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Title: Comparison of effects of light anesthetics (diethyl ether and CO_2) on hypothalamic paraventricular nucleus D_1 and D_2 dopamine receptors- and glucosensitive neurons-induced food intake in 18 hours food-deprived conscious rats

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Abstract

Carbon dioxide (CO_2) and diethyl ether are administered as light anesthetics. However, little is known about side effects of its actions on experimental data. In addition, in all our previous works on regulatory mechanisms of hypothalamus in food intake including the effect of PVN D_1 and D_2 dopamine receptors and glucosensitive neurons, the drug injections were performed under brief diethyl ether anesthesia. In current study, we address the hypothesis which demonstrates that CO_2 and diethyl ether as light anesthetic agents affect the stimulatory effect of PVN dopamine receptors and glucosensitive neurons in feeding behavior.

Male Wistar rats were implanted with guide cannula directed to the PVN. Glucose (0.8 µg), SKF38393 (D1 agonist, 0.5 µg), quinpirole (D_2 agonist, 0.3 µg) and saline (0.3 µl) were microinjected into the PVN and food intake was measured over 1 hour. Our results showed that CO_2 but not diethyl ether decreased food intake compared to intact animals. The PVN injections of glucose, SKF38393 and quinpirole increased food intake under brief diethyl ether anesthesia. In contrast, the PVN microinjected glucose- and dopamine receptors agonists-induced food intake inhibited under light CO_2 anesthesia. Our results suggest that brief exposure to CO_2 and diethyl ether as light anesthetic agents may affect PVN glucosensing neurons- and dopamine receptors- induced food intake in fasted rats.

Key words: CO_2, diethyl ether, PVN, food intake, dopamine receptors, glucosensing neurons

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Introduction

In experimental approaches, inhalation of CO_2 or diethyl ether is used as light anesthetic agents. For example, in our previous studies that we considered the role of the ventromedial (Eliassi, Nazari, & Naghdi, 2009) and paraventricular (Chaleek, Kermani, Eliassi, & Haghparast, 20sthe12**;** Kermani et al., 2012) hypothalamic orexin-1 receptors in regulation of gastric acid secretion, the VMH or PVN drug injections were performed under brief diethyl ether anesthesia. In addition, Zaringhalam, Tekieh, Manaheji, & Akhtari (2013) considered the cellular events during arthritis-induced hyperalgesia under brief CO_2 anesthesia. However, little is known about the effect of light anesthetic agents on experimental results. Van Herck et al. (1991) demonstrated that rat plasma corticosterone and glucose increased after two minutes exposure to diethyl ether anesthesia. Furthermore, Zardooz et al. (2010) showed that a brief exposure to either diethyl ether or CO_2 affected the plasma corticosterone, glucose, and insulin levels in fed and/or fasted rats. These data and others (Tanaka, Nabatame, & Tanifuji, 2005) support that light anesthetic agents affect the experimental data.

Recently, the effects of carbon dioxide (CO_2) on insects and plants behavior have been shown. For example, Shahid, Hill and Ignell (2014**)** demonstrated that the take-off and source contact behavior of *Aedes aegypti* (female yellow fever mosquitoes) is impeded at elevated background levels of CO_2 as a result of masking of the stimulus signal. Furthermore, saprophagous insects often use CO_2 as a cue for finding food (Kojima, 2015) and elevated atmospheric CO_2 increased fiber fractions of a mammalian herbivore, *microtus ochrogaster* (Habeck & Lindroth, 2013).

To control the homeostatic feeding motivation, a number of neurons project to hypothalamic paraventricular nucleus (PVN) (Morton, Cummings, Baskin, Barsh, & Schwartz, 2006; Saper et al. 2002; Schwartz et al., 2000). Dopamine is also considered to be the main catecholamine in the brain and serves an important regulatory role in the control of feeding behavior (Szczypka, Rainey, & Palmiter, 2000; Steele et al., 2010). Dopamine signaling is mediated by five receptors, termed D_1-D_5 receptors. It has been shown that administration of D_2 receptor agonist decreases plasma leptin levels in an obese woman and increases food intake (Kim, Shin, Kim, Lee, & Baik, 2005). Furthermore,

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according to Yu and Kim (2012) study, D_4 receptors in the PVN may be a pharmacological target for obesity. Very recently, we also described the D_1 and D_2 dopamine receptors and also glucosensitive neurons in the hypothalamic paraventricular nucleus (PVN) increased food intake in 18 hours food-deprived rats (data is preparing to be submitted). In our experiments, dopamine agonists, antagonists and glucose were injected into the hypothalamic paraventricular nucleus under light diethyl ether anesthesia. However, little is known about the effect of brief diethyl ether or CO_2 anesthesia on experimental food intake results. Therefore, in this study, we hypothesized if inhalation of diethyl ether and CO_2 as light anesthetic agents is able to affect food intake in conscious rats. Furthermore, we considered and compared the effect of these two anesthetic agents on PVN D_1 and D_2 dopamine receptors- and glucose-induced food intake in 18 hours food deprived rats.

