Blood sugar, serum insulin and serum non-esterified fatty acid levels during thiopentone anaesthesia in dogs

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The purpose of this study was to determine the effect of thiopentone anaesthesia on glucose metabolism. Blood sugar (BS), serum immunoreactive insulin (IRI) and serum non-esterified fatty acid (NEFA) concentrations were measured during the course of (1) an intravenous glucose tolerance test (IVGTT), and (2) an intravenous insulin test (ITT), in conscious and anaesthetized fasted dogs. The IVGTTs were repeated in dogs under alpha- or beta-adrenergic blockade, induced by phentolamine or propranolol. During the IVGTT, the anaesthetized dogs showed glucose intolerance (blood sugar levels were higher than in the control group) and little serum IRI response to hyperglycaemia was detected. An attenuated initial decrease and a slower rebound of NEFA concentration was observed in anaesthetized animals than in controls. Phentolamine administration ($5 \text{ mg} \cdot \text{kg}^{-1} \text{ iv}$) partly restored the IRI response without affecting the BS levels; propranolol ($1 \text{ mg} \cdot \text{kg}^{-1} \text{ iv}$) had no effect. Anaesthetized dogs showed a moderate resistance

to insulin induced hypoglycaemic action and a lack of serum NEFA response during counter-regulation of hypoglycaemia, while in conscious controls an intense rebound was observed. Hyperinsulinaemia after iv insulin administration was longer in anaesthetized dogs than in controls. The insulin distribution space was 78% of body weight and insulin $t_{1/2}$ in blood group compared with 54% and 16 min, in controls. We conclude that thiopentone provokes disturbances in glucose and serum NEFA metabolisms and abolishes the serum IRI response to hyperglycaemia. These effects are influenced by extrapancreatic factors regulating serum IRI levels and by an alpha-adrenergic mechanism, via the inhibition of insulin secretion.

Key words

ANAESTHETICS, INTRAVENOUS: thiopentone;
ANAESTHETIC TECHNIQUES: intravenous;
HORMONES: insulin;
METABOLISM: fatty acids, glucose;
SYMPATHETIC NERVOUS SYSTEM: alpha-adrenergic antagonists, phentolamine, beta-adrenergic antagonists, propranolol.

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Cette étude porte sur les effets de l'anesthésie au thiopental sur la glycémie, l'insulinémie et la concentration des acides gras non-estérifiés (AGNE) sur des chiens en réponse à un test d'hyperglycémie provoquée et à une injection intraveineuse d'insuline. Le groupe contrôle se compose de chiens éveillés. Chez les chiens anesthésiés, on constate après le test d'hyperglycémie provoquée, une intolérance au glucose, une absence de réponse insulinaire 5 min après l'injection du glucose ($29 \pm 15 \mu\text{U} \cdot \text{ml}^{-1}$ comparativement à $85 \pm 8 \mu\text{U} \cdot \text{ml}^{-1}$ pour les contrôles) et une baisse moins importante des AGNE. Un bloc β -adrénergique au propranolol n'a pas modifié ces résultats. Cependant, l'administration de phentolamine (blocage α -adrénergique) rétablit partiellement la réponse insulinaire sans modifier la glycémie. Chez le chien anesthésié, l'injection intraveineuse d'insuline entraîne une résistance aux actions hypoglycémiantes de l'insuline et lipolytique des hormones anti-insuliniques. L'insuline injectée lors du test d'hyperglycémie provoquée avait une demi-vie plus longue (27,5 min contre 16 min) et un volume de distribution plus grand (78% contre 54%) chez les chiens anesthésiés que chez les contrôles. On peut conclure que la réponse insulinaire à l'hyperglycémie diminue chez les chiens anesthésiés au thiopental. En toute probabilité, on peut attribuer ces résultats à une association de facteurs extrapancréatiques auxquels s'ajoute un facteur α -adrénergique inhibiteur de la sécrétion insulinaire.

