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Early Derangements of Arteriovenous Anastomotic and Capillary Blood Flow in the Canine Hindlimb Induced by Supplemental Pentobarbital Anesthesia

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Vasoactive effects of supplemental pentobarbital anesthesia in the canine hindlimb microcirculation were documented in two groups of animals previously anesthetized with 30 mg/kg pentobarbital: Group I with a 5 mg/kg intravenous (iv) bolus of pentobarbital (n = 8) and Group II with a 5 mg/kg 2-min iv infusion of pentobarbital (n = 7). In Group I, measurements at baseline (BL) and 5, 15, 20, and 30 min (min) following pentobarbital administration included cardiac output, mean arterial pressure, total peripheral vascular resistance, common femoral artery flow (CFAQ) and resistance (CFAR), percentage hindlimb arteriovenous anastomotic shunt (AVA%), absolute shunt flow (AVAQ), and hindlimb nutrient capillary flow (NCQ). In Group II these same measurements were made, but the study was continued until all hindlimb hemodynamic parameters returned to control values. CFAQ, AVA%, AVAQ, and NCQ were significantly increased, and CFAR was decreased in both groups. CFAQ and NCQ remained significantly elevated at 30 min in Group I. In Group II CFAR, AVA%, and AVAQ remained elevated at 30 min, but did return to BL by 40 min, as did all other hindlimb hemodynamic parameters measured. Pentobarbital resulted in both AVA and arteriolar dilation, with an increase in the percentage total flow distributed to AVAs. These alterations of microcirculatory flow should be considered during investigations of the distribution of peripheral blood flow, as well as during metabolic studies assessing arteriovenous substrate differences, if interpretative errors are to be avoided.

This investigation was undertaken to define the microcirculatory effects of supplemental pentobarbital, an agent commonly used during hemodynamic studies in experimental animals. Previous studies have suggested that this agent may increase arteriovenous shunting [6, 8]. Although many canine hindlimb shunt studies have employed this anesthetic, to our knowledge, its specific effect on arteriovenous anastomoses has not been defined [5, 9, 10, 12, 13]. In the current investigation, alterations in hindlimb arteriovenous shunting induced by supplemental pentobarbital were quantitated using a radioactive microsphere technique.

MATERIALS AND METHODS

Fifteen adult mongrel dogs, weighing 21 to 37 kg, were anesthetized with an intravenous bolus of pentobarbital (30 mg/kg). Animals were intubated and mechanically ventilated maintaining arterial pH, pCO_2, and pO_2 within physiologic ranges. Esophageal temperature remained stable and normal throughout the study. All dogs received lactated Ringer's solution (10 ml/kg) intravenously during the experiment. A carotid arterial catheter, a transjugular central venous catheter, and a 7-Fr thermodilution Swan–Ganz catheter were appropriately positioned in each animal. Systemic arterial and central venous pressures were continuously monitored. A nonoccluding squarewave electromagnetic flow probe was placed about the common femoral artery for continuous measurement of total femoral blood flow (CFAQ) using a square wave flowmeter. A NaI scintillation detector calibrated for the detection of technetium-99m, was fixed in position over the upper lung fields. Freshly prepared ^{99m}Tc-

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labeled albumin microsopheres with a mean diameter of 20 μm (range 15–35 μm) were used in this experiment (3M Co., Minneapolis). Spheres of this size, when injected in the femoral artery, are trapped in the hindlimb capillary microcirculation unless they pass through arteriovenous anastomoses, whereupon they ultimately lodge in the lung [10]. A 22-gauge Teflon catheter was advanced into the common femoral artery for retrograde intraarterial microsphere administration. Radioactivity in individual syringes containing less than 0.1 ml of the suspension of microspheres (25,000 to 37,500 spheres) was measured before and after injection. Syringes were flushed three times with 0.3 cc of autologous blood. Such injections did not alter total common femoral artery flow. The radioactivity actually injected was determined taking into consideration the decay of ^{99m}Tc as well as the residual radioactivity in the syringes after injection. Injection catheter radioactivity was measured, and found to be insignificant. Three minutes following each microsphere injection, the incremental change in radioactivity over the lung was quantified. Femoral venous injections of a known amount of microspheres were used to determine "100% shunt," measured as change in counts per minute (Δcpm) of radioactivity over the lung field per millicurie radioactivity injected.

