The Effects of a New Adrenergic Antagonist on the Mesenteric Circulation (35968)

DAVID G. REYNOLDS AND KENNETH G. SWAN
(Introduced by S. B. Formal)
Department of Gastroenterology, Division of Medicine, Walter Reed Army Institute of Research, Washington, D. C. 20012

The "target organ" theory of irreversible shock (1, 2) includes the premise that hypotension, regardless of etiology, triggers the release of endogenous catecholamines. These agents are vasoconstrictors in the gut. Sustained intestinal ischemia is associated with escape of enterotoxins into the systemic circulation. The hypotensive episode is made worse and a vicious cycle is established. Alpha adrenergic blockade has been advocated, therapeutically, to interrupt this sequence of events, and the drug of choice has been phenoxybenzamine (1, 3). A new alpha adrenergic antagonist, S-2 [5-(aminopentyl) amino] ethyl phosphorothioic acid (Walter Reed Compound 2823), has been reported to confer greater protection than phenoxybenzamine in experimental shock (4–7). Since phenoxybenzamine has been shown to reverse the mesenteric vasoconstrictor effects of epinephrine but not norepinephrine (8), the ability of the new adrenergic antagonist to protect the gut from the endogenous catecholamines becomes pertinent to this target organ theory.

The present study was designed to assess the alpha adrenergic receptor blocking properties of WR-2823 in the mesenteric circulation of anesthetized dogs. The effects of the four classical adrenergic amines (norepinephrine, epinephrine, isoproterenol, and phenylephrine) on mesenteric hemodynamics were compared before and after alpha adrenergic blockade with WR-2823 and beta adrenergic blockade with propranolol.

Materials and Methods. Mongrel dogs of either sex, weighing 15–25 kg, were used in these studies. Under Nembutal anesthesia, 30 mg kg^{-1}, the superior mesenteric artery was exposed through a midline abdominal incision. The transducer (In Vivo Metric Systems) of an electromagnetic blood flowmeter (Biotronex Laboratory) was placed on the artery near its origin. The flowmeter was calibrated *in vitro* and *in vivo* with whole blood. The first branch of the artery was cannulated with a polyethylene (PE-20) catheter for intra-arterial drug administration. A hydraulic occluder (9) was placed on the artery distal to this branch and used for zero blood flow determinations. The aorta and portal vein were cannulated (PE 160) via distal branches. Pressure recording catheters were attached to transducers (Statham) which were calibrated manometrically before each experiment. Resistance across the mesenteric vascular bed was calculated from the equation: (arterial—portal venous pressure)/blood flow and expressed in peripheral resistance units (PRU).

The following adrenergic agonists were employed in this study: norepinephrine bitartrate (Winthrop), epinephrine hydrochloride (Gold Leaf Pharmacal), isoproterenol hydrochloride (Winthrop), phenylephrine hydrochloride (Winthrop). The drugs were diluted in the saline so that a volume of 0.01 ml kg^{-1} of body weight, would deliver the desired dose. An equal volume of saline was used to flush the catheters following each injection.

Selection of an appropriate dose for the adrenergic agonists was based upon results of four experiments in which the effects of intra-arterial norepinephrine and epinephrine, upon mesenteric blood flow, were compared before and after treatment with WR-2823, 50 mg kg^{-1}, iv (4, 6, 7, 10). Four doses of each catecholamine ranged logarithmically from 10^{-3} to 10^{0} μg kg^{-1}. At a dose of 10^{-1} μg kg^{-1}, the two catecholamines gave the largest reproducible response without significantly

altering central hemodynamic events. This dose was accordingly selected for subsequent studies of the four agonists (mol wt range, 169–211). An additional dose of epinephrine, 1.0 μg kg^{-1}, was injected intravenously to verify that WR-2823 induced "epinephrine reversal" of arterial pressure.

In five experiments, the effects of the four adrenergic amines upon mesenteric hemodynamics were compared before and after adrenergic blockade. Adrenergic agonists were injected in the following sequence: norepinephrine, epinephrine, isoproterenol, and phenylephrine. This sequence was repeated after alpha adrenergic antagonism with WR-2823 and again after beta receptor blockade with propranolol (Ayerst Laboratories, 0.5 mg kg^{-1}, iv).

In each series of experiments sufficient time was allowed between injections for all measured parameters to return to the preinjection levels. Sixty minutes were allowed for alpha adrenergic blockade to take effect and 15 min for beta adrenergic blockade (8).

The results were expressed in terms of the mean ± standard error and their significance was assessed with the t test for paired data (11).

