenalin concentration after 48hr of food deprivation, however, it was not significant (Mustonen, Saarela, Pyykonen, & Nieminen, 2005d). It can, however, be proposed that the increases in noradrenalin concentration is a part of the stress response following food deprivation. The decrease in the noradrenalin level after formalin test, may be associated with decreased stimulation of the sympathetic nervous system and, thus, to energy saving during food deprivation. Based on the previous studies, we suggest that increased noradrenalin concentration might induce the antinociceptive effect that happens after fasting in formalin test. We have no financial or other conflicts of interest.

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