Percentage hindlimb shunt was calculated by the standard formula:

$$\text{Percentage shunt (AVA\%)} = \frac{\Delta\text{cpm over chest with intraarterial injection/dose of intraarterial injection}}{\Delta\text{cpm over chest with venous injection/dose of venous injection}} \times 100.$$

The volume of blood flow through shunts (AVAQ) was calculated as AVAQ = CFAQ × AVA%shunt. The volume of blood flow not passing through arteriovenous anastomoses, and therefore available for capillary exchange as nutrient capillary flow (NCQ), was calculated: NCQ = CFAQ − AVAQ.

Two experimental groups were evaluated. Forty minutes after induction of pentobarbital anesthesia hemodynamic parameters appeared stable. Baseline studies were then obtained. Group I subjects (n = 8) then received an intravenous bolus of pentobarbital (5 mg/kg) and hemodynamic studies were continued for the next 30 min. Group II subjects (n = 7) were studied after a 2-min continuous infusion of the same dose of supplemental pentobarbital, with experimental observations continuing for 40 min. In both Groups I and II the following parameters were measured at baseline (BL) and 5, 15, 20, and 30 min (and 40 min in Group II) after pentobarbital injection: cardiac output (CO), central venous pressure (CVP), mean arterial pressure (MAP), systemic vascular resistance (SVR = MAP − CVP/CO × 79.9 dyn-sec/cm^5), and common femoral artery resistance (CFAR = MAP/CFAQ (liters/min) × 79.9, dyn-sec/cm^5). Percent shunt, shunt flow (AVAQ), and nonshunt nutrient capillary flow (NCQ) were calculated as previously discussed. Data were analyzed by the Wilcoxon signed rank test and paired Student t test.

RESULTS

Effects of pentobarbital on cardiac output differed in the two groups (Table 1). In Group I significant abrupt increases in cardiac output occurred, and the elevations persisted for 30 min after bolus administration. The minor increase in mean cardiac output observed in Group II animals never attained statistical significance.

Mean arterial pressure was significantly decreased only at 5 min in Group I. Although mean arterial pressure decreased in Group II animals, changes from baseline approached but did not attain statistical significance at any time. Systemic vascular resistance was decreased significantly in both groups, indicating

TABLE 1

SYSTEMIC EFFECTS OF PENTOBARBITAL[a]

	Baseline	5 min	15 min	20 min	30 min	40 min
Cardiac output (l/min)						
Group I	3.96 ± 1.11	5.53 ± 2.26*	5.32 ± 1.62*	4.91 ± 1.09*	5.40 ± 1.24**	—
Group II	3.96 ± 0.78	4.19 ± 0.86	4.08 ± 0.86	4.15 ± 0.77	4.38 ± 1.11	4.70 ± 0.88
Mean arterial pressure (mm Hg)						
Group I	140 ± 17	136 ± 19*	137 ± 18	138 ± 18	140 ± 15	—
Group II	141 ± 13	135 ± 17	132 ± 17	133 ± 12.2	133 ± 14	142 ± 12
Systemic vascular resistance (dyn-sec/cm^5)						
Group I	3048 ± 1139	2144 ± 729**	2178 ± 652**	2297 ± 641*	2147 ± 588*	—
Group II	3028 ± 620	2751 ± 573	2778 ± 612	2728 ± 476*	2632 ± 553**	2575 ± 568**

[a] Data described as mean ± 1 SD. Comparisons to control baseline data using Student's t or Wilcoxon tests.
* $P < 0.05$.
** $P < 0.02$.

that pentobarbital caused vasodilatation independent of the speed of administration.

