PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 145, 546–552 (1974)

Lack of Correlation Between Initial Vascular Resistance and Responses to Vasoconstrictor Stimuli in the Perfused Canine Hindpaw[1] (37849)

STANLEY GREENBERG[2] AND WILLIAM R. WILSON

Department of Internal Medicine, Division of Clinical Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242

For most vasoactive constrictor and dilator stimuli the magnitude of the responses is dependent on the initial vascular resistances in the hindlimb, gracilis muscle and the isolated perfused segments of small cutaneous arteries and veins (1–10). These findings resulted in the formulation of the law of initial values (LIV) first expressed by Wilder (2) and subsequently demonstrated by other investigators (3–13). The LIV, as applied to cardiovascular dynamics, states that changes in pressure (resistance) produced by vasoconstrictor stimuli are smallest when initial vascular resistances are high and greatest when initial vascular resistances are low. The change in resistance produced by vasodilator drugs will be least at low initial vascular resistances and greatest at high initial vascular resistances (1–13). The concept of LIV generated the proposal that agonist-induced changes in vascular resistance should be expressed as the relative change in response, that is, as a percentage of the initial vascular resistance rather than the absolute change (2–13).

The validity of the LIV has been challenged since studies performed to evaluate the LIV demonstrated it does not exist in the pressor responses of urethane-anesthetized rats (14), and pentobarbital-treated dogs (15, 16) to various vasoconstrictor stimuli. However, the LIV is found to operate in the cat and dog when the hindlimb or hindquarters are perfused and the flow rate of blood is maintained constant. A recent study demonstrated the LIV to be operant in the pressor responses of the perfused gracilis muscle to serotonin (17).

The canine perfused paw preparation (18) is an extremely useful model for evaluation of the effect of drugs on adrenergic neuroeffector transmission and vascular reactivity (19–22). However, a fundamental problem in utilizing this preparation, as well as many others, is expression and interpretation of data. For example, when Drug A decreases vascular resistance by 50% but only decreases the responses to an agonist by 25%, depending upon whether the response to the agonist is expressed as a percentage of initial perfusion pressure or as the actual magnitude of change in pressure, Drug A either depresses or enhances the pressor response to the agonist. When confronted with two such divergent answers an investigator must expend a lot of time and effort to evaluate the effects of other substances on the preparation to ascertain whether the effect of Drug A on the agonist-induced response was mediated by changes in initial pressure or by the intrinsic action of Drug A. At times a definitive conclusion still cannot be reached concerning the effect of Drug A on the pressor response to an agonist.

The following study was designed to evaluate the effects of the level of initial vascular resistance on the responses of the perfused canine paw to vasoconstrictor and

[1] Supported in part by USPHS grants, NHLI 14388-02 and a Training Grant from the Veterans Administration Hospital (TR-105).

[2] Present address: Department of Physiology, College of Medicine, University of Michigan, Ann Arbor, MI 48104.

vasodilator stimuli. The data clearly demonstrate that over a range of initial pressures from about 80 to 400 mm Hg the LIV is operant for vasodilator stimuli but not for vasoconstrictor stimuli.

Methods. One hundred and fifty three dogs of either sex were anesthetized with pentobarbital sodium (30 mg/kg iv). After endotracheal intubation and initiation of artificial ventilation with a Harvard respirator, the left femoral artery and vein were cannulated for recording of systemic pressure and administration of drugs, respectively.

The right hindpaw was cannulated as previously described (19, 20). Sodium heparin (1000 units/kg) was administered to each animal through the femoral venous catheter. The cranial tibial artery was perfused by the method of Zimmerman and Gomez (18) with a Harvard peristaltic pump with blood taken from the common iliac artery. Flow in the hindpaw averaged 28 ± 0.6 (SEM) ml/min. Isolation of the hindpaw was confirmed by turning off the perfusion pump and observing residual pressure which approached small vein pressure 21 ± 0.3 (SEM) mm Hg. Since flow in the hindpaw was maintained constant, changes in perfusion pressure were equivalent to changes in vascular resistance.