Although it is known that thiopentone administration does not affect the basal blood sugar (BS) concentration in normal man, it has been shown that thiopentone impairs the ability of body tissues to deal with extra glucose introduced into the blood.¹⁻⁶ The mechanisms responsible for the impairment remain unclear.⁷⁻⁹

In normal conscious dogs, a glucose load evokes a hyperglycaemic response¹⁰⁻¹² which in turn induces insulin secretion and inhibits glucagon and growth hormone release.¹³⁻¹⁵ A progressive return of BS to base-line is accompanied by a decrease in serum NEFA. The BS decrease occurring during the second part of the glucose test appears to be the most powerful factor promoting anti-insulin hormone secretion in normal animals.^{14, 16} This causes both BS and serum NEFA levels to return to base line. This counter-regulation is exerted by epinephrine and glucagon but growth hormone is not secreted under these circumstances.¹⁵ Insulin administration causes hypoglycaemia in various mammals including the dog and humans.¹² The mechanisms of this hypoglycaemic effect include dissipation of the injected insulin and stimulation of anti-insulin hormone secretion. In the dog the hormones secreted are mainly epinephrine, glucagon and cortisol,¹⁷⁻¹⁹ which induce BS level return to base-line, and increase serum NEFA. The present studies were carried out to evaluate the effects of thiopentone on several biological variables (heart rate, respiratory rate and mean blood pressure) and glucose metabolism BS, serum immunoreactive insulin (IRI) and serum non-esterified fatty acids (NEFA) under basal conditions, during an *iv* glucose tolerance test (IVGTT), with or without an alpha- or beta-adrenergic blockade, and an *iv* insulin tolerance test (ITT).

Throughout this paper we have referred to "non-esterified fatty acids" (NEFA) instead of "free fatty acids" (FFA) because despite they are not esterified by glycerol, never circulate in a free form, and are always bound to albumin for solubilization.²⁰

Methods

Animals

Forty-eight disinfested, male mongrel dogs, weighing 15-20 kg, fed on dog chow pellets and *ad libitum* water, were used in the experiments. Their use was approved by the Animal Research Committee, Department of Physiology, Medical School, University of Buenos Aires, Argentina.

The animals were kept in individual cages in an animal room of the department, in regular conditions of feeding for three to four weeks before the tests were performed. Meanwhile, they were transferred into the working lab-

oratory for one hour daily for adaptation to new surroundings and the continuous presence of people. During the week preceding the tests, every animal in the basal condition was checked for several biological variables, including blood sedimentation rate, haematocrit, haemoglobin and blood sugar concentrations. Dogs had no clinical or biochemical evidence of disease.

On the day of the experiment, every dog was tested after fasting (18-22 hr). Only normothermic animals were used, (rectal temperatures $<37.8^{\circ}\text{C}$). Twenty-four animals of the total group were kept as conscious, untrained controls; the remaining 24 dogs were anaesthetized one hour before the test performances. Anaesthesia was induced with thiopentone ($12\text{ mg}\cdot\text{kg}^{-1}\text{ iv}$) and was maintained with thiopentone by continuous *iv* infusion ($20\text{ mg}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$). The tracheas were intubated with a cuffed endotracheal tube and the dogs were allowed to breathe spontaneously. To avoid hypoventilation breathing was periodically assisted with positive-pressure ventilation with room air. A #18-ga needle was inserted into a femoral artery to measure the mean arterial pressure, via a damped aneroid manometer. Subsequently another cannula, used for thiopentone injection was inserted into a leg vein to maintain anaesthesia. During the hour after induction of anaesthesia, the heart and respiratory rates were checked every 15 min, and blood samples were taken anaerobically from the femoral artery before and at 30, 60, 90 and 150 min from induction for estimation of pH, PaO_2 , PaCO_2 , haematocrit and plasma bicarbonate concentration. The thiopentone concentration was measured in blood samples taken by venipuncture at the same times.