Major effects on hindlimb hemodynamics were apparent after supplemental pentobarbital administration (Table 2). Common femoral artery flow in Group I increased significantly throughout the period of observation. In Group II, the peak increase in CFAQ occurred at 20 min, but returned toward control values by 30 min. Decreases in hindlimb vascular resistance were observed in both groups, but had returned to levels indistinguishable from control values by 30 min in Group I, and by 40 min in Group II.

Percentage hindlimb shunt more than doubled in each group. In Group I mean percentage shunt increased from 3.2% at baseline to 8.2% at 5 min, and in Group II from a baseline of 2.1 to 6.0% at 20 min. Percentage hindlimb shunt returned to values near control levels by 30 min in Group I, and by 40 min in Group II.

Reflecting this redistribution of blood flow to AVA, absolute shunt flow (AVAQ) more than tripled in both groups. Bolus pentobarbital administration resulted in an early increase of mean AVAQ from 3.9 ml/min baseline to 12.3 ml/min at 5 min with a subsequent return toward control levels by 30 min. In Group II peak shunt flow occurred at 20 min, being similar in magnitude to the maximum shunt flow in the Group I animals, 12.9 ml/min. By 40 min in Group II, AVAQ also had returned close to control values.

Hindlimb nutrient capillary flow (NCQ) in Group I remained significantly elevated compared to baseline values throughout the study period. In Group II, mean NCQ increased from 142 ml/min at baseline to a maximum of 190 ml/min at 20 min, returning toward control levels by 40 min. Maximum derangements in the hindlimb microcircuation occurred in Group I at the initial 5-min observation period, and were noted at 20 min in Group II.

DISCUSSION

The radioactive microsphere technique for study of canine hindlimb arteriovenous anastomoses was first introduced by Lopez-Majano *et al.* [10]. Many investigators have subsequently evaluated the effects of pharmacologic and physiologic manipulations on these naturally occurring arteriovenous shunts, but despite this, the functional significance and physiologic control of AVA remain poorly understood.

Several suggestions that pentobarbital may alter arteriovenous shunting exist in the literature. Kaihara and colleagues reported that radioactivity accumulations in the lungs were

TABLE 2

HINDLIMB EFFECTS OF PENTOBARBITAL[a]

	Baseline	5 min	15 min	20 min	30 min	40 min
Femoral artery flow (CFAQ in ml/min)						
Group I	106 ± 32	157 ± 29***	132 ± 35†	130 ± 36†	122 ± 39*	—
Group II	146 ± 44	165 ± 34	191 ± 62	203 ± 61*	171 ± 50	164 ± 41.5
Femoral vascular resistance (CFAR in dyn-sec/cm^5)						
Group I	114,000 ± 36,000	82,000 ± 15,000***	88,000 ± 23,000***	91,000 ± 27,000***	102,000 ± 36,000	—
Group II	84,000 ± 24,000	68,000 ± 13,000*	60,000 ± 16,000***	59,000 ± 17,000***	68,000 ± 17,000**	74,000 ± 21,000
AVA% shunt						
Group I	3.2 ± 3.2	8.2 ± 6.9***	5.8 ± 5.5*	5.8 ± 5.5	3.1 ± 3.0	—
Group II	2.1 ± 1.6	4.2 ± 2.7**	5.4 ± 4.0**	6.0 ± 3.9**	4.5 ± 3.7**	3.4 ± 2.2
Shunt flow (AVAQ in ml/min)						
Group I	3.9 ± 4.4	12.3 ± 12.0***	8.7 ± 10.0**	8.7 ± 9.8*	4.4 ± 5.02	—
Group II	3.5 ± 3.6	7.0 ± 5.3**	10.9 ± 11.8**	12.9 ± 11.7**	7.9 ± 7.8**	5.9 ± 5.1
Nonshunt nutrient capillary flow (NCQ in ml/min)						
Group I	102 ± 28	124 ± 21**	123 ± 27***	124 ± 30*	117 ± 33*	—
Group II	142 ± 41	158 ± 32	181 ± 57	190 ± 55*	164 ± 48	158 ± 39

[a] Data described as mean ± 1 SD. Comparisons to control baseline data using Wilcoxon or paired t tests.