Method and Materials

Animals

Male 220-250 g Wistar rats (Neuroscience Research Center, Tehran, Iran) were exposed to a 12 : 12 h light: dark cycle at 22–24 ºC. They were deprived of food, but not water, for 18-20 h prior to experiments.

Drugs

Ketamine (Rotex, Levallois-Perret, France) and xylazine (Alfasan, Woerden, The Netherlands) were used to anaesthetize rats. Quinpirole, SKF38393 and glucose were purchased from Sigma (St Louis, MO, USA).

Injection of compounds

Drugs or vehicle were injected in a volume of 0.3 µl into the PVN. The drug injections were performed under brief diethyl ether or CO_2 anesthesia using a Hamilton 0.5 µl. Animals exposed to CO_2 or diethyl ether inhalation for 30 s and obtained full consciousness after 1 minute.

Surgery

After anesthetizing by ketamine and zylazine, animals were fitted with a 23-gauge stainless steel cannula. Cannula was inserted into right PVN according to the stereotaxic atlas of Paxinos & Watson (2007) as follows: lateral: 0.4 mm from midline; dorsoventral: 7 mm from skull surface; anteroposterior: 1.8 mm from the bregma. The injector was extended 1 mm beyond the end of the

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guide cannula. Experimental trials were performed after 7-day recovery period. For histological examination, the brains were fixed in formalin and 100 µm thick sections were taken and examined with light microscopy.

Measurements of food intake

The weight of food pellets used were measured with a Sartorius, TE3135 (Gottingen, Germany), with d = 0.001 mg accuracy. Feeding trials normally conducted from Saturday to Wednesday between 9:00 and 12:00 h. On the test day fasted rats were transported to the laboratory at least 1 h before the beginning of the feeding trial. After injecting the test compound, the rats were placed in a clear plastic cage and allowed access to a pre-measured amount of their regular lab feeding chow. The amount of food and crumbs left in the test cages was measured. Rats received no more than two feeding trials per week. All experiments were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.PHNS.REC.1396.33).

Statistical analysis

Results are shown as the mean ± SEM. The differences between two and more than two groups were evaluated by student's t-test and one way ANOVA followed by Tukey's HSD test, respectively. $P < 0.05$ was considered.

Results

Influence of light CO_2 and diethyl ether anesthesia on food intake:

Inhalation of CO_2 but not diethyl ether (30 s) had a significant effect on food intake. After light CO_2 anesthesia, food intake decreased significantly (Fig. 1). Our results indicate a reduction of approximately 20% with a value of 1.71 ± 0.1 g/h from light CO_2 test group compared to 2.1 ± 0.1 g/h for the control group ($n = 5$) ($P < 0.01$). Furthermore, Fig. 1 demonstrated that light diethyl ether anesthesia had no effect on food intake.

Effects of light CO_2 and diethyl ether anesthesia on glucose-induced food intake

Our study showed that the PVN injection of glucose induced dose-dependent increase of gastric acid secretion and glucose 0.8 µg had maximum stimulatory effect (**Chaleek, Kermani, Eliassi, &**

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Haghparast, 2012). Acid secretion is a part of feeding behavior. As shown in Fig. 2, in light CO_2 test group, glucose 0.8 µg did not affect food intake compared to control rats (animals received saline without anesthetic agents). In diethyl ether groups, food intake increased from 2.1 g/h in saline group to 2.6 ± 0.08 g/h in glucose 0.8 µg-treated rats (n = 5) ($P < 0.01$). In the absence of anesthetic agents, however, the magnitude of glucose-induced food intake was approximately 2-fold more, compared to the control group (Fig. 2).

