Experimental Variation in Vascular Response Studies[1] (36929)

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(Introduced by J. B. Scott)

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In studies of vascular responses in dogs, we (1) and other investigators (2) have noted a considerable degree of experimental variation, which has impaired interpretation of the data. In the present study, we investigated two possible sources of this variation, genetic makeup, and age.

Methods. Seventeen dogs were selected from a highly inbred colony of Labrador retrievers (3) and were either litter-mates, siblings, or half-siblings. The dogs were healthy, male, aged 8–10 mo, and of stable body weight, averaging 25.5 kg (range 21.8–30.0 kg). Sixty-four mongrel control dogs were obtained from the pound; all were male, in good health, weighing an average of 22.8 kg (range 17.7–27.3 kg) and of varying and undetermined age. The mongrel dogs were "conditioned" for at least 4 wk before study, including routine immunizations and removal of endo- and ectoparasites. Under pentobarbital anesthesia (35 mg/kg, administered intravenously), mechanical ventilation, and systemic anticoagulation (heparin, 10,000–15,000 USP units), the pump-perfused forelimb vascular bed was prepared and pump pressure and brachial venous pressure were monitored as previously described (1). Rate of blood flow through the forelimb was held constant at 100 ml/min in all dogs. Laboratory temperature was maintained at 26–27°. Level of anesthesia was adjusted until the corneal reflex disappeared. The respirator was set to maintain measured systemic arterial blood pH at 7.39–7.41. After a 5 min period to allow the establishment of steady state conditions, paired infusions were made into the pump tubing upstream to the pump and the effect on pump pressure was measured. Each pair of infusions, administered at 37°, included an infusion of a vasoactive solution preceded by a control infusion of isosmolar (287 mOsm/kg) sodium chloride solution. The vasoactive infusion and the paired control infusion were delivered at 5 ml/min (except in the case of hypoosmolar NaCl and its control infusion, which were delivered at 8 ml/min). Vasoactive solutions were brought to volume with isosmolar NaCl solution.

Vasoactive solutions infused were: (a) solutions containing isosmolar KCl, delivering 0.460 mEq K^+/min and producing calculated increase in forelimb arterial plasma potassium concentration of 8.4 mEq/liter; (b) solutions containing isosmolar $MgCl_2$, delivering 0.473 mEq Mg^{2+}/min and producing calculated increase in forelimb arterial plasma magnesium concentration of 8.6 mEq/liter; (c) solutions containing isosmolar $CaCl_2$, delivering 0.230 or 0.460 mEq Ca^{2+}/min, and producing calculated increases in forelimb arterial plasma calcium concentration of 4.2 or 8.4 mEq/liter, respectively; (d) hyperosmolar solutions of 50% dextrose in water, diluted with isosmolar NaCl solution to achieve infusate osmolalities of 824 mOsm/kg, delivering 2.68 mOsm of solute/min, and producing calculated increase in forelimb arterial plasma osmolality of 48.7 mOsm/kg; (e) hypoosmolar (145 mOsm/kg) NaCl in water, delivered at 8 ml/min, producing calculated decrease in forelimb arterial plasma osmolality of 20.9 mOsm/kg; and (f) methacholine chloride (Mecholyl chloride, Merck, Sharp, and

[1] Supported by grants from the National Institutes of Health, National Heart and Lung Institute (HE11354 and 7-K3-HE31,793) and from the Michigan Heart Association.

TABLE I. Mean Resistance Values.

Agent	Group[a]	N	Initial resistance (IR)[b]	Change in resistance (ΔR) ± SD[b]	Value F	Value p
Methacholine chloride (1 μg/min)	L	7	5.08	−1.50 ± 0.59	1.373	>.1
	M	20	6.59	−2.31 ± 0.81		
Isosmolar KCl (0.460 mEq K^+/min)	L	7	5.31	−0.47 ± 0.35	2.086	>.1
	M	16	6.90	−1.25 ± 0.73		
Isosmolar $MgCl_2$ (0.473 mEq Mg^{2+}/min)	L	7	5.52	−0.12 ± 0.30	2.033	>.1
	M	20	6.85	−0.74 ± 0.61		
Isosmolar $CaCl_2$ (0.230 mEq Ca^{2+}/min)	L	7	5.75	+0.51 ± 0.54	1.074	>.1
	M	20	7.40	+1.07 ± 0.58		
Isosmolar $CaCl_2$ (0.460 mEq Ca^{2+}/min)	L	7	6.30	+1.04 ± 0.38	2.421	>.1
	M	12	8.34	+2.05 ± 0.92		
Hyperosmolar dextrose (2.68 mOsm/min)	L	10	5.87	−0.59 ± 0.24	4.083	<.025
	M	10	7.66	−0.68 ± 0.98		
Hypoosmolar NaCl (145 mOsm/kg, 8 ml/min)	L	7	5.60	+0.10 ± 0.28	1.037	>.1
	M	27	6.88	+0.33 ± 0.27		

[a] L = Labrador; M = mongrel.

[b] mm Hg/ml flow/100 g forelimb wt/min.

Dohme) diluted in isosmolar NaCl solution to deliver 1 μg/min, expressed as the salt.