Experimental design

The 24 conscious and 24 anaesthetized dogs were grouped as follows:

- 1 The study of the action of thiopentone anaesthesia upon BS, serum IRI and serum NEFA during IVGTT, was performed 90 min after induction of anaesthesia, and comprised six conscious and six anaesthetized dogs.
- 2 The study of the action of thiopentone anaesthesia upon BS, serum IRI and serum NEFA during ITT, was performed 60 min after induction of anaesthesia, comprised six conscious and six anaesthetized dogs.
- 3 The study of the action of thiopentone anaesthesia upon BS and serum IRI during IVGTT in dogs with or without alpha- or beta-adrenergic blockades, comprised the six conscious and six anaesthetized non-blocked dogs mentioned in group #1, together with six conscious and six anaesthetized dogs under alpha- or beta-adrenergic blockade.

TABLE 1 Action of alpha- or beta-adrenergic blockade and isoproterenol infusion on serum immunoreactive insulin concentration in anaesthetized dogs. Anaesthesia was induced 30 min before 0 time. Isoproterenol infusion began just after 30 min. Mean $\pm$ SEM values are given. Statistical analysis: ANOVA (treatments, times and blocks) with comparisons. Number of animals: 6 per group.

Blockade	Time elapsed from phentolamine or propranolol administration (min)					
	0	30 $\downarrow$ Isop	45	60	75	90
Control	15 $\pm$ 10	1 $\pm$ 1	39 $\pm$ 15	75 $\pm$ 24*	67 $\pm$ 27*	54 $\pm$ 23
Alpha	11 $\pm$ 8	10 $\pm$ 4	133 $\pm$ 34†¶	146 $\pm$ 63†§	159 $\pm$ 61†§	113 $\pm$ 47†§
Beta	1 $\pm$ 1	1 $\pm$ 1	2 $\pm$ 1¶	0 $\pm$ 0	10 $\pm$ 9¶	2 $\pm$ 1¶

*, †, ‡: $P < 0.05$; $P < 0.01$; $P < 0.001$. Comparisons with the value at 0 time.

§, ¶, ||: $P < 0.05$; $P < 0.01$; $P < 0.001$. Blocked vs non-blocked.

Sympathetic blockade

Phentolamine hydrochloride (5 mg $\cdot$ kg⁻¹ iv) and propranolol chlorhydrate (1 mg $\cdot$ kg⁻¹ iv) were used for alpha- or beta-adrenergic blockade. Blockade was carried out 60 min after induction of anaesthesia, and 30 min before the start of the tests.

The effectiveness of alpha- or beta-adrenergic blockades were tested in another group of anaesthetized dogs. The dosages of phentolamine and propranolol employed increased or reduced the insulin response to isoproterenol (0.02 μ g $\cdot$ kg⁻¹ $\cdot$ min⁻¹ for 55 min) (Table I).

Tests

The IVGTT consisted of glucose (1 g $\cdot$ kg⁻¹ iv), in an aqueous solution 20 g $\cdot$ 100 ml⁻¹. Blood samples were taken before injection and at 5, 15, 25, 45, 60 and 90 min thereafter. The ITT consisted of glucagon free insulin injection (0.25 IU $\cdot$ kg⁻¹ iv in 5 ml saline). Glucagon free crystalline insulin, potency 22 IU from Lilly Laboratories, Argentine, was used. Blood samples (4 ml) were taken before injection and at 15, 20, 25, 30, 35, 45, 60 min and every 15 min over the next two hours. A polyethylene catheter inserted into a foreleg vein was used for blood withdrawal. Glucose and insulin were injected into hind-limb veins. A small portion of each sample was used for BS determination. Serum for IRI assay and NEFA measurement was obtained by blood clotting at room temperature for one hour, followed by centrifugation (5 min, 2500 rpm) and storage at -70° C until measurement.

Assays

Sodium oxalate prevented blood sample from clotting during sedimentation rate and haematocrit measurement.

