Physiol. Res. 59: 973-978, 2010

Plasma Corticosterone, Insulin and Glucose Changes Induced by Brief Exposure to Isoflurane, Diethyl Ether and CO_2 in Male Rats

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Received September 27, 2009
Accepted March 26, 2010
On-line June 9, 2010

Summary

The impact of anesthetic agents on endocrine and metabolic factors is an important issue. The present study has compared the effects of a short-term exposure to diethyl ether, isoflurane, or CO_2 on plasma corticosterone, insulin and glucose concentrations since the duration of anesthetic exposure may have an effect on those factors. Male rats were divided into fed and fasted groups. The experimental rats were briefly exposed to diethyl ether, isoflurane, or CO_2 (the degree of anesthesia was identical), while a control group was not exposed to the anesthetics. In the fed rats, diethyl ether exposure increased the levels of plasma glucose. CO_2 exposure decreased plasma corticosterone and increased plasma glucose levels. Isoflurane exposure caused no changes in plasma corticosterone, glucose, or insulin levels. In the fasted rats, diethyl ether exposure increased plasma corticosterone and reduced plasma insulin levels. The plasma corticosterone and insulin levels were significantly increased by CO_2 exposure. Isoflurane exposure decreased plasma insulin levels. A brief exposure to either diethyl ether or CO_2 changed the plasma corticosterone, glucose, and insulin levels in fed and/or fasted rats. However, isoflurane exposure had the least effect on the concentration of these factors in both the fed and fasted states.

Key words

Anesthesia • Anesthetic agents • Corticosterone • Glucose • Insulin

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Introduction

Plasma corticosterone levels in rodents have been considered an important index of stress (Vachon and Moreau 2001, Abelson *et al.* 2005). However, studies indicate that plasma corticosterone and/or other metabolic parameters such as glucose and insulin concentrations could be affected by factors (e.g. anesthetic agents) other than typical stressors (Winder *et al.* 1983, Nishiyama *et al.* 2005).

In one study, rats were exposed to diethyl ether anesthesia. After two minutes of exposure the corneal reflex had disappeared, and the animals were removed and subjected to orbital puncture. The results showed a pronounced increase in plasma corticosterone with a slight increase in plasma glucose (Van Herck *et al.* 1991). Conversely, with a longer duration of diethyl ether exposure (30 min), fasting plasma glucose and insulin increased significantly in rats starved for 24 h (Aynsley-Green *et al.* 1973). In a clinical study on human subjects, 15 min of anesthesia with isoflurane or sevoflurane caused a significant increase in plasma glucose, but marked decrease of plasma insulin levels (Tanaka *et al.* 2005). However, a longer duration of anesthesia with isoflurane or sevoflurane (10 h) caused an increase in plasma cortisol and glucose levels, but had no effect on plasma insulin concentration in human subjects

(Nishiyama *et al*. 2005). Zuurbier *et al.* (2008) obtained the same results in rats following 30 min of anesthesia with isoflurane or sevoflurane.

Thus, it is evident that exposure to anesthetics may affect experimental results, with differences in the exposure duration leading to further variations. Therefore, the results obtained from relatively short procedures (e.g. retro-orbital blood sampling), which require a short period of exposure to anesthesia, may also be difficult to interpret due to the additional effects of the anesthetic agents on corticosterone or other metabolic parameters such as plasma insulin and glucose.

In this regard, the present study has been designed to further clarify the effects of a brief exposure to commonly used inhaled anesthetics (isoflurane, diethyl ether and CO_2, which are also used for short laboratory procedures), on plasma levels of corticosterone, glucose, and insulin. The blood samples were acquired using the retro-orbital puncture technique allowing for a rapid sampling procedure.

Methods

Animals

Male Wistar rats weighing 180-210 g (Pasteur Institute, Tehran, Iran) were used throughout this study (n=7-9 per group). The animals were housed two per cage at 22±2 °C, and the regular 12 h dark/light cycle was kept constant (light on at 0700 h and off at 1900 h). The animals had access to food and water *ad libitum*. For the studies using fasted rats, food was withdrawn for 16 h (from 1630 P.M. to 0830 A.M.) before the start of the experiment. All experimental procedures were conducted in accordance with the Committee's Guidelines and Regulations for Animal Care and were approved by the animal care and use committee of the Shahid Beheshti University of Medical Sciences, Neuroscience Research Center.

