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Calorigenic Effects of Glucagon and Epinephrine in Anesthetized Dogs[1] (37923)

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In the dog, injections of glucagon and of epinephrine have different effects upon plasma free fatty acid concenration (FFA). Glucagon causes a small, transient FFA elevation, followed by a decrease (1); epinephrine causes marked elevation of FFA (2); Whitty *et al.* (3) have suggested that the initial rise of FFA after glucagon may be due to rapid stimulation of adipose tissue lipolysis, although they note the possibility that the rise may be due, in part, to catecholamine release. These authors ascribe the FFA decrease to glucagon-induced hyperglycemia and/or insulin release. However, glucagon has no lipolytic effect *in vitro* on the adipose tissue of the dog (4, 5) and insulin does not appear to be necessary for the FFA decrease because cysteins treated glucagon also produces FFA decrease in pancreatectomized dogs (3, 6).

The uptake of FFA by the tissues is determined by the plasma FFA level (7–9) and it has been suggested (7) that the calorigenic effect of catecholamines is due in part to the mobilization and subsequent oxidation of FFA. Since glucagon and epinephrine have different effects on plasma FFA concentration in the dog, a comparison of the effects of these two hormones on O_2 consumption in this animal would be expected to give information regarding the role of FFA mobilization in the mechanism of the calorgenic effect. This is the objective of the present study.

Methods. Five groups of mongrel (male and female) dogs weighing 10–20 kg were used. They were housed in individual cages and fed *ad lib.* a commercial low-fat dog food, which was withheld 16–18 hr before the experiment. Anesthesia was induced by iv injection of sodium pentobarbital (26 mg/kg; Diabutal, Diamond Laboratories, Inc., Des Moines, IA). Throughout the experiment, additional anesthetic was given so that the eyelid reflex could hardly be elicited.

Blood samples were taken from the femoral vein. Blood glucose was determined according to Nelson (10), blood lactate according to Barker and Summerson (11), and plasma FFA according to a modification (12) of the method of Trout *et al.* (13), using 0.02 *M* NaOH in isopropanol for the titration.

Oxygen uptake was monitored with a close-circuit metabolic apparatus. A tracheal tube was connected to a two-way valve located between the inlet and the outlet of a 5-liter spirometer (Sanborn Co., Cambridge, MA) provided with a canister containing Baralyme (Collins, Inc., Braintree, MA) for the absorption of CO_2. The height of the spirometer bell was recorded on the electrosensitive paper of a slowly moving Boehnke kymograph (Eberbach Corp., Ann Arbor, MI). Oxygen uptake was calculated from the slope of the tracing and converted to dry volume at 0° and 760 mm Hg using the spirometer temperature. Calorigenic effect is expressed as O_2 con-

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sumed in excess of the mean preinfusion value, for the hour after beginning of infusion.

The temperature of the dog was measured with a thermistor (Yellow Springs Instrument Co., Yellow Springs, OH) inserted about 10–15 cm into the dog's rectum.

All infusions were made through a catheter inserted into the cephalic vein using a motor-driven syringe pump (model 600-910, Harvard Apparatus Co., Dover, MA). Epinephrine (Adrenaline chloride, Parke Davis & Co., Detroit, MI) was diluted to the desired concentration with saline. Crystalline glucagon (lot 258–234 B-167-1, Eli Lilly Co., Indianapolis, IN) was dissolved in 0.02 *M* glycine buffer, pH 9.5.

Fresh dilutions of epinephrine and fresh solutions of glucagon were prepared daily just before the experiment. Control dogs received an infusion of 0.02 *M* glycine buffer. Each of the groups of dogs received a different infusion, namely: glycine buffer (control), epinephrine (3.0 μg/kg/min), and glucagon (1.0, 3.0, and 11.0 μg/kg/min). The duration of the infusions was 10 min.

The data were analyzed by analysis of variance using appropriate Newman–Keul's procedures (14). Differences were considered significant when $P < 0.05$.