Partial dose–response curves to intra-arterial (ia) nitroglycerin (100 μg), norepinephrine (0.1 to 40 μg), tyramine (50 to 200 μg) and angiotensin (0.1 to 1.0 μg) were obtained and the responses are expressed in terms of change in resistance (Δ pressure/flow = Δ resistance). The change in resistance was plotted against the initial resistance and the slopes, regression lines, and correlation coefficients (r) calculated (23). Similar experiments were performed with stimulation of the sciatic nerve (varying frequencies, 20 V, 2 msec duration, 2 msec delay for 15 sec) utilizing an American Electronic Instrument stimulator and Harvard standard bipolar electrodes. Initial perfusion pressures were altered by intra-arterial infusions of prostaglandin B_2 (50–3200 ng/kg/min ia), prostaglandin B_1 (50 and 200 ng/kg/min ia) and nitroglycerin (1.0 ng/kg/min ia).

Statistical analysis. Data were analyzed with regression analyses and analyses of variance (23). Means were compared with students paired or grouped t test. A level of $P < 0.05$ was chosen for statistical significance.

Results. Responses to nitroglycerin. The absolute and relative changes in resistance to intra-arterial (ia) injections of one dose of nitroglycerin (100 μg) for a representative sample of the total number of dogs are plotted as a function of the initial resistance in Fig. 1. The slope of the regression line and correlation coefficient for all 39 dogs was significant when the data were expressed as the absolute change in resistance but not when the dilator responses to nitroglycerin were expressed as a percentage change in resistance (Fig. 1). The absolute magnitude of the dilator responses

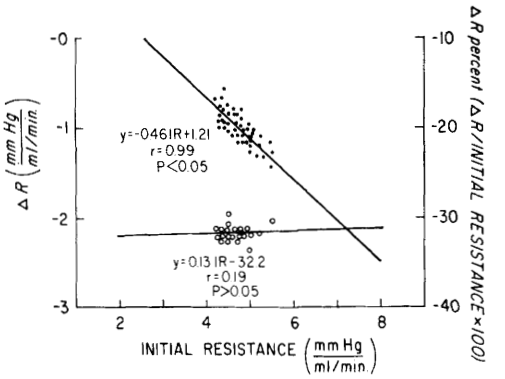

FIG. 1. Relationship between initial resistance and the magnitude of *nitroglycerin*-induced (100 μg ia) *decreases* in vascular resistance of the perfused canine paw. The ordinate represents the peak change in resistance ($\triangle R$, closed symbols) and the percentage change in resistance ($\triangle R$ percent, open symbols) after nitroglycerin. The abscissa represents the initial resistance (IR) in normally innervated canine hindpaws when initial perfusion pressure was set to match systemic pressure. The slope, regression line and correlation coefficient (r) represent the responses from 39 animals. The individual points are the data from a representative sample. The lower line has fewer points to avoid overlap. Note the significant regression and correlation between $\triangle R$ and IR but the absence of any significant correlation between percentage $\triangle R$ and IR.

to nitroglycerin was dependent on the level of initial vascular resistance but not when the data were expressed as the percentage change from the initial vascular resistance.

Responses to constrictor stimuli. The absolute changes in resistance to ia injections of 2 dose levels of norepinephrine in a representative sample of the total number of dogs are plotted as a function of the initial resistance in Fig. 2 (left panel). The slopes of the regression lines and correlation coefficients for all 153 dogs were nonsignificant over the resistances normally observed in this preparation. Similar findings were observed with pressor responses to tyramine (Fig. 2).