Results. Dose–response study. As the dose of either norepinephrine or epinephrine was increased, there was a progressive decrease in mesenteric blood flow (Fig. 1). Norepinephrine was a significantly ($p<.02$) greater vasoconstrictor than epinephrine at a dose of 10^{-1} μg kg^{-1}. Following alpha adrenergic blockade with WR-2823, the dose–response curves for each amine were shifted to the right. At all but the highest dose of agonist, the vasoconstrictor responses to each amine were significantly ($p<.01$) decreased by adrenergic blockade. WR-2823 caused epinephrine reversal of both arterial pressure and mesenteric blood flow in these experiments. Before adrenergic blockade (Fig. 1, inset) epinephrine (1.0 μg kg^{-1}, iv) increased arterial pressure 18 ± 13 mm Hg above the control value ($p<.05$). Following WR-2823 epinephrine decreased pressure 34 ± 7 mm Hg below control ($p<.02$). Intra-arterial injection of the two lowest doses of epinephrine produced small vasodilator responses following WR-2823. Reversal of flow was not seen with higher doses of epinephrine or with any dose of norepinephrine.

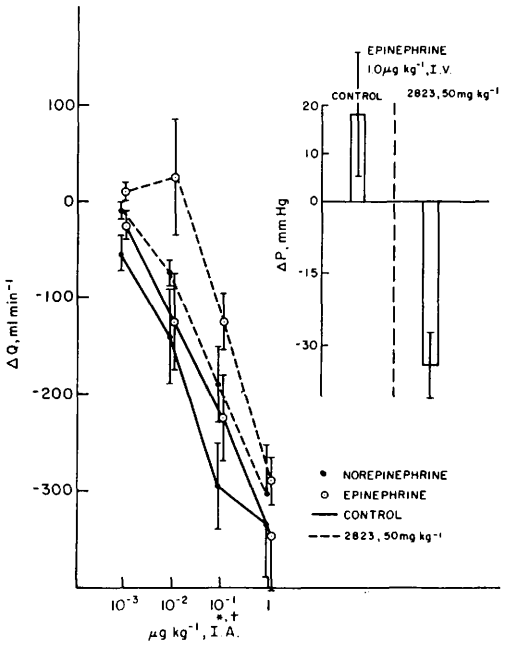

FIG. 1. Effects of norepinephrine and epinephrine on superior mesenteric artery blood flow before and after treatment with WR-2823 (50 mg kg^{-1}): (ordinate) the absolute change in blood flow; and (abscissa) drug dosage in logarithmic progression; (inset) the aortic pressure response to intravenous epinephrine before and after WR-2823 treatment; (*) norepinephrine responses significantly greater than epinephrine responses both before ($p<.02$) and after ($p<.05$) WR-2823. Each point is mean ± SE of 4 experiments in 4 animals.

Catecholamine Injection Study. During the control period mesenteric blood flow was 415 ± 53 ml min^{-1}, arterial pressure was 126 ± 5 mm Hg, and portal pressure was 6.2 ± 0.7 mm Hg. Calculated resistance was 0.29 PRU (Fig. 2). The intra-arterial injection of norepinephrine reduced blood flow to 90 ± 25 ml min^{-1} in 15 sec; blood flow then increased to 537 ± 69 ml min^{-1} at 45 sec. Epinephrine decreased blood flow from 385 ± 45 to 96 ± 23 ml min^{-1}, and this decrease in flow was followed by an increase to 490 ± 68 ml min^{-1} in the same time periods. Isoproterenol caused an initial decrease in flow (337 ± 40 to 274 ± 42 ml min^{-1}) at 10 sec following which flow increased to 615 ± 68