Results. Control experiments. As shown in Fig. 1, the O_2 uptake was practically constant throughout the experiment. Statistical analysis showed no significant difference in O_2 uptake between the preinfusion period and the values measured during the hour following the beginning of the glycine buffer infusion. Rectal temperature was also constant.

Plasma FFA showed a slow rise after the beginning of the infusion. The FFA level 2 hr after the beginning of infusion was significantly higher than the mean preinfusion value. Blood sugar rose significantly during the preinfusion period but showed no significant change after the beginning of the infusion. Blood lactate decreased significantly during the preinfusion period. None of the lactate values after the beginning of the infusion was significantly different from the level at the beginning of the infusion.

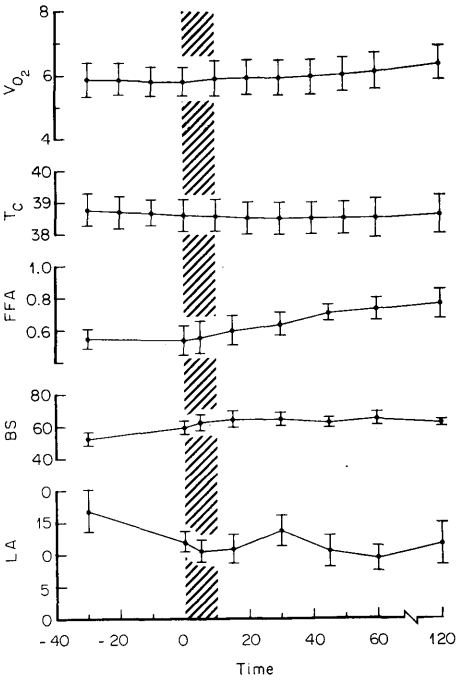

FIG. 1. Effect of glycine buffer infusion (control experiments) in anesthetized dogs. Shaded area represents infusion period. Ordinate: V_{O_2}, oxygen consumption (ml O_2/kg/min); T_C, rectal temperature (°C); FFA, plasma free fatty acids (mEq/liter), BS, blood sugar (mg/100 ml); LA, blood lactate (mg/100 ml). Abscissa, time in minutes. Means ± 1 standard error for 6 dogs.

Epinephrine experiments. Infusion of epinephrine (30 μg/kg in 10 min) produced a calorigenic response, as shown by the peak of the O_2 consumption curve (Fig. 2). O_2 uptake at 20 min was significantly higher than the mean preinfusion value. The mean elevation was 34% above the mean preinfusion value. The calorigenic effect, expressed as O_2 consumed in excess of the mean preinfusion value, for the hour after beginning of infusion, was 45 ml/kg (SE ± 5). Rectal temperature showed a slight nonsignificant decrease throughout the experiment.

Plasma FFA showed a significant elevation 15 min after the beginning of the infusion. The mean value at this time was 141% above the mean preinfusion value. Blood sugar rose and showed its highest level also at 15 min. The mean elevation

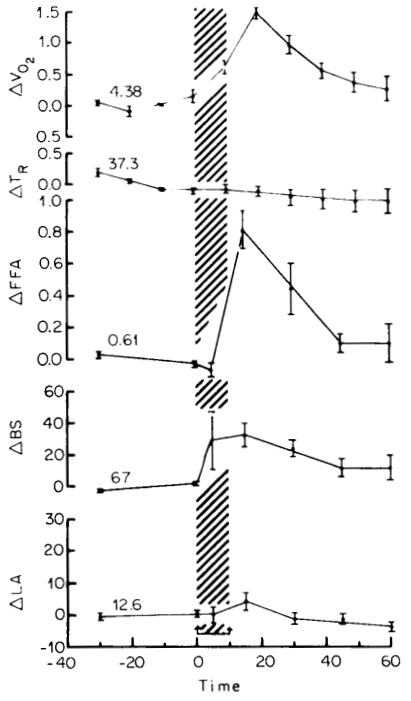

FIG. 2. Effect of epinephrine infusion (30 μg/kg in 10 min) in anesthetized dogs. Points represent differences between the observed values and the mean of the corresponding measurements during the preinfusion period. Mean preinfusion values are given by the figures at the left-hand side of the shaded area, which represents the period of infusion. Ordinate: Δ V_{O_2}, oxygen consumption differences (ml O_2/kg/min); Δ T_R, rectal temperature differences (°C); Δ FFA, free fatty acids differences (mEq/liter); Δ BS, blood sugar differences (mg/100 ml); Δ LA, blood lactate differences (mg/100 ml). Abscissa, time in minutes. Means ± standard error for 4 dogs.