The animals were randomly divided into two groups, fed and fasted. Each group was subdivided into two groups, control and experimental. In the control group, blood was obtained without anesthesia, whereas in the experimental group, the animals were exposed to isoflurane, diethyl ether, or CO_2. The reduction of respiratory rate (nearly 50 %) and loss of the righting reflex (a reflex which maintains the animal's normal standing position and head upright) were considered to be signs of deep anesthesia in the experimental group (Yale University IACUC)[1]. At the end of the experiments, the animals were euthanized by CO_2 (NIH Guidelines)[2].

Open drop method

The method used to deliver isoflurane or diethyl ether to the rats is described in detail in the policies and guidelines of Institutional Animal Care & Use Committee (Yale University IACUC). In short, a cotton ball soaked in the exact amount of isoflurane (1.25 ml/l) or diethyl ether (2.75 ml/l) was placed in a transparent glass desiccator, under a screen to avoid any skin irritation to the rat caused by a contact with the soaked cotton. Each rat was monitored after being placed inside the desiccator with a tightly closed lid. A reduction in the animal's respiratory rate (nearly 50 %) and loss of the righting reflex were indicative of a state of deep anesthesia (Yale University IACUC), which occurred nearly 2 min after isoflurane and 4 min after diethyl ether exposure. The rat was immediately removed from the desiccator as soon as these signs were observed. If no response to a toe pinch was seen, the retro-orbital blood sampling was performed immediately after removing the rat from the desiccator.

In order to anesthetize the rats with CO_2, a vacuum desiccator connected to a CO_2 cylinder was used. After placing the rat inside the desiccator, CO_2 (100 %), with a constant pressure (50 kg/cm^2) and flow rate of 7 l/min, was dispersed into the desiccator. The rat was removed from the desiccator once the signs of deep anesthesia (in about 1 min from starting CO_2flow) were observed. The blood sampling procedure followed immediately.

One milliliter of blood was collected in an Eppendorf tube containing 5 µl heparin (5000 IU/ml) (Chalkley *et al.* 2002), and centrifuged at 3000×g for 5 min (Toleikis and Godin 1995). The plasma was collected and stored at –74 °C for measurements of corticosterone, glucose, and insulin.

Drugs

Isoflurane (Nicholas Piramal, UK), diethyl ether (Merck, Germany), and CO_2in gaseous form (Iran-Oxygen Co.), were used as inhaled anesthetics.

[1] Policies and guidelines, Institutional Animal Care & Use Committee (IACUC). Yale University. Last Modified: November 21, 2005.

[2] Guidelines for euthanasia of rodents using carbon dioxide, NIH Guidelines, December 9, 2001.

Table 1. Plasma corticosterone concentrations in fed and fasted rats of control group (non-anesthetized) and in rats under isoflurane, diethyl ether and CO_2 anesthesia.

Group	Corticosterone concentration (nmol/ml)			
	Control	Isoflurane	Diethyl ether	CO_2
Fed	1.85 ± 0.21	1.50 ± 0.06	1.59 ± 0.10	$0.93 \pm 0.10^{***\,\P}$
Fasted	1.66 ± 0.22	1.84 ± 0.14	$2.65 \pm 0.17^{***\,\S}$	$2.18 \pm 0.15^{*}$

Data are mean ± S.E.M. (n=7-9 per group). * P<0.05, *** P<0.001, significant difference versus control; ¶ P<0.001, significant difference versus diethyl ether and isoflurane anesthesia; § P<0.001, significant difference versus isoflurane anesthesia.

Table 2. Plasma glucose concentrations in fed and fasted rats of control group (non-anesthetized) and in rats under isoflurane, diethyl ether and CO_2 anesthesia.