Effects of increases or decreases in resistance on responses to constrictor stimuli. Initial vascular resistances normally present in the perfused paw preparation were found to have no influence on the absolute magnitude of agonist-induced increases in vascular resistance. Therefore, we evaluated the effect of prostaglandin B_2 and norepinephrine-induced increases in vascular resistance on the pressor responses to ia

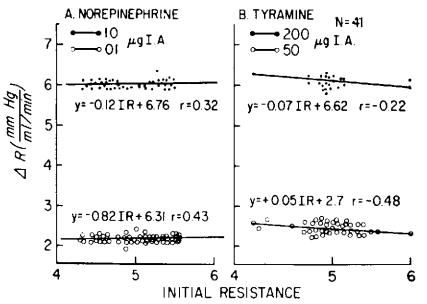

FIG. 2. Relationship between initial resistance and the magnitude of *constrictor*-induced *increases* in vascular resistance in the perfused canine paw preparation. The ordinate represents the peak change in resistance ($\triangle R$) after each agonist. The abscissa represents the initial resistance (IR) in normally innervated canine hindpaws when initial perfusion pressure was set to match mean systemic pressure. Each point represents one response. The slope and regression line represent the responses to NE and tyramine in 153 and 41 animals, respectively The individual points are the data from representative samples. *r* is correlation coefficient. Note the *absence* of any significant regression or correlation between $\triangle R$ and IR.

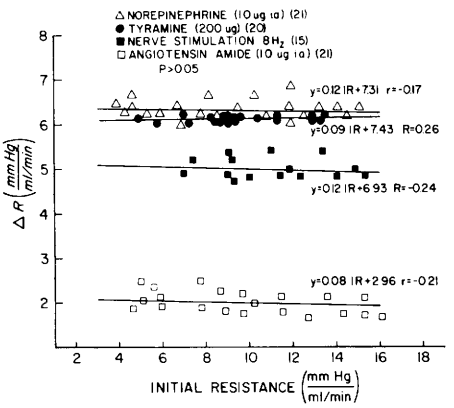

FIG 3. Relationship between initial resistance and the magnitude of *constrictor*-induced *increases* in vascular resistances in the normally innervated perfused canine paw preparation. The ordinate represents the peak change in resistance ($\triangle R$) after nerve stimulation or each agonist. The abscissa represents the initial resistance (IR) in normally innervated canine hindpaws when initial resistance is decreased by ia prostaglandin B_1 (50 and 100 μg/min) or increased by ia norepinephrine (1–10 μg/min) or prostaglandin B_2 (50–3200 μg/min). The slope and regression line represent the responses from the number of animals as shown in parentheses. Note the *absence* of any significant regression or correlation between $\triangle R$ and IR.

tyramine, norepinephrine, nerve stimulation and angiotensin. The data are summarized in Fig. 3. It is quite clear that when initial vascular resistance is increased by intra-arterial infusions of prostaglandin B_2 (norepinephrine and tyramine only) or increased by intra-arterial infusions of prostaglandin B_2 (norepinephrine and tyramine only) or increased by intra-arterial infusions of norepinephrine (all agonists), the absolute magnitude of changes in vascular resistance induced by tyramine, norepinephrine, sympathetic nerve stimulation and angiotensin is unaltered.

Intra-arterial injection of 40 μg of norepinephrine produced approximately a 300 mm Hg rise in perfusion pressure (287 ± 15 mm Hg; SEM). Intra-arterial infusions of prostaglandin B_2 and B_1 do not affect the pressor responses of the perfused hindpaw to norepinephrine (20). We, therefore, increased perfusion pressure to 400–500

