Effect of Adrenergic Blocking Agents on Response of Rabbit Arterial and Venous Strips to Catecholamines[1] (34704)

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The effect of catecholamines on isolated aortic strips has been well characterized by Furchgott and Bhadrakom (1). In addition, Furchgott (2) showed that the *alpha*-adrenergic blocking agent, Dibenamine, was able to block the constrictor responses to epinephrine (E), norepinephrine (NE), and isoproterenol (ISO). This observation indicates that *alpha*-receptors are involved in the constrictor action of the three catecholamines. Although Wurzel *et al.* (3) have shown that dichloroisoproterenol inhibits the constrictor effect of the three catecholamines, the evidence of Pruss *et al.* (4) indicates that this inhibition is not specific for *beta*-receptors.

Since the effects of catecholamines and the antagonism of these effects by adrenergic blocking agents have not been systematically studied on isolated vein strips, such a study forms the basis of this report. Aortic strips have been included for comparison.

Materials and Methods. Male, colored rabbits, of unknown breed, weighing 2–3 kg, were killed by the rapid iv injection of 5 ml of a pentobarbital sodium solution containing 35 mg/ml. The thoracic aorta and abdominal vena cava were removed and helically-cut strips were prepared as described by Furchgott and Bhadrakom (1). All strips were 2.0–2.5 cm in length and 2.0–4.0 mm in width prior to mounting. Strips were mounted in 50-ml Craver-Anderson isolated organ baths and maintained at 37° by water circulated in the outer chamber. Tension was measured by a force-displacement transducer (Grass FT.03) and recorded on a Grass Polygraph (Model 5). An initial tension of 4 g was placed on the aortic strips and 1 g on the vena caval strips. The strips were suspended in Krebs–bicarbonate solution containing glucose, in a concentration of 2 g/liter, and continually gassed with a mixture of 95% oxygen–5% CO_2.

Drug solutions were prepared daily using 0.9% NaCl solution for all dilutions. The volume of drug solution added to the tissue bath was 0.5 ml.

Concentration–response data were obtained by tenfold increases in the concentration of drugs in the bath. After attainment of peak effect, drugs were washed out of the bath by an overflow method and the tension was allowed to return to the base line. This required approximately 5 min for low doses and up to 1 hr for high doses. The number of vascular strips used for each concentration studied is indicated over each bar of Fig. 1.

Experiments with catecholamine antagonists were done as follows: phenoxybenzamine was added to the bath (final concentration of 0.01 μg/ml), washed out 30 min later, and then the concentration–response curve for a particular catecholamine was determined. Pronethalol, however, was present in the bath (final concentration 0.1 μg/ml) before and during the determination of the catecholamine concentration–response curve.

The concentrations of the following drugs are expressed as the free base: *l*-norepinephrine bitartrate monohydrate, *l*-epinephrine bitartrate and *dl*-isoproterenol HCl. The concentrations of phenoxybenzamine HCl[3] and

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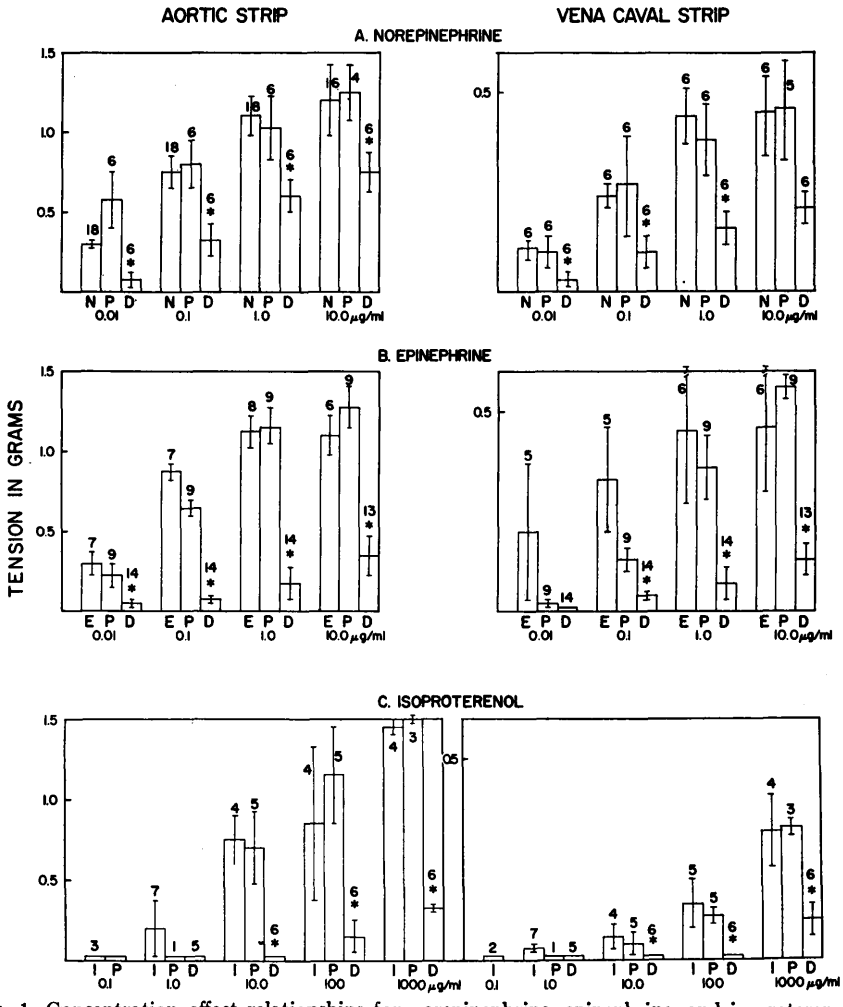