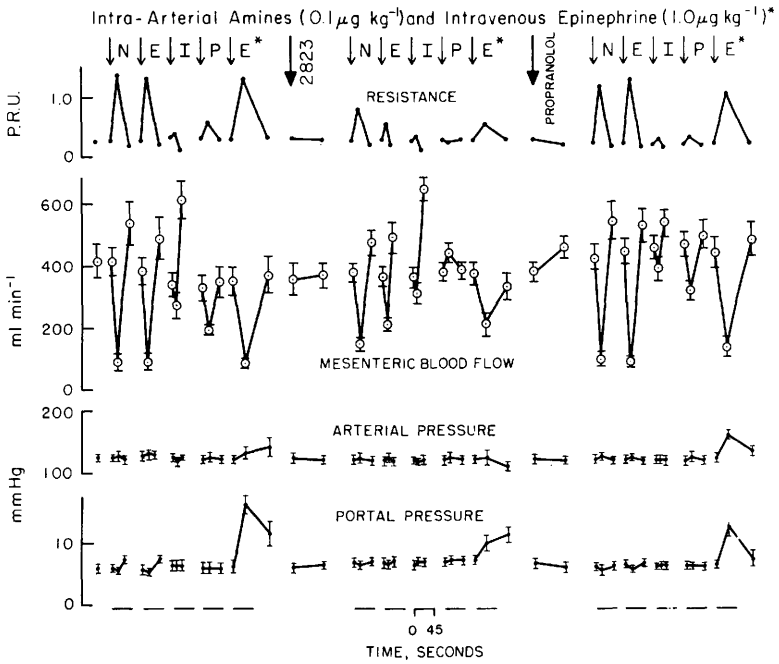

FIG. 2. Mesenteric hemodynamic responses to intra-arterial injections of norepinephrine (N), epinephrine (E), isoproterenol (I), phenylephrine (P), and intravenous injection of epinephrine (E*). Data are presented before and after alpha adrenergic (WR-2823, 50 mg kg^{-1}) and beta adrenergic (propranolol, 0.5 mg kg^{-1}) blockade. Mesenteric blood flow, arterial and portal pressure values represent the mean ± SE of 5 experiments in 5 animals. Vascular resistance was calculated.

ml min^{-1} at 20 sec. Phenylephrine injection reduced blood flow from 331 ± 38 ml min^{-1} to 198 ± 13 ml min^{-1} in 15 sec. There was no secondary dilator response.

The intra-arterial injections did not significantly affect arterial pressure; slight changes in portal pressure were observed and reflected the marked changes in blood flow. Calculated resistances thus reflected changes in blood flow. In contrast the intravenous injection of epinephrine (1.0 μg kg^{-1}) caused mesenteric vasoconstriction, as well as a significant increase in both arterial and portal pressures.

Post alpha adrenergic blockade with WR-2823. Following alpha receptor blockade with WR-2823, the vasoconstrictor response to norepinephrine was significantly ($p<.01$) less than that observed before adrenergic blockade. Blood flow decreased from 380 ± 31 ml min^{-1} to 149 ± 6 ml min^{-1} at 15 sec and then increased to 474 ± 41 ml min^{-1}. Not only was the magnitude of the epinephrine response reduced but it was shortened to approach the 10 and 20 sec time intervals characteristic of the response to isoproterenol. Epinephrine reversal of blood flow was not observed in this series of experiments. The initial vasoconstrictor phase of the response to isoproterenol was attenuated by WR-2823; potentiation of the dilator component of the response was not observed, however. The intra-arterial injection of phenylephrine, following WR-2823, evoked a slight but significant ($p<.05$) increase in blood flow (383 ± 30 to 439 ± 28 ml min^{-1}). The vasoconstrictor response which followed the intravenous injection of epinephrine was significantly ($p<.05$) less than that seen prior to WR-2823 treatment. This large dose of epinephrine reversed arterial pressure in a magnitude similar to that observed in the dose–response study (Fig. 1). The changes in arterial pressure recorded in Fig. 2 are smaller than the maximal responses observed because each point indicates a value for pressure which coincides in time with the change in flow in order to calculate accurately the simultane-

ous value for resistance.

Post alpha and beta blockade responses. Epinephrine reversal of blood pressure persisted throughout the series of injections which followed WR-2823. The final injection (epinephrine, 1.0 μg kg^{-1}, iv) caused a vasodepressor response which lowered arterial pressure significantly ($p<.02$) below the control value. Since WR-2823 has a prolonged duration of action (7), addition of propranolol, in an appropriate dose (8), should have resulted in blockade of both receptors. In this setting, norepinephrine, epinephrine, and phenylephrine, along with intravenous epinephrine, vasoconstrictor responses simulated those observed during the control period. The intra-arterial injection of isoproterenol exhibited "reversal" of blood flow. That is, blood flow was reduced from 457 $\pm$ 37 to 389 $\pm$ 40 ml min^{-1}. The apparent restoration of alpha receptor activity associated with beta receptor blockade is indicated by the calculated resistance changes which approached those observed before adrenergic blockade.

Discussion. The alpha adrenergic blocking properties of WR-2823 have been previously documented (4, 6, 7, 10). Our demonstration that WR-2823 reverses the arterial pressure response to epinephrine confirms this earlier work. These effects of the new compound on arterial pressure indicate that, as an alpha adrenergic antagonist, WR-2823 compares favorably with phenoxybenzamine. Comparison of the mesenteric circulatory effects of the two drugs indicates that the new agent confers less protection from the vasoconstrictor effect of the catecholamines, than does phenoxybenzamine. In a previous report (8), we demonstrated epinephrine reversal of canine mesenteric blood flow. The phenomenon was observed over a wide range of doses of the agonist by pretreatment with phenoxybenzamine. In the present study, this phenomenon was seen only at low doses of epinephrine and the dilator response that followed was considerably less than that seen with phenoxybenzamine. Both adrenergic antagonists attenuated the vasoconstrictor effects of norepinephrine and the degree of attenuation in the two studies was comparable.

