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Myocardial Metabolism during Pentobarbital Anesthesia in Dogs

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Myocardial substrate utilization and hemodynamics were determined in dogs before and one hour and two hours after intravenous administration of sodium pentobarbital. Although total body O_2 consumption decreased, along with arterial O_2 concentration, there was no significant change in mean arterial pressure or cardiac output. Arterial free fatty acid (FFA) concentration and myocardial FFA utilization were sharply diminished, but FFA remained the major energy source for the heart. Arterial glucose concentrations decreased significantly after pentobarbital, but myocardial uptake of glucose remained unchanged. Neither arterial lactic acid concentration nor myocardial lactate uptake was affected. Myocardial pyruvate uptake, by contrast, while remaining quantitatively the least of the contributors of energy measured, was more than doubled one hour after pentobarbital injection despite the fact that arterial pyruvate concentration did not increase. It is concluded that pentobarbital reduces the FFA utilization of both the whole animal and of its myocardium. The energy consumption of the whole animal is similarly diminished, whereas the energy used by myocardium is unchanged, other substrates evidently being substituted for FFA as energy sources. (Key words: Heart, metabolism, pentobarbital; Hypnotics, pentobarbital; Anesthetics, intravenous, pentobarbital; Metabolism, myocardial.)

TO STUDY myocardial metabolism without the influence of anesthesia,[1] we developed a procedure to measure both myocardial substrate utilization and hemodynamic changes in the intact, unnarcotized, untranquilized dog.[2] Results obtained with this technique differed from those reported by others who used animals anesthetized with barbiturates, with or without surgical manipulation.[3,4,5] The present study was designed to investigate those differences, by comparing each of a series of dogs with itself while conscious and after anesthetization with the frequently used barbiturate, sodium pentobarbital.

Methods

Healthy mongrel dogs (10 to 15 kg) of either sex were trained to lie quietly on a table. Several days prior to the experiment, polyethylene catheters were inserted in the right jugular vein and carotid artery under light morphine–pentothal anesthesia, as described previously.[2] On the day of the experiment, after an 18-hour fast, a #7 woven nylon Goodale-Luben heart catheter for coronary sinus blood sampling was introduced into the sinus of the conscious dog through the polyethylene jugular catheter by fluoroscopy[6] and kept patent by a slow saline drip. No anticoagulant was used. Mixed venous blood was sampled by a Goodale-Lubin heart catheter inserted by fluoroscopy at the same time into the pulmonary artery via a second polyethylene jugular catheter; it also was kept patent by a slow saline drip. Arterial blood samples and pressure were obtained from the carotid catheter.

Palmitic-1-^{14}C acid (specific activity 40 μCi/μmole), bound to human serum albumin was infused continuously throughout the experiment via an Intracath catheter in either the saphenous or the cephalic vein. In a previous study it was noted that a two-hour infusion period was needed to produce a constant specific activity or steady state of palmitate entering and leaving the heart,[6] and therefore a two-hour infusion always preceded the first or control blood sample. Simultaneous arterial and coronary sinus control blood

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samples were then collected, following which anesthesia was induced by injecting 26 mg/kg sodium pentobarbital intravenously. The effects of pentobarbital were assessed by drawing additional blood samples one and two hours later; during this time the dogs were not conscious and had little or no involuntary movement.

Blood oxygen and carbon dioxide contents were determined by Van Slyke technique.[7] Free fatty acid,[8] pyruvic acid,[9] lactic acid,[10] and glucose[11] concentrations were determined as described previously.[6] Total body oxygen consumption and CO_2 production were determined using a Noyons Diaferometer as described previously[12]; from them, respiratory quotient was calculated. Cardiac output was calculated from total-body O_2 consumption and arterio-pulmonary artery O_2 difference (direct Fick method). Arterial blood pressure was measured using a P24 Statham transducer in a Model 350 Sanborn recorder and the mean arterial blood pressure electronically derived. Myocardial efficiency was determined according to the equation of Bing and Michal,[22] and was calculated as follows:

$$\text{Myocardial efficiency (per cent)} = \frac{\text{Cardiac work}}{\text{O}_2 \text{ consumption used for work}} \times 100$$

Cardiac work (kg m/min) = cardiac output (l/min) × mean arterial blood pressure (mm Hg) × .0136

O_2 consumption used for work (kg m/min) = $\dot{V}_{O_2}$ (l/min) × caloric equivalent of O_2 with respect to RQ × 426.85 × 0.806.

where 426.85 is the conversion factor of calories to kg m and 0.806 is the fraction of O_2 used in the contractile work of the heart alone.