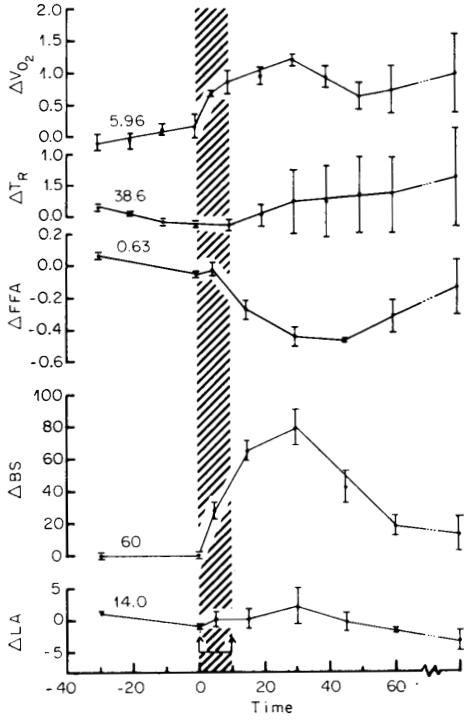

FIG. 3. Effect of glucagon infusion (10 μg/kg in 10 min) in anesthetized dogs. Symbols and units as in Fig. 2. Means ± 1 standard error for 4 dogs.

was 49% above the mean control value and was significant. Blood lactate showed a mean elevation of 34% above the mean preinfusion level, 15 min after the beginning of the infusion, but the elevation was not significant.

Glucagon experiments. Three different doses of glucagon (1.0, 3.0, and 11.0 μg/kg/min) were infused, each for a period of 10 min. Total amounts of glucagon infused were therefore 10.0, 30.0, and 110.0 μg/kg. The results are presented in Figs. 3, 4, and 5.

In all cases, there was a sharp increase in O_2 uptake after the beginning of the infusion, followed by a plateau and a slow decrease. For the 10.0 μg/kg dose, only the 30-min value was significantly elevated above the mean preinfusion value. For the 30 and 110 μg/kg doses, all the values from 10 min to 2 hr after the beginning of the infusion were significantly elevated.

The maximal elevations of O_2 uptake for each of the three doses were respectively 19, 25, and 30% above the corresponding mean preinfusion value. The calorigenic effects, as defined above (mean ± SE), were 49 ± 6, 66 ± 7, and 78 ± 15 ml/kg, respectively, for the hour after beginning the infusion of glucagon. This result shows that the calorigenic effect of 10.0 μg/kg of glucagon (49 ml O_2/kg/hr) is about the same as that of 30 μg/kg of epinephrine (45 ml O_2/kg/hr).

The infusion of 110 μg of glucagon/kg

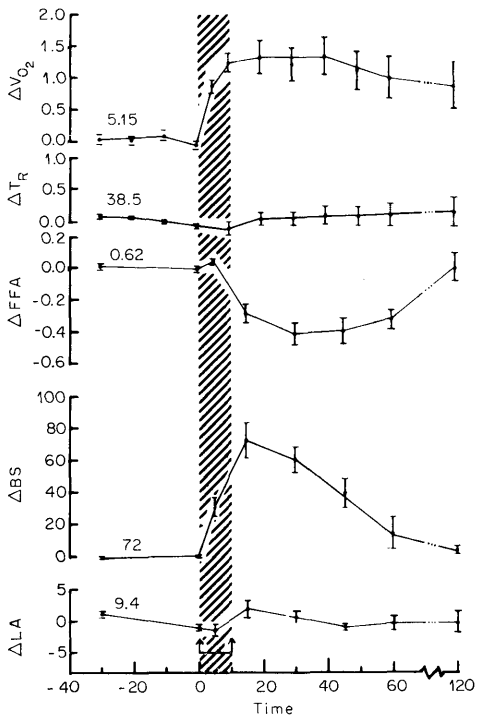

FIG. 4. Effect of glucagon infusion (30 μg/kg in 10 min) in anesthetized dogs. Symbols and units as in Fig. 2. Means ± 1 standard error for 5 dogs.

