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Effect of Ether, Sodium Pentobarbital and Chloral Hydrate Anesthesia on Rat Plasma Metabolite Concentrations

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The effect of ethyl ether, pentobarbital sodium (Nembutal®) and chloral hydrate anesthesia on the concentrations of glucose, lactate, glycerol, insulin, individual amino acids and urea have been studied in rat plasma, as well as the glycogen content in the animal liver and striated muscle. Control animals were injected with saline solution. Two samples were taken from each animal at 15 and 30 minutes from the beginning of anesthesia. These samples were obtained from the cut tail's tip and from the neck wound produced by beheading the animal. Results show considerable differences between the three sets of animals and their controls as well as in their tail versus neck values. The overall effect of the three anesthetics used were roughly the same in spite of their different actions upon the different parameters studied. This was most marked in the aminograms. Significant changes induced were rather similar in number for all. The metabolite concentration profile in each situation may be used as a reference for possible artifacts induced by any experimental approach using these anesthetics.

The stress induced from blood collection or tissue sampling produces several metabolic changes in experimental animals. This is probably mediated by an increased catecholamine release (2, 7) which affects both lipidic and glucidic metabolism (16). This problem is not overcome by using general anesthetics which are known to affect metabolic parameters in different ways (11, 12, 20, 21, 24). Hormonal (5, 20, 21, 23) glycogen and metabolite levels in the tissues are also known

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to be altered by anesthesia (9, 22, 25). However, the effect of anesthetics on protein and amino acid metabolism has been sparsely studied, and few data are available on the changes induced in plasma amino acid concentrations (1).

As a continuation of these studies and with the purpose of achieving an adequate control of the changes induced by anesthesia in experimental metabolic studies of amino acid metabolism, we have determined the comparative effects of three anesthetics — ethyl ether, nembutal and chloral hydrate — on several glucidic, lipidic and amino acid metabolites in the plasma of the rat.

Materials and Methods

Fed female virgin rats of Wistar stock whose initial weights are shown in figure 1 were housed in collective cages in a light cycle and temperature controlled room (12 hours on, 12 hours off; 23 ± 2° C). Four groups of seven animals each were used. Animals belonging to group *C* were injected i.p. with 0.9 % NaCl (2.5 ml/kg of body weight) and kept caged on the laboratory bench. These animals were bled from the tail's tip 15 min later and beheaded exactly 30 min after the saline injection.

Animals in group *E* were injected i.p. with saline and immediately placed in a large glass beaker containing an ether-soaked cotton swab protected with a plastic grid on the bottom covered with a glass plate. After 3 min, the slightly anesthetized animals were removed from the beaker, placed prone and forced to breathe a water-saturated ether: CO_2/O_2 (5:95 %) mixture through a plastic face-piece, in order to keep the rat under deep anesthesia without anoxia. The anesthetized animals were bled from the tail at 15 min and decapitated at 30 min after the saline injection.

Animals in group *N* were injected i.p. with a pentobarbital sodium solution in water (Nembutal®, Sigma), 16 mg/ml at a dosis of 2.5 ml (40 mg)/kg body weight. After 15 min, they were bled from the tail and 30 min after the injection were decapitated.

Animals in group *CH* were injected i.p. with a chloral hydrate (Merck) solution in water (200 mg/ml) at a dosis of 2.5 ml (500 mg)/kg body weight. They were bled and beheaded exactly as the other three groups.

The state of anesthesia was checked at 14 and 19 min after injection in all the animals. In every case the leg retrieval and winkling reflexes were suppressed and respiratory movements were slow and deep.

In all groups, blood obtained from the tail was collected in dry, heparinized china plates within less than 1 min after the cutting of the tail's tip. With decapitation, blood was collected from the neck into dry heparinized beakers. Pieces of liver and leg striated muscle were removed within less than 15 s after decapitation and immediately frozen in liquid nitrogen for glycogen determination.