* $P < 0.05$.

** $P < 0.025$.

*** $P < 0.01$.

† $P < 0.001$.

significantly higher with left atrial injection of radioactive microspheres in dogs anesthetized with 25 to 30 mg/kg of pentobarbital, than occurred with similar microsphere injections in unanesthetized dogs [8]. These investigators speculated that this might result from "diversion of these spheres around systemic vascular beds" [8]. Rhodes and his coauthors reported existence of "a wider variation in percent shunting in pentobarbital anesthetized dogs" [12]. Another investigation on regional distribution of blood flow during pentobarbital administration noted marked increases in lung microsphere entrapment and decreased skeletal muscle blood flow [6]. Although these investigators speculated that systemic arteriovenous shunting resulted from pentobarbital administration, shunting was never quantitated by direct measurement.

Results of our study indicate that pentobarbital acts as an arterial dilator when administered by intravenous bolus or slow infusion in dogs already anesthetized with this drug. Hemodynamic responses to pentobarbital appear similar when administered to animals already anesthetized with this barbiturate or when administered to dogs previously anesthetized with other agents such as choloralose or urethane [11]. Lowering of arterial pressure and systemic vascular resistance following administration of 5 mg/kg of pentobarbital are comparable with drug induced vasodilation which has been previously reported [6, 11]. Because the effects of other anesthetic agents on hindlimb arteriovenous shunt are unknown, and because of the pervasive use of pentobarbital anesthesia in research related to arteriovenous anastomotic blood flow, pentobarbital was used to induce anesthesia in this study. This dose of pentobarbital was chosen for study because it is a frequently used anesthetic supplement in canine experiments.

An important microcirculatory derangement induced by supplemental pentobarbital anesthetic is an increase in percent of hindlimb shunt. This phenomenon may be due to relaxation or active dilation of AVAs. Accordingly, increased volume flow through such shunts may theoretically result from either "opening" of the AVA, or by increases in volume flow through AVA following proximal arterial dilation [9]. Both phenemona may have been operative in our experiments. Pentobarbital also increased hindlimb nutrient capillary blood flow, which may result from arterial dilation. Previous "vital" studies have suggested that pentobarbital causes venodilation as assessed by increased venular size, in addition to arterial dilation [7]. The increased venular size, reported in the former study, may reflect increased arteriovenous shunting, greater flow with arteriolar dilation, or primary venodilation. Specific mechanisms by which pentobarbital causes vasodilation are unclear, but may include inhibition of vasoconstriction by endogenous epinephrine, by acting as a "calcium entry blocker," or by inhibition of vasomotion [1, 2].

Results of the present investigation may explain some of the reported variability in percent hindlimb shunt and earlier controversy regarding the normal baseline percent shunt in canine hindlimbs when pentobarbital was the anesthetic utilized, since times of anesthetic administration and shunt measurement were probably not controlled [3, 4, 10, 12]. It has been previously suggested this variability may have been due to the lack of control of arterial blood gases [3, 4]. Experiments from our laboratories have suggested that this is not true [13].

It is apparent that intravenous pentobarbital alters both systemic and hindlimb hemodynamics when administered as a supplemental dose in the previously anesthetized dog. All hindlimb hemodynamic parameters (CFAQ, CFAR, AVA%, AVAQ, and NCQ) in this study returned to the level of controls by 40 min when the drug was administered slowly. Alterations in systemic hemodynamics were somewhat less abrupt and less sustained with the slower administration compared to the bolus administration of supplemental pentobarbital anesthesia. Using pentobarbital anesthesia in studies of extremity shunting utilizing radioactive microspheres is acceptable if the vasoactive effects of the drug are

considered. The effects of other anesthetic agents on arteriovenous shunting are unknown. One may conclude from our data that experimental measurements in arteriovenous shunt studies as well as in metabolic studies investigating arteriovenous substrate differences should not begin, and baseline values should not be obtained, until 30 to 40 min following initial or supplemental administration of pentobarbital, if interpretive errors are to be avoided.

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