Plasma corticosterone, insulin and glucose changes induced by brief exposure to isoflurane, diethyl ether and CO_2 in male rats

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Short Title: Inhaled anaesthetics and metabolic changes

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Summary

The impact of anaesthetic 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 the plasma corticosterone, insulin and glucose concentrations since duration of the anaesthetic exposure may have an affect on those factors.

Male rats were grouped into fed and fasted. The experimental rats were briefly exposed to diethyl ether, isoflurane, or CO_2 (the plane of anaesthesia was identical), while a control group had no exposure to the anaesthetics.

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 change 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: Anaesthesia. Anaesthetic agents. Corticosterone. Glucose. Insulin

Introduction

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

In one study, rats were exposed to diethyl ether anaesthesia. 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 24h starved rats (Aynsley – Green *et al*. 1973). In a clinical study on human subjects, 15 minutes of anaesthesia with isoflurane or sevoflurane caused a significant increase in plasma glucose, yet markedly decreased plasma insulin levels (Tanaka *et al*. 2005). However, a longer duration of anaesthesia with isoflurane or sevoflurane (10 hours) 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. obtained the same results in rats following 30 minutes of anaesthesia with isoflurane or sevoflurane (Zuurbier *et al*. 2008).

Thus, it is evident that exposure to anaesthetics may affect experimental results, with differences in the exposure duration leading to further variation. Therefore, the results obtained from relatively short procedures (e.g. retro-orbital blood sampling), which require a short period of exposure to anaesthesia, may also be difficult to interpret

due to the additional effects of the anaesthetic agents on corticosterone or other metabolic factors (e.g. 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 anaesthetics (isoflurane, diethyl ether and CO_2, which are also used in the laboratory for short procedures), on the levels of plasma 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/group). The animals were housed 2/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 anaesthesia, 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 anaesthesia in the

experimental group (Yale University IACUC)[1]. At the end of the experiments, the animals were euthanized by CO_2 (NIH Guidelines)[2].

Experimental Procedure

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 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 anaesthesia (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 the indications 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, CO2 (100%), with a

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.

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 anaesthesia (in about 1 min from starting CO2 flow) were observed. The blood sampling procedure followed immediately.

One millilitre 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 CO2 in gaseous form (Iran-Oxygen Co.), were used as inhaled anaesthetics.

Assessments

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

Statistical analyses

All data are expressed as the mean ± SEM of plasma corticosterone, glucose and insulin concentrations. One-way and two-way analysis of variance were performed and supported by an LSD test. P values below 0.05 ($P<0.05$) 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 anaesthesia 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 belonging to each group of anaesthetic 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 ($P<0.001$) anaesthesia compared with the controls (Table 2). In the fed group, diethyl ether anaesthesia caused a significant elevation of plasma glucose concentrations as compared to isoflurane ($P<0.001$) and CO_2 ($P<0.01$) anaesthesia (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 the 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 anaesthesia 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 anaesthesia, 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 anaesthesia 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 anaesthetics. The results of this study indicated that a brief exposure to diethyl ether and CO2 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 anaesthetics.

In this study, the control (nonanaesthetized) 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 anaesthetic 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 seen to be elevated concomitantly under diethyl ether anaesthesia 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 (Bojkova *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). In an interesting study, Ansley-Green and his colleagues 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 (Aynsley-Green *et al*. 1973), which contradicts our results. One explanation for this difference could be the time of anaesthetic exposure, which was lower in our study (e.g. 4 min compared with 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 anaesthetic. 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 anaesthesia is in agreement with the results of an experiment which was done by Altholtz and colleagues in rats using the same anaesthetic (Altholtz *et al*. 2006). The insulin elevation in the same group of rats was observed to be euglycaemia due to the acute hyperglycaemic effect of corticosterone secretion induced by the CO_2. However, in the fed rats under CO_2 anaesthesia, 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 CO2 exposure (Winder *et al.* 1983).

Under 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 anaesthetic exposure duration is the reason for the differences between our results and those from previous studies. In contrast to our study, Saha and colleagues have demonstrated an increase in plasma glucose concentration in fed animals under isoflurane anaesthesia (Saha *et al.* 2005) 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 and colleagues have also shown a plasma glucose increase and decrease in plasma insulin under isoflurane or sevoflurane anaesthesia in patients subjected to minor surgery (Tanaka *et al.* 2005). The decrease in fasting plasma insulin, which is 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 factors are anaesthetic and situation (i.e. fed or fasted)- dependent. Therefore, the results of this study suggest that the metabolic concentration profile obtained in each situation may be used as a reference for possible artefacts induced by any experimental approach using these anaesthetics.

Acknowledgment: 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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hemodynamics and without major surgical stress in fed rats. *Anesth Analg* **106**:135-142, 2008.

Table Legends

Table 1: Plasma corticosterone concentrations in the rats of control group (nonanaesthetized) and the groups under isoflurane, diethyl ether and CO_2 anesthesia, in fed and fasted states.

Each point represents mean ±SEM of plasma corticosterone concentration. (n=7-9/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; (in the same group).

Table 2: Plasma glucose concentrations in the rats of control group (nonanaesthetized) and the groups under isoflurane, diethyl ether and CO_2 anesthesia, in fed and fasted states.

Each point represents mean ±SEM of plasma glucose concentration. (n= 8-9/group) *P<0.05, ***P<0.001 significant difference versus control; ΨP<0.01, ¶P<0.001 significant difference versus CO_2 and isoflurane anesthesia respectively; (in the same group).

Table 3: Plasma insulin concentrations in the rats of control group (nonanaesthetized) and the groups under isoflurane, diethyl ether and CO_2 anesthesia, in fed and fasted states.

Each point represents mean ±SEM of plasma insulin concentration. (n=7-9/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; (in the same group).

Table1

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 ± 0.10$^{***\,¶}$
Fasted	1.66 ± 0.22	1.84 ± 0.14	2.65 ± 0.17$^{***\,§}$	2.18 ± 0.15*

Table 2

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

Table 3

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***§