

COMPARISON OF EFFECTS OF DECAPITATION AND ANESTHESIA ON METABOLIC AND
HORMONAL PARAMETERS IN SPRAGUE-DAWLEY RATS

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Summary

The modes of euthanasia by either anesthesia or by decapitation were compared by assessing several metabolic and hormonal parameters from plasma and hormone receptors from liver plasma membranes. Two different anesthetics were used. Compared to decapitation, euthanasia by anesthesia significantly increased plasma glucose and triglyceride levels but not plasma cholesterol. Plasma insulin was also significantly increased by anesthetics. No significant differences were observed in plasma glucagon levels or insulin and glucagon receptors from liver plasma membranes between rats euthanized by decapitation and anesthesia. Glucagon receptors were however, affected by dietary carbohydrates. It is concluded that in studies involving measurements of metabolic and hormonal parameters, the use of anesthesia is to be avoided for euthanasia and that decapitation should be the method of choice.

In most studies involving experimental animals, euthanasia is normally carried out by either decapitation or using anesthesia. Recently, serious objections to euthanasia by decapitation have been raised. Several laboratories have studied acute as well as chronic effects of different anesthetics on metabolic and hormonal parameters in rats (1-9). However, the comparison of euthanasia by anesthesia with decapitation is lacking. Dutkiewicz and Chelstowski (10) compared the effects of ketamine and thiopental anesthesia on rat heart mitochondrial function and observed that decapitation caused some changes in the functional integrity of the mitochondria. Anesthetics affect brain as well as CNS system thereby altering the production and release of neuropeptides. Neuropeptides have been shown to be involved in carbohydrate and lipid metabolism. Hence it is anticipated that euthanasia by using anesthetics would alter the levels of metabolites in plasma and other tissues. Anesthetics also affect the pancreas (8) by altering the concentrations of pancreatic hormones such as insulin, glucagon and somatostatin which are also involved in carbohydrate and lipid metabolism. Therefore it is important to compare the effects of anesthesia on numerous metabolites and hormones. In the present study we compared the effects of amytal, pentobarbital and decapitation on plasma glucose, triglyceride, cholesterol, insulin and glucagon in rats fed different diets.

In order to see whether similar effects of anesthesia and decapitation are observed under different metabolic conditions the rats were fed either one of two synthetic diets differing in the source of carbohydrates or laboratory

chow. Since brain exclusively utilizes glucose as a source of energy, anesthesia may also alter glucose metabolism in the brain. Anesthetics affect brain membrane receptors. They may also affect receptors from other tissues. Chronic treatment of rats with phenobarbital has been reported to decrease receptors for epidermal growth factor and insulin in liver golgi membranes (5). We therefore studied the effect of anesthesia on liver membrane receptors for insulin and glucagon. The present study sheds some light on the effect of anesthesia on metabolic and hormonal parameters. This in turn will help interpret the results of metabolic studies and will help identify a method of choice for euthanasia when measurements of specific metabolic and hormonal parameters are performed.

Material and Methods

Two separate experiments were carried out. In first experiment, 24 weanling male Sprague-Dawley rats weighing between 35-40 gm were fed ad libitum synthetic diets containing either 54% starch (12 rats) or 54% sucrose (12 rats) as carbohydrate along with 10% casein, 10% lactalbumin, 16% fat, 5.9% cellulose, 4% salt mix, 3.1% AIN-76 and 1% vitamin mix. The rats were housed in individual cages with a reverse dark-light cycle of 12 hours light. The rats were fed the diets for 6 weeks. At the end of the feeding period and after an overnight fast, 6 rats on each diet were anesthetized by amytal, 100 mg/kg BW (sodium amobarbital, Eli Lilly and Co., Indianapolis, IN). Blood was collected from the abdominal aorta. The remaining rats were decapitated. In both cases blood was collected in tubes containing 10.5 mg EDTA and 1000 U Trasylol (FBA Pharmaceuticals, Inc., N.Y.). Plasma was separated, aliquoted and stored at -70°C until analyzed. Plasma was analysed enzymatically for glucose (11), triglyceride (12) and cholesterol (13) with the Centrifichem automated system (Baker Instrument, Pleasantville, N.Y.). Immunoreactive glucagon was measured using Unger's 04A antibody by the method previously described (14). Immunoreactive insulin was determined with a kit from Immunonuclear Corporation, Stillwater, MN (Cat. No. 0600). Rat insulin (gift from Eli Lilly and Co., Indianapolis, IN) was used as standard. Membranes were prepared from liver according to the procedure of Neville (15) and insulin and glucagon receptors were measured as previously described (16). Native porcine insulin and glucagon were gifts from Eli Lilly and Co., Indianapolis, IN. Hormone binding data were analyzed by Scatchard plots (17) and competition inhibition plots (18) to assess number and affinity of the receptors. Statistical analysis was carried out by randomized complete block analysis of variance (ANOVA) to evaluate the effects diet and method of euthanasia (19).