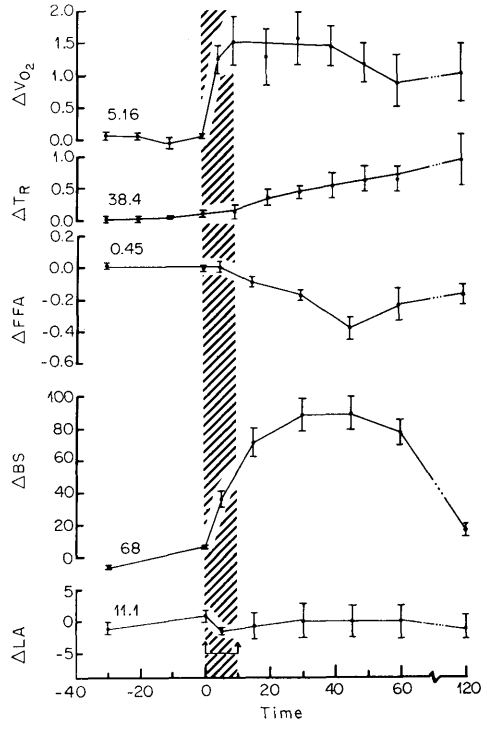

FIG. 5. Effect of glucagon infusion (110 μg/kg in 10 min) in anesthetized dogs. Symbols and units as in Fig. 2. Means ± 1 standard error for 4 dogs.

caused a slight, not significant, increase in the rectal temperature (av. 0.7° at 60 min). No temperature change was observed in the other two groups. of dogs. The anesthesia of one of the dogs receiving glucagon at the dose of 10 μg/kg became light during the experiment and the dog had a marked increase in rectal temperature which accounts for the large SE in the temperature data for this group (Fig. 3).

Plasma FFA showed a significant decrease 15 min after beginning the infusion of glucagon for the 10 and 30 μg/kg dose groups. The FFA decrease for the 110 μg/kg dose was slower than that observed with the other two doses. The FFA level was significantly decreased after 30 min of infusing 110 μg/kg, reached the lowest level measured at 45 min, and did not return to control level 2 hr after the start of the infusion.

Blood sugar increased during glucagon infusion. The highest values observed were 132, 103, and 132% above the mean preinfusion level 30, 15, and 30 min after infusing 10, 30, and 110 μg/kg, respectively. Sixty minutes after beginning of infusion with the two lower glucagon doses, the blood sugar was not significantly different from the corresponding mean preinfusion level. With the highest dose (110 μg/kg) the blood sugar was still significantly elevated above mean control level 2 hr after beginning the glucagon infusion.

There were no significant changes in the blood lactate concentration in any of the groups of dogs treated with glucagon.

The excess oxygen consumption for the hour following the beginning of the infusion was significantly correlated with the logarithm of the glucagon dose given in 10 min of infusion ($r = +0.49$, $N = 19$). The relationship between dose (μg/kg) and calorigenic effect (ml O_2/kg in excess of

preinfusion value, for the hour after beginning infusion) is described by the regression equation:

$$\text{ml } O_2/\text{kg} = 27.7\ (\log \mu\text{g glucagon/kg}) + 22.7.$$

Discussion. Our results confirm the calorigenic effect of epinephrine in the dog, first described by La Franca (15), and are in agreement with previous work regarding the effect of this hormone on plasma FFA (16) and blood sugar (17). No elevation of blood lactate after epinephrine injection was observed in this study. Other authors (18, 19) using similar doses of epinephrine reported large increases in blood lactate.