Arterial blood samples were analyzed for pH, PaCO₂ and PaO₂.²¹ Plasma bicarbonate concentration was derived from the Siggard-Andersen and Engel nomogram.²² Blood was assayed for thiopentone according to the method of Jailer and Goldbaum, modified by Goldbaum.^{23, 24} Blood sugar, serum IRI and serum NEFA during IVGTT and ITT were measured in a Technicon

Autoanalyzer,²⁵ using a commercial kit for radioimmunoassay (CNEA, Argentine), and assayed according to the method of Itaya and Ui.²⁶

Statistical evaluation

The estimations of blood pressure, of heart and respiratory rates, during anaesthesia were compared with respective basal values according to a three factor (time, block and blockade) ANOVA.²⁷ The values of pH, PaCO₂, PaO₂ and sodium bicarbonate concentration in the anaesthetized dogs were compared with those in conscious controls according to a two factor (time and block) ANOVA.²⁷

A three factor (treatment, time and block) ANOVA was used to study results from IVGTT and ITT. Semilog equations representing glucose and insulin disappearance from blood in conscious and anaesthetized dogs during IVGTT and ITT, respectively, were calculated. These equations' general form was: $y = \log_e V = a + Kt$, where V: concentration of the variable under study, a: ordinate axis; intercept, K: constant (slope), t: time from glucose or insulin load, respectively. The straight lines were compared for "y" means and slopes using Student t test.²⁸ Glucose and insulin distribution spaces (DS) were calculated according to the formulae employed in the indicator dilution principle: DS % body wt = 10000/a, where a: ordinate axis intercept by straight line representing glucose and insulin disappearance from blood during IVGTT and ITT, respectively. Glucose and insulin half-life time ($t_{1/2}$) in blood was calculated: $t_{1/2}$ min = 0.69/K, where K is the slope of the straight lines mentioned above. Differences were considered significant when $P < 0.05$.

Results

Biological data of fasted dogs (n = 48)

The mean ($\pm$ SEM) blood sedimentation rate was 8 $\pm$ 3 mm $\cdot$ hr⁻¹; haemoglobin concentration 14 $\pm$ 2 g $\cdot$ dl⁻¹; haematocrit 46 $\pm$ 2 ml $\cdot$ dl⁻¹; and blood sugar concentration was 4 $\pm$ 0.3 mM $\cdot$ L⁻¹.

TABLE II Influence of thiopentone anaesthesia on haemodynamic variables and respiratory rate in normal dogs. Anaesthesia was induced at time 0. Phentolamine or propranolol were injected *iv* just after 60 min. Mean $\pm$ SEM values are given. Statistical analysis: ANOVA (treatments, times, blocks). Groups: A = non-blocked dogs; B = alpha-adrenergic blockade; C = beta-adrenergic blockade. Number of animals per group: Six.

Variables		Time after induction of anaesthesia, minutes						
		0	15	30	45	60	90	120
Heart rate bt $\cdot$ min ⁻¹	A	126 $\pm$ 6	122 $\pm$ 4	119 $\pm$ 4	115 $\pm$ 7	116 $\pm$ 6	114 $\pm$ 5*	112 $\pm$ 3*
	B	131 $\pm$ 7	124 $\pm$ 6	121 $\pm$ 5	120 $\pm$ 7	118 $\pm$ 5*	127 $\pm$ 4	130 $\pm$ 4
	C	124 $\pm$ 7	118 $\pm$ 4	122 $\pm$ 4	120 $\pm$ 6	119 $\pm$ 4	101 $\pm$ 4††	98 $\pm$ 3††
Mean blood pressure KPa	A	19.7 $\pm$ 1.5	17.3 $\pm$ 2	16 $\pm$ 1*	16.5 $\pm$ 0.8*	17.7 $\pm$ 0.9	16.5 $\pm$ 0.7*	17.3 $\pm$ 0.8
	B	18.3 $\pm$ 1.6	16.5 $\pm$ 1.3	15.7 $\pm$ 1.3	16.6 $\pm$ 0.7	17.8 $\pm$ 0.7	15.8 $\pm$ 1.1	15.7 $\pm$ 0.9
	C	17.7 $\pm$ 1	16.8 $\pm$ 0.7	16 $\pm$ 1.6	16.7 $\pm$ 0.8	17.5 $\pm$ 0.8	16.3 $\pm$ 0.9	16 $\pm$ 1.1
Respiratory rate bth $\cdot$ min ⁻¹	A	27 $\pm$ 3	16 $\pm$ 1†	15 $\pm$ 3†	17 $\pm$ 1†	14 $\pm$ 1†	15 $\pm$ 1†	13 $\pm$ 2†
	B	25 $\pm$ 2	17 $\pm$ 2†	14 $\pm$ 3†	15 $\pm$ 1†	13 $\pm$ 2†	14 $\pm$ 1†	13 $\pm$ 2†
	C	24 $\pm$ 2	16 $\pm$ 2†	13 $\pm$ 2†	16 $\pm$ 1†	12 $\pm$ 2†	18 $\pm$ 2*	16 $\pm$ 1†