Since each anesthetic has a different effect, two anesthetics, Amytal and pentobarbital were used in the second experiment. Eighteen weanling male Sprague-Dawley rats weighing between 35-40 gm were fed laboratory chow diet (Ralston-Purina) for 4 weeks. After an overnight fast, 6 rats each were euthanized by either amytal (100mg/kg BW), pentobarbital (40 mg/kg BW) or decapitation. The collection of blood and the analytical procedures were performed as described for experiment 1.

Results

Table 1 shows the effect of dietary carbohydrates and method of euthanasia on plasma levels of glucose, triglyceride, cholesterol, insulin and glucagon and insulin and glucagon binding to liver plasma membranes. Dietary carbohydrates had no significant effect on any of the plasma metabolites or hormones, but, dietary sucrose compared to starch significantly increased glucagon binding to liver plasma membranes. Sucrose fed rats also had larger liver than rats fed starch (data not shown). Compared to decapitation, the use of anesthesia significantly increased plasma glucose and insulin levels but

TABLE 1

Effect of Dietary Carbohydrates and Mode of Euthanasia on Plasma Metabolites and Hormones and Hormone Binding to Liver Plasma Membranes (Experiment 1)[†].

Euthanasia dietary carbohydrate	Amytal		Decapitation	
	starch	sucrose	starch	sucrose
Glucose, mmol/L	7.8 ± 0.4	8.9 ± 0.6	5.1 ± 0.3	5.1 ± 0.4
Triglyceride, mmol/L	0.57 ± 0.04	0.58 ± 0.07	0.85 ± 0.12	0.94 ± 0.08
Cholesterol, mmol/L	1.58 ± 0.17	1.47 ± 0.12	1.79 ± 0.13	1.78 ± 0.13
Insulin, pmol/L	172 ± 27	136 ± 10	88 ± 4	102 ± 16
Glucagon, pmol/L	42.2 ± 2.6	43.8 ± 3.3	27.7 ± 1.5	34.7 ± 3.1
Insulin Binding % [*]	4.2 ± 0.6	4.2 ± 0.3	5.3 ± 1.3	5.1 ± 0.6
Glucagon Binding % ^{**}	37.4 ± 2.8	43.2 ± 4.4	40.0 ± 1.7	42.3 ± 1.5
ANOVA				
	diet	euthanasia		
Glucose, mmol/L	NS	p<0.001		
Triglyceride, mmol/L	NS	p<0.001		
Cholesterol, mmol/L	NS	NS		
Insulin, pmol/L	NS	p<0.01		
Glucagon, pmol/L	NS	NS (p<0.07)		
Insulin Binding % [*]	NS	NS		
Glucagon Binding % ^{**}	p<0.05	NS		

[†]Mean ± SEM of 6 rats.

^{*}% binding/50 µg protein.