As described in the results, glucagon infusion has a significant calorigenic effect in the dog. This result compares with observations in rats (20, 21), newborn rabbits (22), and man (23). Tibblin (24) has reported that glucagon (50 μg/kg) restores to normal level the reduced O_2 consumption of hypovolemic dogs, but we found no previous report describing the calorigenic effect of glucagon in normal dogs.

The effects of glucagon on the various blood components measured in this study are in agreement with previous observations demonstrating that, in the dog, glucagon causes of FFA (3, 6, 25–27), elevation of blood sugar (3, 19), and no change of blood lactate (19).

There are some evident differences between the calorigenic effects of glucagon and epinephrine observed in our experiments. The increase of O_2 uptake produced by glucagon occurs faster and is more marked and sustained than that produced by epinephrine, as shown by Figs. 2–5. Taking into consideration the molecular weight of the hormones, it may be calculated that the calorigenic response associated with the infusion of 1 nmole of glucagon is about 63 times greater than that of 1 nmole of epinephrine. This is in contrast with the observations of Davidson *et al.* in the rat (20). In this animal the calorigenic effect of 1 nmole of glucagon was only 1.9 times greater than that of 1 nmole of epinephrine.

There is a remarkable difference between epinephrine and glucagon in regard to the relationship between their calorigenic effects and their effects on FFA mobilization. The calorigenic effect of epinephrine was associated with the well-known elevation of FFA produced by this hormone in the dog, whereas the calorigenic effect of glucagon was associated with a decrease of FFA. It has been proposed (7) that the elevation of metabolic rate caused by epinephrine may be directly attributable to the elevation of FFA. However, in view of the fact that nicotinic acid inhibits completely the FFA-mobilizing effect of norepinephrine, but only partially its calorigenic effect, Steinberg concluded that the calorigenic effect of catecholamines is "at most only partially dependent on the simultaneous FFA mobilization" (16). It is clearly shown by our results that the calorigenic effect of glucagon occurs in the dog in the absence of any increase of plasma FFA. That the calorigenic effect of glucagon is independent of FFA mobilization is further supported by observations showing that this hormone has adipokinetic effect (28) but no calorigenic effect (21) in the mouse.

The fact that glucagon causes an elevation of O_2 consumption and a decrease of plasma FFA concentration in the dog suggests the possibility that the calorigenic effect of this hormone is related to increased utilization and oxidation of circulating FFA. Several lines of evidence (29–33) support the view that glucagon stimulates the rate of oxidation of long-chain fatty acids by the liver. Tibblin has demonstrated that the increase in whole-body O_2 consumption produced by glucagon in hypovolemic dogs is accounted for by the increased O_2 uptake of the liver and the superior mesenteric region (24, 34). We suggest, therefore, that the calorigenic effect and the decrease in FFA concentration produced by glucagon in the dog are mainly due to the effect of this hormone stimulating fatty-acid oxidation by the liver.

Summary. The effect of infusing for 10 min either epinephrine or glucagon on oxygen consumption, plasma FFA, blood sugar, and blood lactate was studied in fasting anesthetized dogs. Calorigenic effect is expressed as excess oxygen consumption (ml/

kg) above the mean preinfusion level for the hour following the beginning of the infusion. Infusion of epinephrine (30 μg/kg in 10 min) caused mean calorigenic effect of 45 ml O_2/kg (SE ± 5, $P < 0.05$). Glucagon at doses of 10, 30, and 110 μg/kg in 10 min produced calorigenic effects (means ± SE) of 49 ± 6, 66 ± 7, and 78 ± 15 ml/kg, respectively, which were significant ($P < 0.05$).

The calorigenic effect of infusing 10 μg/kg of glucagon compares with that obtained by infusing 30 μg/kg of epinephrine. Taking into account the molecular weight of the two hormones, the calorigenic effect per nmole of glucagon infused is 63 times greater than that produced by 1 nmole of epinephrine.

It is suggested that the calorigenic effect, and the decrease of plasma FFA produced by glucagon in the dog, are mainly due to stimulation by this hormone of the oxidation of fatty acids by the liver.

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