*, †: $P < 0.05$; $P < 0.01$. Comparisons with the value at 0 time.

††: $P < 0.01$. Comparisons vs 60 min.

TABLE III Blood pH, PaO₂, PaCO₂, sodium bicarbonate and thiopentone concentrations in normal dogs. Anaesthesia was induced at time 0; thiopentone induction dose: 12 mg $\cdot$ kg⁻¹. Mean $\pm$ SEM values are given. Statistical analysis: ANOVA (times and blocks); Number of animals per group = 12.

Variable	Conscious	Anaesthetized (Time after induction of anaesthesia, minutes)			
		30	60	90	150
pH	7.38 $\pm$ 0.04	7.31 $\pm$ 0.03	7.34 $\pm$ 0.03	7.32 $\pm$ 0.04	7.35 $\pm$ 0.04
PaO ₂ (kPa)	11.6 $\pm$ 0.5	10.8 $\pm$ 0.5	11.3 $\pm$ 0.4	11.1 $\pm$ 0.7	10.7 $\pm$ 0.5
PaCO ₂ (kPa)	5.6 $\pm$ 0.4	6.4 $\pm$ 0.3*	6.1 $\pm$ 0.4	6.3 $\pm$ 0.4	5.7 $\pm$ 0.7
Bicarbonate (mmol $\cdot$ L ⁻¹)	26 $\pm$ 3	21 $\pm$ 3	23 $\pm$ 4	21 $\pm$ 4	25 $\pm$ 2
Thiopentone (μ g $\cdot$ ml ⁻¹)	-	31.8 $\pm$ 3	34.6 $\pm$ 4	37.1 $\pm$ 4	29.8 $\pm$ 5

* $P < 0.05$ anaesthetized vs conscious

Thiopentone anaesthetized dogs

Table II. Heart and respiratory rates decreased after induction of thiopentone anaesthesia ($P < 0.05$).

Arterial blood pressure decreased slightly immediately after induction of anaesthesia and continued at this level throughout the study.

Table III. Blood pH and PaCO₂ were transiently decreased and increased respectively by anaesthesia; conversely, PaO₂ and plasma sodium bicarbonate concentration were not affected.

Thiopentone concentration in plasma, remained with little changes over the three hours of anaesthesia.

Therefore, all variables had stabilized 60 min after induction of thiopentone anaesthesia.

Thiopentone anaesthesia and metabolism during IVGTT
Basal BS, serum IRI and serum NEFA concentrations in conscious and anaesthetized dogs were similar (Figure 1). Glucose administration induced hyperglycaemia, which lasted longer in the anaesthetized dogs than in the

conscious controls. Glucose disappeared from the blood exponentially in both groups. Thus, the semilog relationship of BS to time from glucose load was linear ($P < 0.001$) in the anaesthetized ($r = -0.75$) and in the control ($r = -0.83$) groups throughout the study. In the anaesthetized dogs ($y = \log_e BS = 5.854 - 0.0141 t$) and in the conscious controls ($y = \log_e BS = 5.3956 - 0.0189 t$) the equations had similar slopes ($P > 0.05$) but their "y" general means differed ($P < 0.001$). The glucose space was 28% body weight in the anaesthetized dogs and 45% in the conscious dogs. Glucose half-life times in circulation were 49 and 36.5 min, respectively.

Hyperglycaemia evoked an increase in serum IRI in conscious dogs which was not seen in anaesthetized dogs ($P < 0.01$).