Since coronary blood flow (CBF) measured by the antipyrine method[13] expresses flow per 100 g of left ventricle, all calculations derived from coronary flow measurements were related to 100 g of left ventricle. Uptake of substrates by the heart was determined by multiplying the arterio–coronary sinus difference by the CBF. Myocardial removal derived from FFA-^{14}C uptake was also calculated from the equation of Gold *et al.*[14]:

$$\frac{(A - V)\ \text{FFA-}^{14}\text{C (dpm/ml)} \times \text{plasma flow (ml/100 g per min)}}{\text{A FFASA (dpm/}\mu\text{Eq)}}$$

where A = arterial concentration, V = venous concentration, and SA = specific activity. Myocardial FFA oxidation to CO_2 was determined[14] by the equation

$$\frac{(V - A)\ ^{14}CO_2\ \text{(dpm/ml)}}{\text{A FFASA (dpm/}\mu\text{Eq)}} = \mu\text{Eq/ml blood}$$

This value, multiplied by the CBF, gives the rate of FFA oxidation. The contribution of FFA to total myocardial CO_2 production was calculated using the following formula (assuming an average chain length of 17 for FFA):

$$\frac{\text{FFA oxidized } (\mu\text{Eq/min}) \times 17 \times 100}{CO_2 \text{ produced } (\mu\text{moles/min})} = \text{per cent } CO_2 \text{ from FFA}$$

Total-body turnover of plasma FFA (rate of entry and exit) was calculated from the infusion rate of palmitate-^{14}C divided by the SA, according to the equation of Steele[15] used in our previous studies.

Since each animal served as its own control, statistical analysis was conducted for dependent samples and the mean changes calculated. Student's t test was used to determine the significance of change. Friedman's nonparametric two-way analysis of variance[16] was used to test whether the difference in uptake of pyruvate was due to the pentobarbital. Analysis of variance[17] was used to determine by means of a single test if all three groups, control, one-hour, and two-hour samples, differed significantly in percentages

of CO_2 derived from plasma FFA oxidation. Likewise, analysis of covariance[17] was used to study the relationship between CO_2 production and the arterial FFA levels in the three groups. Within-class correlations were preferred to individual partial correlations in order to compare the three groups with each other.[18]

Results

There was a tendency for mean arterial blood pressure and cardiac output to decrease one and two hours after administration of sodium pentobarbital (table 1), but the decrease was not statistically significant. Myocardial efficiency,[22] however, decreased markedly at two hours to 14.82 per cent ($P < 0.001$) from a control value of 19.68 per cent.

Arterial O_2 content was 8.33 mM/l in the control condition (table 2); the decreases of 2.99 mM/l at one hour and 2.85 mmoles at two hours were highly significant ($P < 0.01$ and $P < 0.001$, respectively). CO_2 content, initially 16.77 mM/l in the control situation, rose 1.96 mM/l one hour ($P < 0.01$) and 0.92 mM/l two hours ($P < 0.01$) after pentobarbital administration. While total-body O_2 consumption decreased, the ventilatory RQ did not change significantly from control values. Sodium pentobarbital appeared to decrease myocardial O_2 consump-

TABLE 1. Hemodynamic Changes after Administration of Sodium Pentobarbital*

	Cardiac Output (l/min)	Coronary Blood Flow (ml/100 g·min)	Efficiency (Per Cent)	Mean Arterial Pressure (mm Hg)
Control	4.43 (±1.04)	161.5 (±28.1)	19.68 (±2.97)	148 (±8)
1 hour (mean change from control)	−0.85 (±0.76)	+23.5 (±35.5)	+5.27 (±7.29)	−13 (±7)
2 hours (mean change from control)	−2.48 (±1.03)	+41.5 (±36.9)	−4.86 (±0.46)	−26 (±12)
P	NS	NS	<0.001	NS

* Mean values ± SEM; n = 5 dogs; + denotes increase; − denotes decrease.
NS = not significant.