^{**}% binding/25 µg protein.

decreased plasma triglycerides. Although anesthetized rats had lower concentrations of plasma cholesterol and glucagon, the values were not significantly different from those rats that were decapitated. Plasma glucagon and insulin and glucagon binding were not significantly affected by anesthesia. When binding data were analyzed by Scatchard plots and competition-inhibition plots, no significant differences were observed in either receptor number or affinity for insulin and glucagon receptors between rats euthanized by decapitation or anesthesia (data not shown). However, sucrose fed rats, compared with those fed starch had higher number of glucagon receptors in anesthetized as well as decapitated rats. Thus, increased glucagon binding in sucrose fed rats is due to an increase in the number of receptors.

In chow fed rats, no significant differences were observed in rats euthanized by either amyral or pentobarbital (Table 2). Therefore, the data from the two groups of rats were combined and compared with the data from the decapitated rats (19). Anesthetized rats had significantly higher plasma glucose and insulin but lower plasma triglycerides as compared to rats sacrificed by decapitation. No significant differences were observed in insulin or glucagon receptor numbers or affinity between any of the groups (data not shown). Regardless of the nature of diet, similar differences were observed in all parameters between anesthetized rats and decapitated rats.

Table 2

Effect of Mode of Euthanasia on Plasma Metabolites and Hormones Membranes in Laboratory Chow Fed Rats (Experiment 2)[†].

Euthanasia	Amytal	Pentobarbital	Decapitation	ANOVA*
Glucose, mmol/L	8.7 ± 0.6	8.0 ± 0.5	6.6 ± 0.2	p<0.03
Triglyceride, mmol/L	0.91 ± 0.14	0.86 ± 0.14	1.11 ± 0.12	p<0.05
Cholesterol, mmol/L	1.97 ± 0.27	2.07 ± 0.20	2.05 ± 0.13	NS
Insulin, pmol/L	104 ± 9	113 ± 26	67 ± 14	p<0.03
Glucagon, pmol/L	30.6 ± 3.2	31.7 ± 4.5	32.5 ± 4.7	NS
Insulin Binding %	7.9 ± 1.2	10.7 ± 1.8	8.2 ± 1.0	NS
Glucagon Binding %	30.4 ± 3.2	32.5 ± 6.1	29.5 ± 4.4	NS

[†]Mean ± SEM of rats.

*Duncans Multiple Range test showed no differences between rats sacrificed by amytal or pentobarbital, therefore for ANOVA the two groups were combined and compared with decapitated rats.

Discussion

In the present study, significant differences were observed in several metabolic and hormonal parameters between rats euthanized by anesthesia compared to decapitated rats regardless of the type of the diet.

Both amytal and pentobarbital significantly increased plasma glucose levels. Penicaud et al. observed a transient hyperglycemia 3 minutes after treatment with pentobarbital (6). The increase appeared to be due to the increased glucose production and the decreased glucose clearance. In anesthetized rats peripheral glucose utilization was decreased in brain and postural muscles but not in nonpostural muscles or adipose tissue in (6). It is important to note that in the present study increased plasma glucose occurred in spite of significantly elevated plasma insulin. It is possible that elevated glucose stimulated insulin secretion since insulin concentration decreases over time as seen with pentobarbital anesthesia. However, a direct effect on pancreas cannot be ruled out. Increased glucose cannot be accounted for by changes in glucagon since glucagon was neither elevated in the present study nor decreased as observed in another study (6). Similarly there were no significant changes in either insulin or glucagon receptors. It is possible that other counter regulatory hormones to insulin such as growth hormone, corticosterone or catecholamines could be responsible. A decrease in glucose utilization has also been observed in peripheral tissue (20) as well as in brain (21) with halothane anesthesia. Besides glucose, fructose-2,6-bisphosphate (22) and several other metabolites (23) are altered in liver and brain by use of anesthesia prior to decapitation.