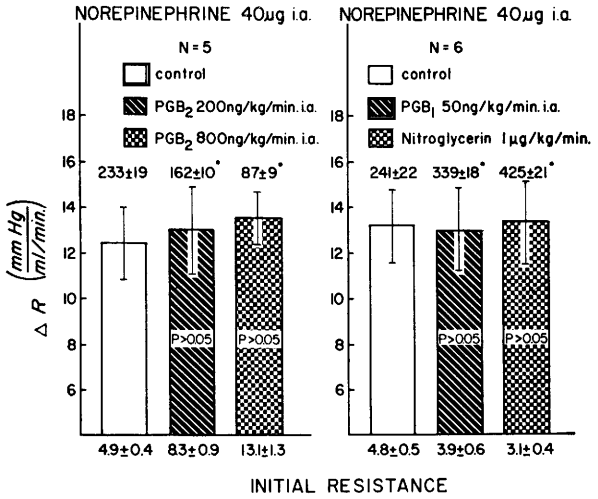

FIG. 4. Relationship between initial resistance and the magnitude of *constrictor*-induced *increases* in vascular resistances in the perfused canine paw preparation. The bars represent the peak changes in resistance ($\triangle R$) after norepinephrine. The value on the top of the bar represents the change in response to NE expressed as a percentage of the initial resistance. The abscissa represents the initial resistance (IR) in normally innervated canine hindpaws when initial resistance is increased by ia infusions of prostaglandin B_2 (left panel) or decreased by ia infusions of PGB_1 (right panel) or nitroglycerin (1.0 µg/kg/min ia). (*) The percentage change in resistance differs significantly ($P < 0.05$) from control despite the obvious similarity of the magnitude of the responses.

mm Hg with graded infusion rates of PGB_2 and administered ia injections of NE. It is quite clear from Fig. 4 (left panel) that the absolute magnitude of NE-induced increases in resistance remains constant despite increases in initial vascular resistance. A typical record of this experiment is illustrated in Fig. 5. When initial vascular resistances were decreased by ia infusions of prostaglandin B_1 and nitroglycerin, the pressor responses to ia norepinephrine did not significantly differ from control responses (Fig. 4, right panel).

Discussion. The data presented here clearly demonstrate that nitroglycerin-induced vasodilation is directly proportional to the initial vascular resistance of the blood perfused canine hindpaw under conditions of constant flow (Fig. 1). This finding is in agreement with those of other investigators utilizing perfused preparations and the intact animal for measurement of responses to dilator substances (2–13). Nitroglycerin relaxes arteriolar smooth muscle (24). The greater the degree of initial smooth muscle shortening the greater will be the capability of the muscle to dilate. However, the relative (%) decrease in resistance appears to

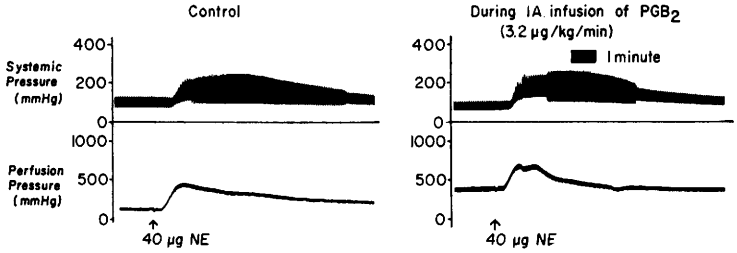

FIG. 5. A typical record illustrating the lack of effect of PGB_2-induced increases in vascular resistance on the pressor response to norepinephrine (40 µg ia). Top: systemic pressure; bottom: perfusion pressure.

remain constant over a wide range of initial resistances. Therefore, in agreement with many investigators, we conclude that dilator responses of the canine perfused paw preparation must be expressed as a percentage change from base line pressure or resistance.

Large changes in perfusion pressure or resistance may spontaneously occur as a result of changes in the level of anesthesia, drug-induced vasoconstriction or other pharmacologic interventions. Studies performed to test the LIV in skeletal and isolated cutaneous arteries demonstrated augmentation of agonist-induced vasoconstriction at low initial vascular resistances and a diminution at high initial vascular resistances (2–13). The data presented in Figs. 2–5 clearly demonstrate that the actual changes in resistance produced by vasoconstrictor stimuli in the perfused canine hindpaw are independent of the initial vascular resistance. If the LIV is applied to these responses then an inverse relationship between response and initial vascular resistance must exist since the former remains constant and the latter increases. However, in this instance, the data would be normalized to fit the LIV rather than invocation of the LIV to explain the data. This makes interpretation of the data tenuous at best and may lead to erroneous conclusions.