TABLE 2. Respiratory Changes after Administration of Sodium Pentobarbital*

	Arterial		Myocardial O_2 Consumption (mmoles/100 g·min)	Myocardial RQ	Total Body O_2 Consumption (ml/min)	Ventilatory RQ
	O_2 (mmoles/liter)	CO_2				
Control	8.33 (±.23)	16.77 (±.59)	1.088 (±.171)	.730 (±.038)	172 (±10)	.856 (±.036)
1 hour (mean change)	−2.99 (±.67)	+1.96 (±.37)	−.390 (±.215)	+.043 (±.076)	−62 (±8)	−.083 (±.052)
P	<0.01	<0.01	NS	NS	<0.001	NS
2 hours (mean change)	−2.85 (±.23)	+0.92 (±.34)	−.201 (±.217)	+.002 (±.081)	−52 (±11)	−.046 (±.043)
P	<0.001	<0.01	NS	NS	<0.01	NS

* Mean values ± SEM; n = 6 dogs; + denotes increase; − denotes decrease.
NS = not significant.

TABLE 3. Changes in Plasma Level and Myocardial Uptake of Various Substrates after Administration of Sodium Pentobarbital

	Arterial Pyruvic Acid (mg/100 ml)	Pyruvic Acid Uptake (mg/100 g/min)	Arterial Lactic Acid (mg/100 ml)	Lactic Acid Uptake (mg/100 g/min)	Arterial Glucose (mg/100 ml)	Glucose Uptake (mg/100 g/min)
Control	0.996 (±0.275)	0.524 (±0.340)	10.95 (±2.68)	5.535 (±1.744)	116.7 (±6.5)	6.377 (±1.195)
1 hour (mean change)	+0.107 (±0.349)	+0.661 (±0.218)	+0.09 (±2.47)	+3.369 (±1.995)	−18.98 (±2.75)	+0.052 (±1.664)
P	NS	<0.05	NS	NS	<0.001	NS
2 hours (mean change)	−0.154 (±0.243)	+0.082 (±0.135)	−1.68 (±2.05)	+0.922 (±0.990)	−22.02 (±3.68)	−3.039 (±2.540)
P	NS	NS	NS	NS	<0.01	NS

* Mean values ± SEM; n = 6 dogs; + denotes increase; − denotes decrease.
NS = not significant.

tion at one hour, but this decrease was not significant. Myocardial RQ remained unchanged.

Changes in plasma concentrations and myocardial uptake of various substrates are given in table 3. Arterial pyruvic acid concentrations showed little or no change, although one hour after anesthesia myocardial uptake of pyruvate more than doubled from a control value of 0.524 mg/100 g/min (significant at $P < 0.05$). By two hours pyruvate uptake was not significantly different from the control value using Student's t test. However, Friedman's nonparametric two-way analysis of variance[16] showed that the differences between uptake of pyruvate in the control condition and at one hour and two hours were significant at $P = 0.042$. In all cases the greatest uptake was observed in the one-hour sample.

Neither lactic acid concentration nor its myocardial uptake was altered significantly by sodium pentobarbital. Arterial glucose concentration, on the other hand, decreased 19 mg/100 ml ($P < 0.001$) at one hour and 22 mg/100 ml at two hours from a control value of 116.7 mg/100 ml, both changes being significant. Despite these decreases in plasma

TABLE 4. Changes in FFA Metabolism after Administration of Sodium Pentobarbital*

	Arterial Plasma FFA (μEq/ml)	Total Body CO_2 from Plasma FFA (Per Cent)	Total Body FFA Turnover (μEq/min)	Myocardial FFA Removal (μEq/100 g/min)	Myocardial FFA Oxidation (μEq/100 g/min)
Control	0.861 (±0.138)	64.87 (±0.876)	555.7 (±39.4)	38.41 (±7.34)	31.89 (±9.03)
1 hour (mean change)	−0.545 (±0.095)	−45.41 (±5.90)	−314.7 (±47.5)	−19.70 (±7.19)	−19.62 (±8.04)
P	<0.01	<0.001	<0.001	<0.05	.1 > *P* > .05
2 hours (mean change)	−0.532 (±0.124)	−50.85 (±10.90)	−392.4 (±87.9)	−18.23 (±5.20)	−15.09 (±17.81)
P	<0.01	<0.01	<0.01	<0.02	NS