Serum NEFA concentration decreased in both groups. In the conscious dogs, the decrease was statistically significant only at 25 min, and was followed by an intense rebound. In the anaesthetized dogs, the decrease was more prolonged than in the conscious controls, and the

FIGURE 1 Effect of thiopentone anaesthesia on changes in blood sugar, serum immunoreactive insulin and serum non-esterified fatty acids during intravenous glucose tolerance test in normal dogs.

subsequent rebound was small. Serum NEFA concentration at the end of the test was less than in the conscious animals ($P < 0.01$).

Alpha-adrenergic blockade with phentolamine

The hyperglycaemic response to glucose in the conscious dogs was not affected by alpha-adrenergic blockade with phentolamine (NS, Figure 2A). There was a more intense (5-60 min, $P < 0.01$) response in the anaesthetized dogs, than in the conscious dogs, which was not modified by phentolamine pretreatment.

The serum IRI response to glucose in the conscious dogs was not changed by phentolamine ($P = \text{NS}$, Figure 2C). Anaesthesia abolished the response ($P < 0.05$) but it was partially restored by alpha-adrenergic blockade ($P < 0.05$ at 15 min).

Beta-adrenergic blockade with propranolol

The hyperglycaemic response to glucose in conscious and anaesthetized dogs was not affected by beta-adrenergic blockade (Figure 3). Hyperglycaemia was increased by thiopentone anaesthesia ($P < 0.01$ between 5 and 60 min, $P < 0.05$ at 90 min), but it was not further affected by beta-adrenergic blockade.

In conscious dogs the serum IRI response to exogenous glucose was not affected by propranolol pretreatment, but the response was abolished under thiopentone anaesthesia

FIGURE 2 Effect of alpha-adrenergic blockade with phentolamine on blood sugar and serum immunoreactive insulin concentrations in conscious and thiopentone anaesthetized dogs during intravenous glucose tolerance tests.

FIGURE 3 Effect of beta-adrenergic blockade with propranolol on blood sugar and serum immunoreactive insulin concentrations in conscious and thiopentone anaesthetized dogs, during intravenous glucose tolerance test.

($P < 0.05$). This effect was not further modified by adrenergic blockade with propranolol.

Thiopentone anaesthesia and metabolism during ITT

Insulin administration caused hypoglycaemia in both groups of animals ($P < 0.01$), but was less intense in the anaesthetized group ($P < 0.01$, Figure 4). Serum

FIGURE 4 Effect of thiopentone anaesthesia on blood sugar, serum immunoreactive insulin and serum free fatty acid levels in dogs during an intravenous insulin tolerance test.

IRI concentration increased ($P < 0.01$) after insulin. The rise was much more prolonged in the anaesthetized dogs than in the conscious controls. Hyperinsulinaemia was more intense ($P < 0.01$) in the anaesthetized group only at 60 and 90 min. The semilog relationship of serum IRI to time was linear in the anaesthetized ($r = -0.75$) and conscious dogs ($r = -0.88$, $P < 0.001$). The equations representing insulin disappearance from blood in anaesthetized dogs ($y = \log_e \text{ serum IRI} = 5.7603 - 0.0251 t$) and in conscious controls ($y = \log_e \text{ serum IRI} = 6.1293 - 0.0432 t$) were different in slope ($P < 0.001$) and, in "y" general means (4.935 and 4.7096, respectively, $P < 0.01$). In the anaesthetized group, insulin half-life time ($t_{1/2}$) in blood was 27.5 min and insulin distribution space was 78% body wt., compared with 16 min and 54%, respectively, in the conscious controls.

In conscious dogs, serum NEFA concentration increased in response to insulin ($P < 0.05$), but returned to the basal level after 75 min. This response was abolished in anaesthetized animals; so that the serum NEFA concentration in this group was below that found in the conscious controls.

Discussion

These results show that, in dogs, thiopentone anaesthesia provokes disturbances in glucose metabolism and NEFA.

These disturbances were not caused by surgical stress, because we deliberately excluded surgery in the experiments.^{29, 30} Furthermore, the haemodynamic and acid-based variables, which are known to be responsible for alterations in carbohydrate metabolism³¹ did not influence the results.