The effects of the new agent on mesenteric circulatory responses to isoproterenol and phenylephrine are comparable to our observation with phenoxybenzamine (8). Each antagonist caused phenylephrine reversal of mesenteric blood flow and attenuated the initial vasoconstrictor effect of isoproterenol without potentiating the vasodilator effect of the agonist.

The therapeutic implications of this study concern the adrenergic theory of shock. The latter is thought to adversely effect the gut due to the vasoconstrictor properties of endogenously released catecholamines. If adrenergic antagonism ameliorates shock by protecting the gut, then phenoxybenzamine would remain the drug of choice by virtue of its greater ability to block and reverse the effects of epinephrine on the mesenteric circulation. The fact that WR-2823 confers greater protection upon the subject of experimental shock than does phenyoxybenzamine poses a paradox. If the target organ theory of shock is to be validated, then an adrenergic antagonist which best protects the gut from the catecholamines should be the one associated with the least morbidity in experimental shock. The fact that WR-2823 confers greater protection than phenoxybenzamine in experimental shock while at the same time acting as a less effective antagonist to the mesenteric vasoconstrictor action of norepinephrine and epinephrine suggests two possible alternatives: either the target organ theory of irreversibility in shock is inaccurate or else the locus of action of the new compound in protecting the experimental shock model is other than the gut. Recent findings from this laboratory suggest the former alternative is the more likely. Intra-arterial infusions of the endogenous catecholamines into the canine gut are followed by a very brief period of vasoconstriction. Autoregulatory escape from relatively high doses of catecholamines occurs within minutes and restores blood flow to near control levels.

Experimental septic shock in the subhuman primate has been shown in our own laboratory, as well as others (12) to be unaccompanied by changes in mesenteric blood flow despite severe hypotension and death.

Experimental hemorrhagic shock in the same preparation is associated with hypotension of equal magnitude and marked mesenteric vasoconstriction despite plasma catecholamine concentrations that are less than those seen in endotoxin shock. Neither shock model in the primate is accompanied by irreversible changes in the gut as opposed to the findings of hemorrhage and necrosis in the canine model.

Summary. The new alpha adrenergic antagonist, WR-2823, causes epinephrine reversal of blood pressure in the anesthetized dog. The drug attenuates but does not reverse the mesenteric vasoconstrictor effects of norepinephrine and epinephrine. WR-2823 does cause phenylephrine reversal of mesenteric blood flow. Addition of beta adrenergic receptor blockade restores the responses of the mesenteric circulation to all four adrenergic agonists to that observed prior to treatment with WR-2823.

1. Texter, E. C., Chou, C. C., Merrill, L. S., Laureta, H. C., and Frolich, E. D., J. Lab. Clin. Med. **64**, 624 (1964).

2. Lillehei, R. C., Dietzman, R. H., and Movsas, S., Gastroenterology **52**, 468 (1967).

3. Nickerson, M., *in* "Drug Therapy of Shock," Shock: Pathogenesis and Therapy. (K. D. Block, ed.), p. 356. Springer-Verlag, Berlin (1962).

4. Vick, J. A., Pharmacologist **12**, 284 (1970).

5. Vick, J. A., Ciuchta, H. P., and Manthei, J. H., J. Pharmacol. Exp. Ther. **150**, 382 (1965).

6. Vick, J. A., Heiffer, M. H., Webster, W. H., and Jacobus, D. P., Physiologist **12**, 383 (1969).

7. Demaree, G. E., Frost, J. S., and Heiffer, M. H., Proc. Soc. Exp. Biol. Med. **136**, 1332 (1971).

8. Swan, K. G., and Reynolds, D. G., Amer. J. Physiol. **220**, 1779 (1971).

9. Jacobson, E. D., and Swan, K. G., J. Appl. Physiol. **21**, 1400 (1966).

10. Heiffer, M. H., Herman, E. H., Vick, J. A., Demaree, G. E., Mundy, R. L., Reynolds, D. G., and Jacobus, D. F., Fed. Prod., Fed. Amer. Soc. Exp. Biol. **28**, 611 (1969).

11. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed., Iowa State Univ. Press, Ames (1967).

12. Brobmann, G. F., Ulano, H. J., Hinshaw, L. B., and Jacobson, E. D., Amer. J. Physiol. **219**, 1464 (1970).

Received May 24, 1971. P.S.E.B.M., 1971, Vol. 138.