* Mean values ± SEM; n = 6 dogs; + denotes increase; − denotes decrease; FFA = free fatty acid.
NS = not significant.

glucose, there was no change in myocardial glucose uptake from the control value of 6.38 mg/100 g/min at either one hour or two hours.

FFA metabolism was markedly affected by the anesthetic (table 4). A decrease in arterial FFA concentration of 0.545 μEq/ml from the control value of 0.861 μEq/ml at one hour was significant ($P < 0.01$). There was no further change at two hours. Nearly 65 per cent of the total-body CO_2 production was initially derived from plasma FFA. This value decreased to 20 per cent ($P < 0.001$) at one hour, and remained significantly depressed after two hours. FFA turnover in the entire body initially was 555.7 μEq/min. Sodium pentobarbital lowered this by 314.7 μEq/min ($P < 0.001$) at one hour and 392.4 μEq/min at two hours ($P < 0.01$). In the control situation virtually all of the FFA removed by the heart (38.41 μEq/100 g/min) was also oxidized (31.89 μEq/100 g/min) At one hour removal and oxidation had decreased by the same amount (20 μEq/100 g/min). At two hours, myocardial removal was still the same as at one hour.

Discussion

In a recent review, Merin[20] pointed out the dearth of complete metabolic studies of myocardial metabolism during inhalation anesthesia. The same lack exists for barbiturate anesthesia. The present study, wherein conscious, trained, resting dogs, free of anxiety, served as their own controls before being anesthetized offers a physiologic approach to this problem. The various isolated-organ techniques and *in-vitro* methods all suffer from obvious limitations where data for the whole normal animal are needed.

It is generally accepted that pentobarbital decreases total-body O_2 consumption,[19] as we have here observed. Merin states that this metabolic depression is associated with a decreased arterial O_2 concentration, which was also seen in this study. We found, however, that the decrease in total-body O_2 consumption was not associated with or due to a decrease in myocardial O_2 consumption.

It is possible that the hypotension, hypoxia, retention of CO_2, or change in *p*H might bring about an increase in catecholamine release which might have effected the metabolic and hemodynamic changes attributed to pentobarbital. However, the changes in O_2 consumption were opposite in direction to those attributed to catecholamines. In addition, independent variables indicate that significant catecholamine release during anesthesia is unlikely: plasma FFA concentration decreased approximately 60 per cent after pentobarbital anesthetization, plasma glucose concentration also decreased, about 16 per cent, and plasma lactic acid concentration remained unchanged or decreased slightly. Each of these plasma constituents normally increases sharply in response to catecholamines. The hemodynamic data are equally irreconcilable with increased catecholamine release.

We have previously shown a direct relation between arterial FFA concentration and the percentage of total-body CO_2 derived from FFA oxidation in conscious normal dogs.[12] In the present study, a similar relation was seen during anesthesia (fig. 1). The three lines, one hour, two hours, and control, drawn through their respective points, were different from each other. The three lines were parallel but had the same slope. Statistically, by analysis of variance,[17] it was shown that the lines through the control points and through points representing one and two hours were significantly different from each other ($P < 0.001$). Thus, the y intercepts, reflecting these values, are different (22.2, 3.87, and 2.90). Analyses of covariance[17] showed that there was no difference in the slopes of the three lines. In fact, within-class correlation was significant at $P < 0.001$. What this means is that sodium pentobarbital shifted the curve downward so that at the same FFA concentration there is less CO_2 from FFA oxidation. In other words, a comparable increase in FFA level in the pentobarbital-anesthetized dog would produce a much smaller increase in the percentage of CO_2 derived from the FFA than in the unanesthetized state. The anesthetic apparently affects transport into the cell and/or the metabolic degradation of fatty acid. In the present study we have not been able to identify the reason for this.