Effects of light CO_2 and diethyl ether anesthesia on SKF3833 (D_1 receptor agonist) - and quinpirole (D_2 receptor agonist)-induced food intake

In our previous study we showed that PVN-microinjected SKF38393 and quinpirole increased food intake in a dose-dependent manner and the maximum effects were observed at doses 3 and 5 µg, respectively (**data is preparing to be submitted).** PVN injection of SKF38393 ($P < 0.001$) or quinpirole ($P < 0.01$) decreased food intake after light CO_2 anesthesia (Fig. 3 and 4). Compared to CO_2 group, light diethyl ether anesthesia had reverse effect on D1 and D_2 receptors -induced food intake. As shown in Fig. 3 and 4, PVN microinjection of SKF38393 (5 µg) and quinpirole (0.3 µg) increased food intake compared to saline group ($P < 0.001$ and 0.0001, respectively).

Discussion

In this study we have demonstrated that brief inhalation of CO_2, but not diethyl ether, as light anesthetic agents decrease food intake compared to saline-treated rats. Furthermore, in current study we have found that D_1 and D_2 dopamine receptors-induced food intake decreases under light CO_2 anesthesia. However, despite the negative effect of CO_2 on D1 and D_2-induced food intake, we observed D_1 and D_2 agonist increase feeding behavior under brief diethyl ether anesthesia. Our result has also shown that glucose-stimulated food intake remains in high level under light diethyl ether anesthesia as well as intact animals (without light anesthetic agents). However, this effect did not observed in CO_2 group at the same drug condition.

The hypothalamic paraventricular nucleus (PVN) receives a number of central pathways to control the eating behavior (Blouet & Schwartz, 2010; Morton, 2006; Schwartz et al., 2000). These studies have demonstrated that the neuropeptides and neurotransmitters are involved in these phenomena. For

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example, anorectic agent induces its effects through the cerebral release of dopamine, and the consequent activation of D1-like and D2-like receptors (Leibowitz, 1975; Chen, 2001; Kuo, 2002; Kuo, 2003), decreasing the level of hypothalamic NPY (Hsie, Yang, & Kuo, 2005; Kuo, 2005). Furthermore, we have shown that PVN-microinjected SKF38393 (a dopamine D1 agonist) and quinpirole (a dopamine D_2 agonist) increased food intake at doses more than 0.07 µg. These effects were inhibited by D_1 and D_2 dopamine receptor antagonists, SCH23390 and sulpiride, respectively (**data is preparing to be submitted**).

Within the hypothalamus, glucosensitive neurons are found in the arcuate and paraventricular nuclei (Silver & Erecinska, 1998). Our results showed that the PVN-microinjected glucose increased gastric acid secretion at doses of 350-750 nM in 18-24 h fasting conscious rats **(Chaleek, Kermani, Eliassi, & Haghparast, 2012)**. Gastric acid secretion is a part of feeding behavior. Therefore, we suggested that the PVN-glucose sensing neurons might be involved in central regulatory mechanism of acid secretion and the control of energy homeostasis. All our experiments were done under brief diethyl ether anesthesia. Although it is well established that PVN D_1 and D_2 dopamine receptors and glucosensing neurons are involved in regulatory mechanisms of feeding behavior, the specific effects of inhalation of light anesthetic agents including CO_2 and diethyl ether during experimental approaches have remained unexplored. Our results showed that glucose and D_1 and D_2 agonists increased food intake under brief diethyl ether anesthesia whereas light CO_2 inhalation inhibited the effect of glucose and changed the stimulatory effects of D_1 and D_2 agonists to inhibitory effects in feeding behavior. At the present time, we do not know the exact CO_2 and diethyl ether mechanisms on food intake. It is probably that the food intake is decreased under brief anesthesia as a result of masking of the stimulus signal. For example, the PVN and lateral hypothalamus received NPY-containing neuron projections from arcuate nucleus. It has been shown that NPY increased food intake by activating NPY1 and NPY5 receptors within the hypothalamus (Levens & Della-Zuana, 2003; Mashiko et al., 2007). Furthermore, our previous studies indicated the orexin A-induced gastric acid secretion in PVN (Chaleek, Kermani, Eliassi, & Haghparast, 2012) was blocked by ICV administration of NPY1- and NPY5-receptor antagonists (Kermani

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& Eliassi, 2012). Gastric acid secretion is a part of feeding behavior. Meguid et al. (2000) demonstrated that afferent information from the autonomic nervous system, affects gastrointestinal mediators, circulatory concentrations of nutrients and hormones are transmitted to the presynaptic monoaminergic system of the hypothalamus. These presynaptic afferent neurons influence postsynaptic cells by release of dopamine. According to their model, postsynaptic neurons may express both D_1 and D_2 receptors which are involved in food intake by activation of stimulatory and inhibitory food intake neuropeptides including neuropeptide Y (NPY). Therefore, we suggest that masking of first or second order hypothalamic neurons in response to the light anesthetic agents may be one mechanism by which food intake is decreased.