Plasma samples were deproteinized with perchloric acid for the determination of lactate (17) and glycerol (8) by enzymatic procedures. An aliquot of whole blood was deproteinized by the SOMOGYI (26) procedure and used for the estimation of glucose with a glucose-oxidase method (18). Insulin levels were measured (15) in plasma by means of a rat radioimmunoassay kit (supplied by Novo Industri, Copenhagen). Glycogen in liver and muscle was determined after alkaline digestion and ethanol precipitation (14) with subsequent acid hydrolysis and glucose determination (18). Plasma urea concentration was determined with the indophenol method (10). Aliquots of plasma were deproteinized with cold acetone (2) then the supernatants were used for individual radiochemical amino acid determinations using labelled dansyl-chloride and t.l.c. resolution of individual Dns-amino acids

(3); ^{14}C labelled Dns-Cl was obtained from the Radiochemical Centre, Amersham. Non radioactive Dns-Cl and all standards amino acids and enzymes were obtained from Sigma.

Results

Figure 1 shows the weights of the animals used, the changes in liver weight relative to body weight and glycogen content in liver and muscle. It is readily apparent that there was a relative increase in liver size in the anesthetized animals, that was significant for both ether and chloral hydrate anesthetized rats. The liver glycogen content was also higher in all the anesthetized animals than in the controls, but the only significant increase found was for the nembutal-anesthetized rats.

Plasma immunoreactive insulin levels were considerably higher in ether and nembutal-treated rats than in controls. Chloral hydrate also provoked an increase in insulin.

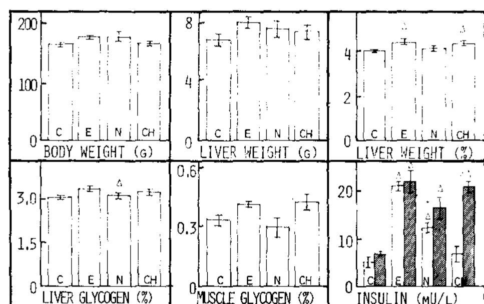

Fig. 1. *Body and liver weight, liver and muscle glycogen and plasma insulin in controls and anesthetized rats.*
All values are the mean ± s.e.m. of 6-7 different animals. C = controls; E = ether anesthetized; N = nembutal anesthetized; CH = chloral hydrate anesthetized. Clear narrow bars refer to plasma 15 minutes (tail) values, dark narrow bars refer to plasma 30 minutes (neck) values. Significance of the differences versus controls: $\Delta = p < 0.05$. Significance of the differences between 15 and 30 minutes (tail versus neck) samples: $\dagger = p < 0.05$.

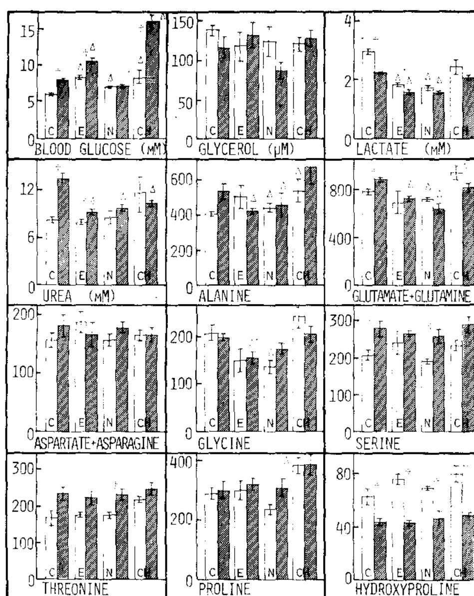

Fig. 2. *Blood glucose and plasma glycerol, lactate, urea and gluconeogenic amino acids and imino acids in control and anesthetized rats.*
Amino acid concentrations are expressed in μmoles/liter. All other symbols are the same as those of figure 1.

Figure 2 shows that ether raised the plasma glucose concentrations, both with regard to the 15 and 30 min intervals, as well as the relative tail/neck blood concentrations differences found in the controls. Nembutal treatment suppressed the tail/neck difference, producing smaller changes in the overall blood glucose pattern. Here, the only significant increase, compared to the control group, was in the 15 minute blood sample. This difference dissapeared in the 30 minute sample. Chloral hydrate followed the same pattern described for ether, but here the effect was more intense as the tail/neck difference (15 min versus 30 min from the be-

ginning of anesthesia) was more than 94 % higher in the second sample, compared to a 29 % increase in controls, and the 26 % and 3 % increases for ether and nembutal respectively.