These results also demonstrate that thiopentone administration abolishes the insulin response to a glucose load in normal dogs despite increasing the hyperglycaemic response.

The regulation of serum IRI levels, is chiefly performed by the BS level and is also under the influence of several pancreatic and extrapancreatic factors. The extrapancreatic factors include: the size of the insulin distribution space and serum insulin clearance. The extrapancreatic factors were studied during the ITT, as proposed by other investigators,³² who had demonstrated that serum clearance of insulin was exponential and similar in normal and depancreatized dogs. The rate of clearance of circulating insulin in the thiopentone anaesthetized dogs was less than in conscious controls, which suggests that thiopentone anaesthesia depresses serum insulin removal by body tissues. Serum insulin clearance was exponential in conscious and in anaesthetized dogs. This can be transformed into linear functions exhibiting highly significant, high correlation coefficients which confirmed the results of Campbell and Rastogi³² in conscious dogs. Theoretically, the 72% increase in serum insulin $t_{1/2}$ observed in the dogs during thiopentone anaesthesia should be accompanied by a proportional enhancement in serum IRI concentrations during ITT. However, only partial enhancement occurred because thiopentone increased the insulin distribution space by approximately 44%. These results do not allow firm conclusions to be made of the action of thiopentone on insulin secretion. It has been demonstrated that pentobarbitone failed to affect directly the insulin release by rat pancreas pieces which had been incubated *in vitro*.⁷ However, partial restoration of the insulin response to hyperglycaemia induced by phentolamine in thiopentone anaesthetized animals, as shown here, suggested that such anaesthesia abolished serum response partly via an alpha-adrenergic mechanism, which is known to depress insulin secretion.³³

Dogs anaesthetized with thiopentone were moderately intolerant to extra glucose (IVGTT) and were resistant to the hypoglycaemic action of insulin (ITT). Serum NEFA concentration decreased in the thiopentone anaesthetized group at the beginning of IVGTT despite the absence of hyperinsulinaemia, likely because high BS levels are lipogenic in canines.³⁴ In contrast, the moderate glucose intolerance occurring in these animals might be related to an abolished insulin response.³⁵⁻³⁷ Nevertheless, the moderate resistance to the hypoglycaemic action of

insulin when insulin availability was high (ITT) makes us suspect that insulin counterregulation has been affected by thiopentone anaesthesia. Blood concentrations of epinephrine, glucagon, ACTH and cortisol are increased by hypoglycaemia in the normal conscious dog.³⁸ Epinephrine and glucagon increase the BS level³⁹⁻⁴⁰ under the permissive action of cortisol; epinephrine also induces rapid lipolysis *in vivo*⁴¹ but glucagon action in these circumstances is doubtful. There are no studies on the influence of thiopentone anaesthesia on the secretion and metabolic actions of most insulin antagonists. We suggest that thiopentone anaesthesia depresses the epinephrine secretory response to hypoglycaemia *in vivo* as demonstrated *in vitro*,⁴² which is in keeping with the depressed serum NEFA observed at the end of IVGTT and ITT. The adrenergic mechanisms appear to play no important role in the regulation of BS level (IVGTT) in conscious and thiopentone anaesthetized dogs because this variable was not affected by alpha- or beta-adrenergic blockade. Since the hyperglycaemic action of glucagon and epinephrine are synergistic it seems that glucagon is responsible for glucose intolerance (IVGTT) and resistance to insulin's hypoglycaemic action (ITT) in thiopentone anaesthetized dogs. Growth hormone did not induce insulin antagonism during IVGTT because it is not secreted.⁴³

We conclude that, in the dog, thiopentone anaesthesia, abolishes the insulin response to hyperglycaemia. This is partly mediated by an alpha-adrenergic mechanism and is influenced by an intense broadening of the insulin distribution space. The behaviour of BS and serum NEFA in the anaesthetized animals during glucose test results partly from the lack of insulin response but, also it appears to be related to changes in insulin antagonism as evidenced in the course of glucose and insulin tests.

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