Since the actual increase in resistance obtained to vasoconstrictor stimuli in the canine perfused paw preparation is independent of the level of initial vascular resistance, normalization of data to account for the changes in baseline resistance would lead, in this preparation, to misinformation concerning the effects of drugs on vascular responses to vasoconstrictor stimuli unless (a) the initial resistance is unaffected by the drugs or pharmacologic interventions, or (b) the direction of the change in absolute magnitude of the response and the normalized data are in agreement.

The present study cannot offer an explanation for the inapplicability of the LIV to the pressor responses of the perfused hindpaw to various vasoconstrictor stimuli. Although a larger total vascular resistance develops when initial resistances are higher, the absolute magnitude of agonist-induced increases in vascular resistance are essentially unchanged. Similar conclusions have been reached by other investigators (21, 22 and P. J. Kadowitz, personal communications) utilizing the perfused canine hindpaw preparation. The canine paw vasculature has many arteriovenous (A–V) anastomoses. Vasoconstrictor stimuli may only contact the smooth muscle in some A–V anastomoses or, more likely, only partially constrict many of them. At higher initial resistances an equivalent dose of agonist may produce the same change in resistance by further shortening of the musculature of these A–V anastomoses. It also is possible that the different vasoconstrictor stimuli may preferentially contract large or small arteries, as in the kidney (25).

The results of the present studies differ from those of Zimmerman, Gomer and Liao (26) and Kadowitz, Sweet and Brody (27) who find that sectioning of the sympathetic chain at L-4 to L-5 reduces the pressor responses to angiotensin. This procedure also reduces the pressor response to prostaglandin B_2 (28) and prostaglandin $F_{2\alpha}$ (29). The constrictor response to these agents is dependent on tonic sympathetic impulse activity to the canine paw. However, the pharmacologic interventions utilized here primarily affect smooth muscle and do not decrease vascular resistance by removal of sympathetic tone. Therefore, while our results are applicable to direct smooth vasodilator substances, they should not be extrapolated to agents which may decrease vascular resistance by interference with normal adrenergic neurotransmission processes.

The absolute magnitude of change in vascular resistance produced by vasoconstrictor substances is constant over a wide range of initial resistances. These results have direct applicability to experiments which evaluate the effect of vasoactive drugs on the responses of the perfused canine paw to norepinephrine and sympathetic nerve stimulation when constant flow perfusion is utilized. When initial vascular resistance changes as a consequence of physi-

ologic or pharmacologic interventions by smooth muscle dilator or vasoconstrictor substances, then increases or decreases in the pressor responses to bolus injections of agonists, such as norepinephrine, tyramine or angiotensin, would not be secondary to the change in initial vascular resistance but would reflect the intrinsic facilitatory or depressant action of the various physiologic or pharmacologic interventions.

Summary. The effect of initial vascular resistance on the vascular responses of the perfused canine hindpaw to nitroglycerin, norepinephrine, tyramine, sympathetic nerve stimulation and angiotensin was evaluated. Initial resistances were altered by (a) vasodilator drugs (nitroglycerin and prostaglandin B_1) and (b) background infusions of norepinephrine and prostaglandin B_2. Over a wide range of initial vascular resistances, the absolute magnitude of dilator responses of the perfused canine hindpaw to nitroglycerin is directly proportional to the level of initial vascular resistance. Over the pressure range of most physiologic and pharmacologic perfusions (80–400 mm Hg; 2.7–18.5 peripheral resistance units), the change in resistance produced by stimulation of the sympathetic nerves and intra-arterial norepinephrine, tyramine and angiotension was independent of the initial vascular resistance and the mode by which these resistances were altered. Although the larger total vascular resistances developed when initial resistances were higher, the magnitude of induced increases in vascular resistance was unchanged. If these results are extrapolated to constant flow perfusion experiments utilizing the canine hindpaw, in which the initial vascular resistance increases as a result of physiologic or pharmacologic interventions, they indicate that changes in response to a given constrictor agonist produced during drug-induced changes in vascular resistance would result from the intrinsic activity of the drug and not secondary to the change in vascular resistance.