There were no changes observed in plasma glycerol concentrations in any of the groups studied.

Plasma lactate concentrations dropped considerably with ether and nembutal anesthesia. Also, the tail/neck differences found in the controls disapeared in all anesthetized groups. No significant lactate alterations occurred with chloral hydrate anesthesia, although a significant lactate drop was found in the ether and nembutal treated groups.

The urea pattern of the controls showed considerable tail/neck differences —an increase of 95 % in the neck sample versus the tail sample. This difference was less marked in the anesthetized animals with only a 17 % difference in the ether and nembutal-treated animals and a reversed pattern of a mere 9 % difference in the chloral hydrate-treated animals. Urea concentrations were therefore significantly altered in the 30 min samples taken from the anesthetized animals.

The controls also showed several tail/neck differences in plasma amino acid concentrations (figs 2 and 3) — 15 versus 30 minutes of the saline injection —, with relative increases with time for serine and glutamate plus glutamine as well as a relative decrease for hydroxyproline and cysteine plus cysteate concentrations. Hydroxyproline and cysteine plus cysteate followed the same pattern in all anesthetized animals and control. In the anesthetized animals there was a significant decrease in tryptophan and phenylalanine concentrations of both ether and chloral hydrate-treated animals.

Ether treated animals showed a relative, time-dependent decrease in the alanine and glycine concentrations and an increase in lysine concentrations. The relative decrease in 15 *versus* 30 min samples in

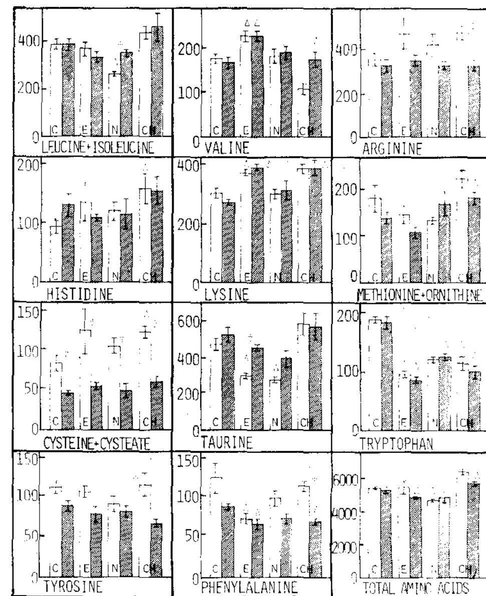

Fig. 3. *Plasma branched chain, basic, sulphur, aromatic and combined total amino acids in control and anesthetized rats.*
Concentrations are expressed in μmoles/liter. All other symbols are the same as those of figure 1.

cysteine plus cysteate was partially compensated by a taurine increase. A significant rise in valine concentration compared to the controls in both times of extraction was also observed.

Nembutal provoked the same general response by the gluconeogenic amino acids as ether, but here the relative 15 versus 30 min rise in glycine, serine and threonine were counteracted by a drop in the glutamate plus glutamine concentrations. There was a relative time-dependent stabilization in leucine plus isoleucine and taurine concentrations.

Chloral hydrate-induced changes were relatively different from those induced by ether and nembutal. Alanine, glutamate plus glutamine, arginine, histidine and cysteine plus cysteate increased significantly in the 15 but not in the 30 min samples,

whereas valine and methionine plus ornithine values increased significantly in the 30 min sample. Lysine showed a significant increase and tryptophan a decrease in both samples.

Discussion

A saline injection provokes in the controls a rise in plasma catecholamines that affects glycogen, glucose and insulin levels. This situation is aggravated by bleeding through the tail's tip, as this is an additional source of stress that generates the lifferences seen in several amino acids was between the 15 (tail) and 30 (neck) min samples, as has been previously studied (4).

The validity of the differences in results between the control animals and anesthetized groups was very clear. When viewed against the background of the combined effects of stress and anesthesia, the results obtained from animals in an increasing state of stress, which anesthesia was supposed to suppress, were quite different. Liver glycogen content, as well as relative liver weights were higher in anesthetized rats than in controls. The data for controls, however, were rather low with regard to the anesthetized groups and approached those for intact rats (4).