In conclusion, the present study demonstrates that light CO_2 but not diethyl ether anesthetics decrease food intake. Our results suggest that dopamine receptors and glucosensing neurons in PVN may be, at least in part, one of targets of light anesthetic agents. Whether these effects result from masking of inhibitory or stimulatory neurons which originate in the PVN or is mediated by fibers of passage is yet to be determined. Current study also suggests that feeding experimental results may be affected by any experimental approach using these anesthetics

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Figure legends

Figure 1 Inhibitory effects of the light anesthetized CO2 and diethyl ether on PVN injection of saline in food intake over a 1 h period. Light anesthetics decreased food intake. Data are mean ± SEM (n = 5

per group). ***P < 0.001 compared to saline group, **P < 0.01compared with saline group (intact animal)

Figure 2 Effects of the light anesthetized CO_2 and diethyl ether on PVN microinjection of glucose (0.8 µg) in food intake over 1 hour. CO_2 inhibited and diethyl ether decreased glucose-induced food intake compared to intact animal. Data are mean ± SEM (n = 6 per group). **P < 0.01, ****P < 0.0001 compared to saline group.

Figure 3 Effects of light CO_2 and diethyl ether anesthetics on PVN-microinjected SKF38393 induced food intake. SKF38393 decreased and increased food intake under brief CO_2 and diethyl ether anesthesia, respectively. ****P < 0.0001 compared to saline groups.

Figure 4 Effects of light CO_2 and diethyl ether anesthetics on PVN microinjection of quinpirole in food intake over 1 hour. Quinpirole decreased and increased food intake under brief CO_2 and diethyl ether anesthesia, respectively. ****P < 0.0001, **P < 0.01compared to saline groups.

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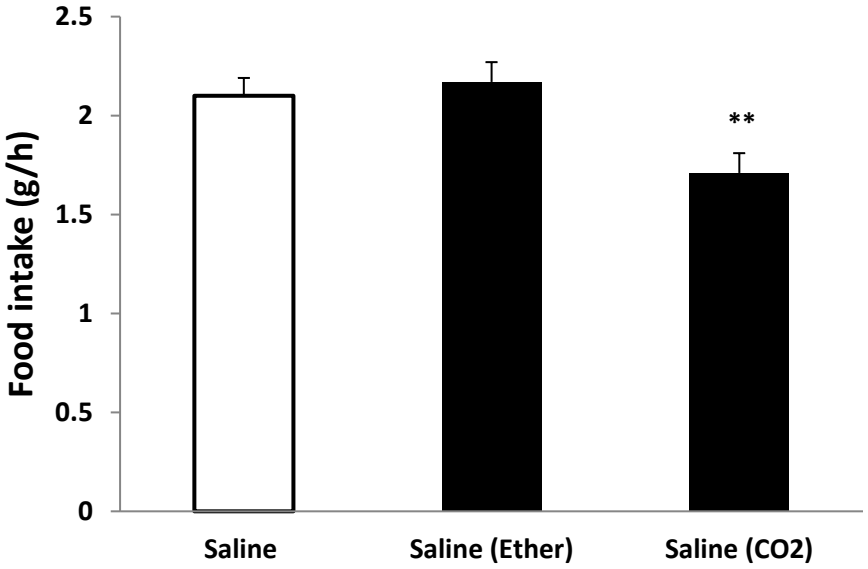

Figure 1

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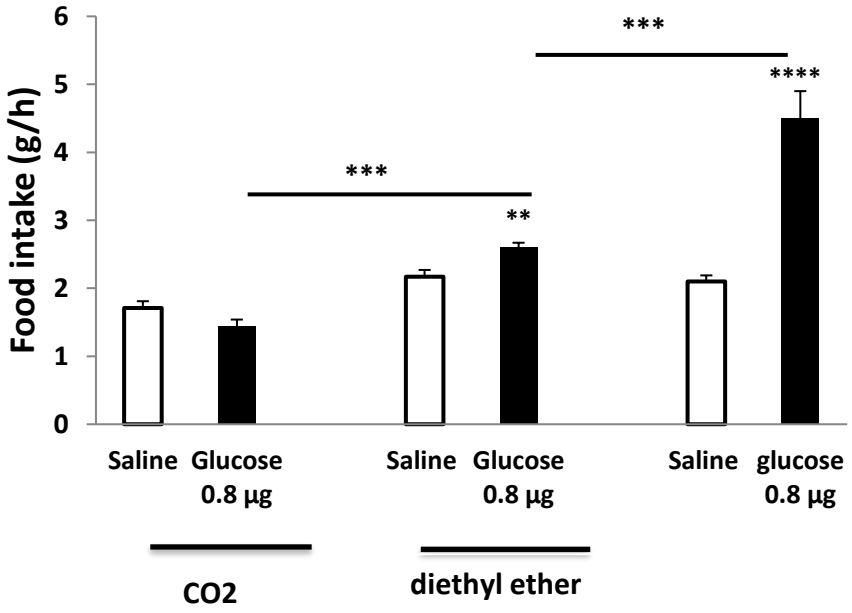