The authors gratefully acknowledge the generous gift of prostaglandins from Drs. J. R. Week and J. E. Pike of the Upjohn Co., Kalamazoo, MI. We also express our appreciation to Dr. M. J. Brody for his helpful suggestions and critical appraisal of this manuscript.

1. Folkow, B., and Oberg, B., Acta. Phsysiol. Scand. **47**, 131 (1959).
2. Wilder, J., "Stimulus and Response: The Law of Initial Values." Bristol, Wright (1967).
3. Kendrick, J. E., Amer, J. Physiol. **208**, 1000 (1965).
4. Folkow, B., and Sivertsson, R., Life Sci. **7**, 1283 (1968).
5. Overbeck, H. W., and Johnston, R. F., Proc. Soc. Exp. Biol. Med. **142**, 1041 (1973).
6. Kendrick, J. E., and Matson, G. L., Proc. Soc. Exp. Biol Med. **142**, 1306 (1973).
7. Davis, D. L., and Dow, P., Amer. J. Physiol. **222**, 415 (1972).
8. Myers, H. A., and Honig, C. R., Amer. J. Physiol. **216**, 1429 (1969).
9. Davis, D. L., and Hammond, M. C., Amer. J. Physiol. **216**, 1292 (1969).
10. Dobrin, P. B., and Rovick, A. A., Amer. J. Physiol. **217**, 1644 (1969).
11. Hammond, M. C., Davis, D. L., and Dow, P., Amer. J. Physiol. **216**, 414 (1969).
12. Hatch, R. C., Hughes, R. W., and Bozivich, H., Amer. J. Physiol. **213**, 1515 (1967).
13. Korol, B., and Brown, M. L., Amer. J. Physiol. **213**, 112 (1967).
14. Phelan, E. L., Erytishir, M. D., and Smirk, K. B. E., Circ. Res. **10**, 817 (1962).
15. Setnikar, I., and Magistretti, M. J., Arch. Int. Pharmacodyn. Ther. **153**, 405 (1965).
16. Page, I. H., and Taylor, R. D., Amer. J. Physiol. **156**, 412 (1949).
17. Emerson, T. E., Jr., Meier, R. D., and Daugherty, R. M., Jr., Proc. Soc. Exp. Biol. Med. **142**, 1185 (1973).
18. Zimmerman, B. G., and Gomez, J., Int. J. Neuropharmacol. **4**, 185 (1965).
19. Greenberg, S., Engelbrecht, J. A., and Wilson, W. R., Proc. Soc. Exp. Biol. Med. **143**, 1008 (1973).
20. Greenberg, S., Engelbrecht, J. A., and Wilson, W. R., J. Clin. Invest. **52**, 248 (1973).
21. Kadowitz, P. J., Sweet, C. S., and Brody, M. J., J. Pharmacol. Exp. Ther. **176**, 538 (1971).
22. Kadowitz, P. J., Sweet, C. S., and Brody, M. J., J. Pharmacol. Exp. Ther. **179**, 563 (1971).
23. Steel, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).
24. Somlyo, A. P., and Somlyo, A. V., Pharmacol. Rev. **20**, 267 (1968).
25. Brody, M. J., and Fischer, H. W., Amer.

J. Physiol. **207**, 495 (1964).

26. Zimmerman, B. G., Gomer, S. K., and Liao, J. C., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **31**, 1344 (1972).

27. Kadowitz, P. J., Sweet, C. S., and Brody, M. J., J. Pharmacol. Exp. Ther. **183**, 275 (1973).

28. Greenberg, S., Engelbrecht, J. A., and Wilson, W. R., Pharmacologist **15**, 208 (1973).

29. Powell, J. R., and Brody, M. J., Pharmacologist **15**, 208 (1973).

Received Sept. 4, 1973. P.S.E.B.M., 1974, Vol. 145.