Order of vasoactive infusions was similar in Labrador and mongrel dogs. Each infusion lasted 4 min, and intravascular pressures were recorded at the first and third minute. There was a 5–10 min period between each pair of infusions to allow the limb resistance to return to base line levels. At conclusion of the experiment, the dog was killed, the forelimb was amputated at the level of the tourniquets and weighed.

Total forelimb vascular resistance was calculated as $(\overline{P}_{BA} - \overline{P}_{LV})/F$, where $\overline{P}_{BA}$ represents mean pump perfusion pressure (mm Hg), $\overline{P}_{LV}$ represents mean brachial venous pressure (mm Hg), and F represents pump flow (ml/min/100 g forelimb wt). Resistance was expressed (mm Hg/ml flow/min/100 g). Response to the equivolume control infusion of isosmolar NaCl was subtracted from response to the paired vasoactive infusion before data were further analyzed. The F test was used to test for homogeneity of variances of responses in Labradors and mongrels (4).

Results. Table I presents observed resistance values. Forelimb weight was greater ($p < .05$) in Labradors than in mongrels (means 696.4 and 618.2 g, respectively). Adjusted for limb weight, mean initial limb resistance (IR) was greater in mongrels than in Labradors ($p < .001$), but variance of IR was similar in the two groups ($p > .1$). As previously reported (1) limb resistance fell in response to methacholine, KCl, $MgCl_2$, and hyperosmolar dextrose; limb resistance rose in response to $CaCl_2$ and hypoosmolar NaCl. In general, responses to these vasoactive agents were greater in mongrels than in Labradors. There was a significant ($p < .05$) positive linear correlation between IR and response (ΔR) to these vasoactive agents, as previously reported (1).

Variances of responses were similar in the two groups of dogs, with the sole exception of a significantly reduced ($p < .025$) variance in the response of Labradors to hyperosmolar dextrose. To illustrate these findings, responses to methacholine are plotted in Fig. 1. In this figure the reduced initial resistances in Labradors, the significant linear correlation between IR and ΔR, and the absence of a reduction in variance of response in the Labradors may be seen.

Discussion. Unexplained and thus uncontrollable experimental variation in cardiovascular response studies has been recognized as a major problem (2, 5). For this reason Page and Taylor (2) studied a variety of

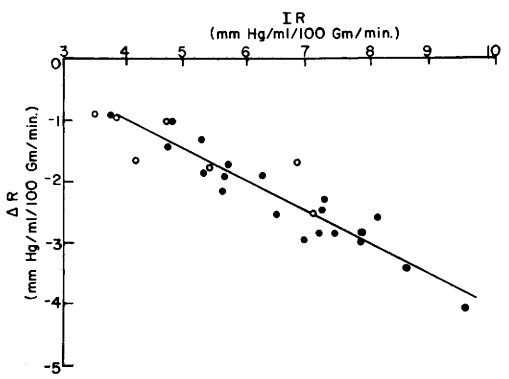

FIG. 1. Limb vascular responses ($\triangle R$) to intrabrachial arterial infusions of methacholine 1 μg/min plotted against level of limb initial vascular resistance (IR). (●) responses in mongrel dogs. (○) indicate responses in Labrador dogs. Regression line is drawn for responses in mongrel dogs only (r [IR vs $\triangle R$] $= 0.939$; $p < .01$).

factors which might influence the stability of responses in dogs, including body weight, depth of anesthesia, temperature, artificial changes in blood volume produced by intravenous infusions of isosmolar NaCl solution or blood, muscular relaxing agents, length of residence in the kennel, and initial blood pressure. None of these variables seemed to have an important effect on cardiovascular responsiveness. Other investigators (6, 7) however, have since reported a significant correlation between initial blood pressure and cardiovascular responsiveness.

Similarly, several investigators (8–10) have found that the level of initial resistance (IR) of a vascular bed influences the magnitude of its response (ΔR) to a variety of vasoactive agents; we (1) also found initial resistance to be a significant and important source of experimental variation. In the present study the relationship between IR and ΔR, rather than a greater vascular smooth muscle "sensitivity," undoubtedly accounted for the greater responses noted in mongrel dogs to the agents tested. Consideration by other investigators of this potentially important source of variation will likely reduce experimental error in future vascular response studies.

We also investigated the effect of two variables, not previously tested, on the stability of vascular responses in dogs. Our results surprisingly indicated that these variables, genetic constitution and age, seem to have little influence on the variability of vascular responses. Accordingly, considering the significantly higher costs for each research-usable dog from an inbred colony, it appears that use of such dogs in preference to mongrels for vascular response studies is not justified.

Summary. To investigate sources of variation in vascular response studies in dogs, forelimb vascular responses to a variety of vasoactive agents were measured in 17 line-bred Labrador dogs of the same age, and in 64 mongrel dogs of varying age. Results indicated that the level of forelimb initial resistance contributed importantly to variance in vascular responses. In contrast there was little evidence that genetic makeup or age influenced experimental error. Thus, variation in the level of initial resistance of a vascular bed must be considered in interpretation of data from vascular response studies. There is little advantage, however, to be gained from use of expensive highly inbred dogs in such studies.

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Received Sept. 15, 1972. P.S.E.B.M., 1972, Vol. 141.