Figure 2

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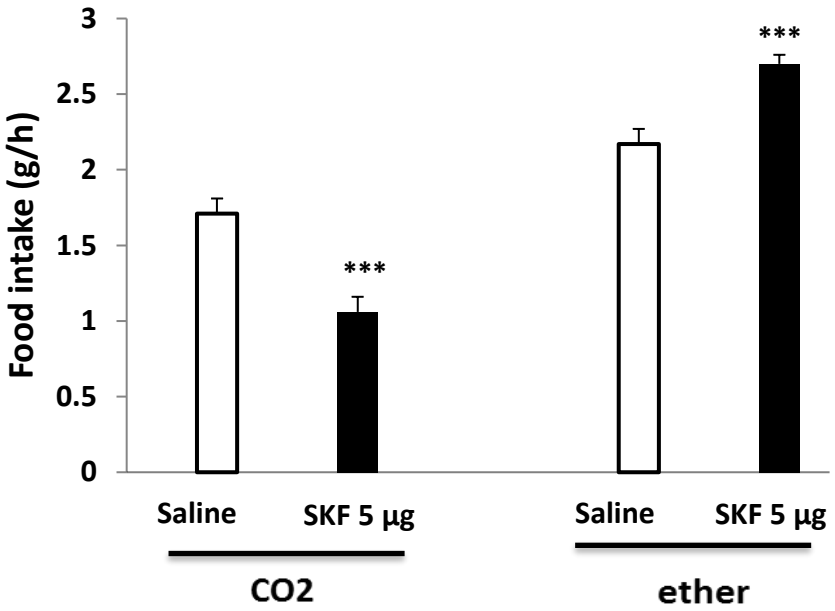

Figure 3

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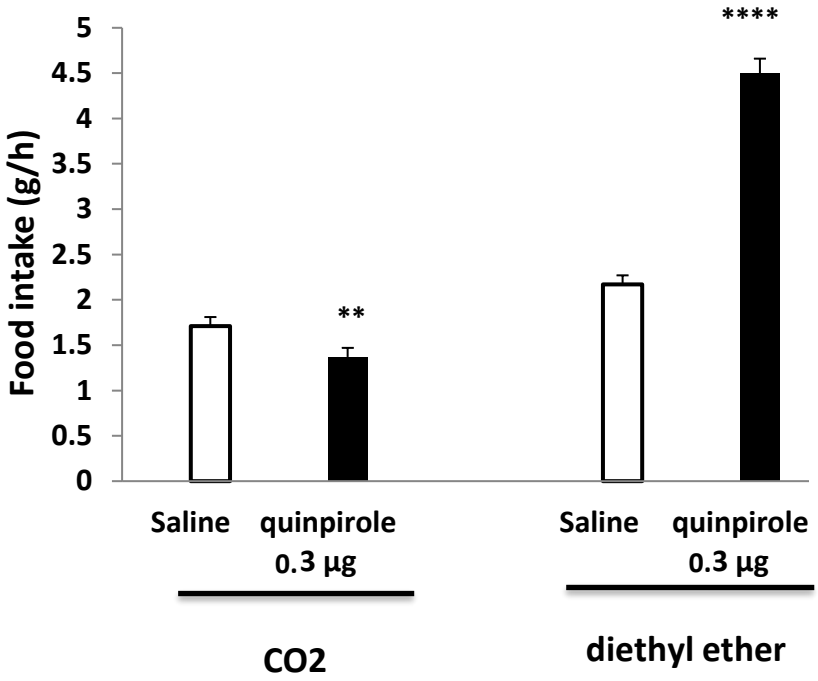

Figure 4

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