Barbiturates decrease skeletal muscle activity during anesthesia and therefore, de-

crease the amount of oxygen used by skeletal muscle in the anesthetized animal. The heart presents a different picture. There was only an insignificant decrease in myocardial O_2 consumption or RQ. Small changes in MABP and cardiac output, which were also not significant alone, when calculated together (work) showed a significant decrease in the work performed by the heart. Since myocardial O_2 consumption decreased only slightly, there was a significant decrease in the efficiency of the heart after two hours (but not after one hour).

Metabolically, myocardial FFA removal and oxidation of FFA continued to account for 40–50 per cent of myocardial O_2 consumption during this time and thus supplied a major portion of the energy for the heart.

If all of the observed myocardial glucose uptake were oxidized, it would account for approximately 20 per cent of the O_2 consumption of the heart. Although the arterial plasma glucose concentrations decreased significantly in response to pentobarbital, the apparently reduced glucose uptake at two hours was not statistically significant.

Lactate was consistently taken up by all hearts. The apparent increase in lactate uptake one hour after anesthetization was not statistically significant.

While pyruvate provides 2–3 per cent of the total energy need of the heart in the unanesthetized state, this value more than doubled one hour after pentobarbital. Jowett and Quastel have reported[1] that barbiturates interfered with oxidation of pyruvate by *in-vitro* heart preparations. Hunter[21] indicated that in man barbiturates retard carbohydrate metabolism by inhibiting pyruvate oxidation. While our finding seems to conflict with these reports, all that is indicated here is that the uptake of pyruvate is increased. It may be that while the cellular oxidative pathways may be blocked, synthesis (of glucose or fatty acid) might be enhanced. Since pyruvate oxidation rates were not measured, what the myocardial uptake reflects is an increase in net transport into the heart cell. The metabolic fate of pyruvate in these experiments is not known. However, from the work of others, we presume oxidation is decreased and, since

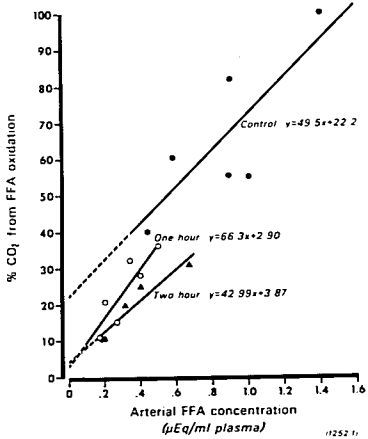

FIG. 1. Relation between percentage of carbon dioxide derived from free fatty acid (FFA) oxidation and arterial FFA concentration during control period and one and two hours after intravenous administration of pentobarbital (26 mg/kg). Solid circle, control; open circle, 1 hour; triangle, 2 hours.

there is little storage of pyruvate, we assume there was an increase in synthesis.

We conclude that the metabolic state of the anesthetized dog is different from that of the conscious dog. The lower plasma concentration of FFA found in the narcotized dog[3,4,14] compared with the conscious animal must be attributed to the effects of the anesthesia. Since the movement of FFA into the tissue depends upon the plasma concentration, a decrease in that concentration would also decrease FFA oxidation rate, and indeed this was found. The decrease in oxygen consumption due to pentobarbital reflects a depressed metabolic state, and the nature of the substrates used by the whole body is changed, with fatty acid becoming a minor component. Myocardial oxygen consumption, on the other hand, does not change as a result of the anesthetic. However, the amount of FFA taken up and oxidized by the heart is depressed, due in part to the lower FFA concentration. One can infer from that that the decrease in

total-body O_2 consumption is largely the result of decreased skeletal muscle metabolism. Pentobarbital depresses tissue oxygen consumption (except in the myocardium) while also decreasing fatty acid mobilization from the fat depots. This may reflect depressed O_2 consumption in the adipose tissue. In other words, if the source of the major substrate component is inhibited, those tissues using fatty acid must use other substrates. These two effects of pentobarbital, depressed tissue O_2 consumption and decreased fatty acid mobilization, as observed in the dog, present a different picture from that seen in the conscious, awake animal. The use of an anesthetic such as pentobarbital introduces several complicating factors that must be borne in mind in the interpretation of results.

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