Group	Glucose concentration (mg/dl)			
	Control	Isoflurane	Diethyl ether	CO_2
Fed	105.58 ± 4.03	118.17 ± 2.02	$144.96 \pm 4.61^{***\,\Psi\,\P}$	$124.28 \pm 11.13^{*}$
Fasted	88.03 ± 2.09	92.83 ± 4.08	98.11 ± 3.25	96.97 ± 4.98

Data are mean ± S.E.M. (n=8-9 per group). * P<0.05, *** P<0.001 significant difference versus control; Ψ P<0.01, ¶ P<0.001 significant difference versus CO_2 and isoflurane anesthesia, respectively.

Hormone assessments

Plasma corticosterone was analyzed by the corticosterone Elisa kit (DRG, Germany). Plasma glucose was assessed using a glucose oxidase method (Pars Azmoon, Iran). Plasma insulin was determined by the rat insulin Elisa kit (Mercodia, Sweden).

Statistical analyses

All data are expressed as the mean ± S.E.M. One-way and two-way analysis of variance were performed and supported by an LSD test. P<0.05 value was considered to be statistically significant.

Results

Plasma corticosterone levels in fed and fasted animals

Plasma corticosterone levels decreased significantly in the fed group under CO_2 anesthesia compared with the control, isoflurane, and diethyl ether groups (P<0.001) (Table 1).

In the fasted animals, diethyl ether increased corticosterone levels significantly as compared to the controls and isoflurane-treated animals (P<0.001). Exposure to CO_2 also increased plasma corticosterone levels compared with the fasted control rats (P<0.05) (Table 1).

Moreover, a two-way ANOVA showed no significant difference between fed and fasted rats of the control group, whereas there were significant differences (P<0.001) between fed and fasted rats in each group subjected to the same anesthetic agents.

Plasma glucose levels in fed and fasted animals

A significant increase in plasma glucose levels was observed in the fed rats under CO_2 (P<0.05) and diethyl ether (P<0.001) anesthesia compared with the controls (Table 2). In the fed group, diethyl ether anesthesia caused a significant elevation of plasma glucose concentrations as compared to isoflurane (P<0.001) and CO_2 (P<0.01) anesthesia (Table 2).

No significant difference was observed between all groups of fasted rats with respect to plasma glucose concentrations (Table 2).

However, plasma glucose concentrations in fed rats were significantly higher than those in the fasted animals in all experimental groups (P<0.001).

Plasma insulin levels in fed and fasted animals

Plasma insulin levels were increased significantly in the fed groups under diethyl ether and CO_2 anesthesia as compared to the isoflurane group (P<0.001) (Table 3).

In the fasted groups of rats, the plasma insulin was significantly increased under CO_2 anesthesia,

Table 3. Plasma insulin concentrations in fed and fasted rats of control group (non-anesthetized) and in rats under isoflurane, diethyl ether and CO_2 anesthesia.

Group	Insulin concentration (µg/l)			
	Control	**Isoflurane**	**Diethyl ether**	**CO_2**
Fed	1.03 ± 0.09	0.71 ± 0.11	1.51 ± 0.3¶	1.42 ± 0.18¶
Fasted	0.71 ± 0.12	0.29 ± 0.08**	0.32 ± 0.06**	1.33 ± 0.22$^{***\ §}$

Data are mean ± S.E.M. (n=7-9 per group). ** P<0.01, *** P<0.001 significant difference versus control; ¶ P<0.001, significant difference versus isoflurane anesthesia; § P<0.001 significant difference versus isoflurane and diethyl ether anesthesia.

compared to the control, diethyl ether, and isoflurane groups of animals (P<0.001) (Table 3). Compared to the control rats, a significant decrease in plasma insulin concentrations was observed under isoflurane and diethyl ether anesthesia in the fasted state (P<0.01) (Table 3).

A two-way ANOVA showed a significant difference between the fed and fasted rats in the isoflurane and diethyl ether groups (P<0.001).

Discussion

The main objective of the present study was to further clarify the effects of a brief exposure to inhaled anesthetics. The results of this study indicated that a brief exposure to diethyl ether and CO_2 may cause significant changes in plasma corticosterone, insulin, and glucose concentrations both in fed and fasted rats compared to isoflurane. These results have highlighted the possibility of changes in endocrine and metabolic factors, even under brief exposure to these anesthetics.