In the present study, a decrease in plasma triglyceride was observed in anesthetized rats compared to decapitated rats. An increase in plasma insulin and no change in glucagon causes relative insulin excess which would favor lipogenesis thereby increasing plasma lipid level. A direct effect of anesthesia on lipolysis in adipose tissue cannot be ruled out. Beside pancreatic and adrenal hormones, lipolysis is also modulated by opioid peptides

especially those derived from proopiomelanocortin. Thus, β -endorphin and lipotropin stimulate lipolysis (24). Decreased triglycerides could be due to increased lipolysis by these opioid peptides secreted from pituitary or hypothalamus under the direct stimulation by anesthetics. Pentobarbital has been shown to decrease the activity of enkephalin neurons in that the conversion of peptide met-enkephalin to tripeptide by enkephalinase is decreased by 60% (4). However, we have not measured opioid peptides in the present study. Butler et al (25) compared the effects of decapitation, pentobarbital and decapitation after anesthesia with CO₂, methoxyflurane and ether on arachidonic acid metabolism in rat and rabbit aorta and observed significant differences among the different methods of euthanasia.

Nazian (7) studied the acute effect of several anesthetics including pentobarbital, halothane and ether on pituitary and adrenal reproductive hormones in intact and castrated male rats. All anesthetics produced some changes in one or more hormones in either intact or castrated rats. Even milder anesthetics like ketamine and halothane appear to alter follicle stimulating hormone, indicating that none of anesthetics was suitable for the study of any reproductive hormone. In proestrous rat pentobarbital innitally stimulated prolactin secretion for 10-30 minutes, after that time, prolactin secretion was blocked (26). Others have also observed significant alteration in plasma levels of pituitary, thyroid, and gonadal hormones in animals euthanized after anesthesia (27, 28). Taborsky et al. (3) reported inhibition of cholinergic input to the pancreas in anesthetized rats thereby decreasing the secretion of pancreatic polypeptide. Similarly halothane has been reported to inhibit insulin release (8). Anesthetics have also been reported to decrease blood flow to some but not all organs and to decrease cardiac output (2). Thus anesthetics affect endocrine as well as non endocrine organs besides brain and CNS.

In the present study we did not observe any effect of pentobarbital or amylal on either insulin or glucagon binding to liver plasma membranes. In general, receptors are either up or down regulated by the plasma hormone levels (18). We have reported decreased insulin and glucagon receptors whenever the hormone levels are elevated (14). However, changes in receptors are not immediate and there is a substantial time lag. In humans, plasma insulin peaks 30-60 minutes after oral glucose load, but insulin binding changes only after 5 hours when plasma insulin concentration returns to fasting level (29). In rats glucagon binding to hepatocytes was decreased only in chronically injected (7 days) rats but not in 2 hour glucagon infused rats even though plasma glucagon levels were several fold higher in both treated groups as compared to controls (14). Thus, the lack of change in insulin or glucagon receptors in the present study was not surprising. Zarembka et al (30) have reported no effect of ether or ketamine anesthesia on rat uterine estrogen and progesterone receptors. However, chronic treatment with phenobarbital for more than 2 weeks has been shown to decrease both insulin as well epidermal growth factor receptors to liver microsomal and golgi fractions (5). The hormone concentrations were not measured, however, in that study. It is unclear whether changes in plasma hormone levels were responsible for the decreased binding.

The effects of carbon dioxide on metabolic and hormonal indices need to be investigated before it can be used for euthanasia. Carbon dioxide would alter the pH of blood and that may affect some metabolic indices. The acidic pH may also affect the secretion of some of the enzymes and/or hormones from different tissues. Carbon dioxide suffocation has been shown to affect arachidonic acid metabolism in animals (25) and substance P in plasma (31).

Thus, the results from these studies indicate that anesthetics affect metabolic and hormonal parameters. Anesthetics also result in changes in

circulation to most organs, which may impact on organ function. Further, different anesthetics have quantitatively and qualitatively different effects on some parameters and that same anesthetic may have quantitatively different effects on different parameters. It is clear that the use of anesthetics, either by injection or inhalation, is not a method of choice for studies involving metabolic and endocrine parameters. The use of anesthesia, however, does not appear to affect hormone receptor measurements. It is debatable whether decapitation produces any more pain and discomfort than injection of anesthesia, or the suffering during early period of inhalation (32-35). Certainly, scientifically, decapitation would provide more uniform and reliable results.

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