High glucose concentrations in the anesthetized animals seem to be related to a decreased glucose utilization through the lycolytic pathways and to an enhancement of gluconeogenesis as suggested by the relative decrease in the concentration of gluconeogenic amino acids found in ether and nembutal-treated rats. Choral hydrate-treated rats behaved in a relatively different way. Here, the overall concentration of gluconeogenic amino acids was considerably increased; the glucose concentration also rose, especially with time, while liver glycogen concentration remained at a high level. Such an amount of glucose being released into the plasma must be the consequence of a lowered uptake and diminished utilization of glucose. This situation triggered a considerable buildup of insulin as a reflex action, as seen in the 15 versus 30 min samples. It was supposed that gluconeogenesis would be lowered or inhibited by chloral hydrate as observed by the unchanged lactate levels, while concentrations of gluconeogenic amino acids in plasma actually increased, indicating a lack of utilization of these substrates by the liver.

It should be pointed out that the anesthetized animals had also been subjected to stress in the initial phases of anesthesia, as the procedure itself provokes a catecholamines increase (6) as well as changes at the splanchnic bed hemodynamics (19). In fact, the parameters determined in this study for the nembutal and ether show a remarkable coincidence.

Plasma insulin increased significantly in both nembutal and ether-treated animals. This was in general agreement with data already in the literature (20, 29). The effect of anesthesia on insulin release is known to be in accord with the dietary status of animals (5, 29). The different effects of anesthetics upon plasma insulin levels might be partly due to the direct result of insulin release and indirect actions mediated by the catecholamines.

Ether provoked a glycine and alanine concentration decrease by affecting the β-adrenergic-induced amino acid release block at peripheral tissues (13). There was an apparent increase in gluconeogenesis from lactate and amino acids in ether and nembutal-treated rats; as glucose concentrations showed a trend to rise, glycogen stores in the liver were kept constant and lactate as well as gluconeogenic amino acids decreased significantly.

Urea concentrations were lower in the anesthetized animals than in the stressed controls when the 30 min samples were considered. This was probably due to the lack of further adrenergic stimulation of proteolysis and amino acid disposal. It could also be supposed that the depres-

sion of respiratory centers caused by anesthesia would induce a relative acidosis that would be partly counteracted through ammonia neutralization by the kidney, resulting in a lower availability of ammonia for urea synthesis.

Tryptophan values decreased with anesthesia. This was a consequence of the activation of tryptophan degradation by the liver (27, 28). ANDA *et al.* (1) indicated a significant rise of both phenylalanine and tyrosine (but not tryptophan) 24 h after anesthesia. These effects were not seen in this short term study.

The practical result of this paper as concerns the selection of the idoneous anesthetic to use in biochemical studies in the rat is still unclear. In spite of the fact that their action patterns on the parameters were not uniform, the overall effect of the three anesthetics was comparable, and the number of significant changes induced by them was very similar. For this reason, the choices of anesthetics to use for experimental work on the rat will be deferred to a selection based primarily on the type of variable to be studied.

The metabolites concentration profile in each situation is given as a reference on the possible artifacts induced in any given experimental approach with the use of anesthetics.

Resumen

Se estudia el efecto de los anestésicos éter etílico, pentobarbital sódico (Nembutal®) e hidrato de cloral, sobre la concentración de distintos metabolitos en el plasma de rata (glucosa, lactato, glicerol, insulina, aminoácidos individuales y urea), así como el contenido en glucógeno del hígado y del músculo estriado. Los animales control fueron inyectados con solución salina. A los 15 y 30 minutos del inicio de la anestesia se sacaron dos muestras de cada animal, una de la herida del cuello producida al decapitar al animal y otra de la punta de la cola. Las diferencias encontradas fueron considerables en los tres grupos de animales, tanto con respecto a los animales control como las muestras del cuello con respecto a las de la cola. El efecto global de los tres anestésicos estudiados puede considerarse equivalente, a pesar de las lógicas diferencias entre los distintos parámetros estudiados, especialmente para los aminoácidos plasmáticos. Las concentraciones de metabolitos en cada situación pueden servir de referencia de los posibles artefactos experimentales introducidos con el uso de anestésicos.

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