In this study, the control (non-anesthetized) group values may be affected by the stress imposed by the procedure (e.g. handling and blood sampling). However, since this intervention could not be avoided, we considered this group as the control group.

Diethyl ether as an anesthetic agent causes noticeable stress responses (Van Herck *et al.* 1991) and has even been used as an actual stressor (Hashimoto *et al.* 1989). In contrast to the results of the current study, plasma concentrations of corticosterone and glucose were elevated concomitantly under diethyl ether anesthesia in other studies (Van Herck *et al.* 1991, De Haan *et al.* 2002). However, the difference may be due to the experimental design (e.g. the duration and type of exposure and also the volume of the diethyl ether). On the other hand, fasting may cause plasma corticosterone elevation (Woodward *et al*. 1991, Chang *et al.* 2002), but it is also dependent on the fasting duration (Bojková *et al.* 2006). It appears that fasting, even for a short duration, combined with diethyl ether, could markedly increase plasma corticosterone levels. Moreover, the significant reduction in plasma glucose levels in the fasted control rats compared to the fed animals may be attributable to the effect of fasting (Guezennec *et al.* 1988). Aynsley-Green *et al*. (1973) showed that in rats that were fasted for 24 h, the fasting plasma glucose and insulin levels rose significantly following a 30 min exposure to diethyl ether using a vaporizer, which contradicts our results. One explanation for this difference could be the time of anesthetic exposure, which was shorter in our study (4 min versus 30 min). This finding shows that the effects of diethyl ether on plasma insulin and glucose levels could be different according to the length of exposure to the anesthetic. Another possible explanation is that plasma corticosterone elevation following diethyl ether exposure in the fasted rats may actually inhibit insulin secretion (Billaudel and Shutter 1982). In the fed rats, increased plasma glucose concentrations under diethyl ether exposure, without significant change in corticosterone, could be the result of an increase in the sympathoadrenal system activity (Carruba *et al*. 1987).

An increase in plasma corticosterone levels in the fasted rats under CO_2 anesthesia is in agreement with the results of an experiment which was done by Altholtz *et al*. (2006) in rats using the same anesthetic. The insulin elevation in the same group of rats was observed to be euglycemia due to the acute hyperglycemic effect of corticosterone secretion induced by the CO_2. However, in the fed rats under CO_2 anesthesia, despite a reduction in plasma corticosterone concentration, the plasma glucose levels increased. This increment of plasma glucose may be due to the increased sympathoadrenal system activity caused by CO_2 exposure (Winder *et al.* 1983).

Under a brief exposure to isoflurane, the lack of changes in plasma corticosterone is in contrast with previous studies which indicated that long-term duration

of isoflurane exposure may increase plasma cortisol in humans (Nishiyama *et al.* 2005) and plasma corticosterone in rats (Altholtz *et al.* 2006). It appears that anesthetic exposure duration is the reason for the differences between our results and those from previous studies. In contrast to our study, Saha *et al.* (2005) have demonstrated an increase in plasma glucose concentration in fed animals under isoflurane anesthesia, which may be due to the increase in plasma norepinephrine as well as increased growth hormone levels induced by isoflurane (Diltoer and Camu 1988). Tanaka *et al.* (2005) have also shown a plasma glucose increase and decrease in plasma insulin under isoflurane or sevoflurane anesthesia in patients subjected to minor surgery. The decrease in fasting plasma insulin, which was also observed in the present study, may be due to the inhibition of glucose-stimulated insulin release induced by isoflurane exposure (Desborough *et al*. 1993) and the fasting state (Pequignot *et al*. 1980).

In conclusion, the results from this study indicate that the measured metabolic parameters are dependent on anesthetics and fed or fasted state. Therefore, the results of our study suggest that the metabolic profile obtained in each situation may be used as a reference for possible artifacts induced by any experimental approach using these anesthetics.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by a grant from Neuroscience Research center, Shahid Beheshti University of Medical Sciences and Applied Neuroscience Research center, Baqyiatallah (a.s.) University of Medical Sciences. The authors would like to express their gratitude to Ms. Mansoureh Farahmand for her help in preparation of the manuscript.

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