FIG. 1. Concentration–effect relationships for norepinephrine, epinephrine, and isoproterenol. The graphs on the left illustrate contractile responses in rabbit aortic strips while those on the right show contractile responses in vena caval strips. Note the change in tension scale between aortic and vena caval strips; (N) norepinephrine; (E) epinephrine; (I) isoproterenol; (P) pronethalol in a concentration of 0.1 μg/ml; and (D) phenoxybenzamine in a concentration of 0.01 μg/ml. The "I" on each bar represents ±1 SE; (*) the difference from the control bar (N, E, or I) is significant at the 0.05 level; the numbers over each bar indicate the number of vascular strips used for each concentration.

pronethalol HCl are expressed as the salts.

Results. A. The effect of catecholamines. In aortic strips, E and NE were nearly equipotent over a concentration range of 0.01 to 10 μg/ml. On the other hand, tension was not developed in the presence of ISO until a concentration of 1 μg/ml was reached and appeared to be still rising at a concentration of 1000 μg/ml. Although strict quantitative comparisons cannot be made because of the incomplete concentration–effect curves, NE and E appeared to be about 100-fold more potent than ISO.

A similar situation existed in the case of vena caval strips. NE and E were equipotent while ISO appeared 3 or 4 log units less potent. These data are illustrated in Fig. 1.

B. Effect of blocking agents on the response to catecholamines. Norepinephrine. A concentration range of 0.01 to 10 μg/ml was studied in both aortic and vena caval strips. A clear maximum of tension development was

not reached with the highest concentration in aortic strips but maximal effects were reached in vena caval strips at a dose of 1 μg/ml. In the presence of pronethalol, 0.1 μg/ml, the relationship between concentration of NE and tension was not altered in either type of strip. After exposure to phenoxybenzamine, 0.01 μg/ml, the responses to all concentrations of NE were reduced in the aortic strips and to all but the highest concentration in the vena caval strips. These results are illustrated in Fig. 1A.

Epinephrine. The results with E were similar to those obtained with NE and are illustrated in Fig. 1B. Concentrations of 0.01 to 10.0 μg/ml produced increasing tension in the aortic strips. Pronethalol did not alter the concentration–effect curves. Preexposure to phenoxybenzamine, however, significantly reduced the tension produced by each concentration of E in the aortic strips and by all but the lowest concentration in the vena caval strips.

Isoproterenol. Over a concentration range of 1 to 1000 μg/ml, ISO produced increased tension in both aortic and vena caval strips though the degree of tension increase differed. This is illustrated in Fig. 1C. Pronethalol did not significantly alter the relationship between concentration and tension in either type of strip. After exposure to phenoxybenzamine, the responses to the three highest doses of ISO were significantly reduced in both the aortic and vena caval strips.

Discussion. The concentration–effect relationships found in aortic strips in this series of experiments are in good agreement with those of Furchgott and Bhadrakom (1). E and NE produced maximal effects at 1.0 and 10.0 μg/ml, while the maximal effect of ISO was not attained until a concentration of 1000 μg/ml was reached.

The concentration–effect relations for E and NE were almost identical in aortic and vena caval strips, whereas the latter appeared less sensitive to ISO than the aortic strip.

Furchgott (2) and Fleisch and Maling (5) have reported a relaxing effect of sympathomimetic amines on aortic strips in the presence of drug induced increase in tone or *alpha*-adrenergic blockade. The latter investigators have shown that the relaxation is blocked by propranolol. In our experiments, the tone of the strips was low, therefore, this *beta*-inhibitory effect was not seen.

On the basis of the results with blocking agents, the receptors involved in the contractile effect of catecholamines on arterial and venous strips may be classified as *alpha*-adrenergic receptors: the contractions produced by all three catecholamines were significantly reduced by the *alpha*-adrenergic blocking agent, phenoxybenzamine and were not significantly nor uniformly affected by the *beta*-adrenergic blocking agent, pronethalol.

The lack of effect of pronethalol raises the question of whether the dose was too low for effective *beta*-adrenergic blockade in this tissue. The dose of pronethalol of 0.1 μg/ml was chosen from earlier investigations which indicated that the concentrations 0.01 to 0.1 μg/ml were sufficient to reduce the response of E to 50% in a number of organs showing beta effects, *e.g.* papillary muscle, tracheal chains, and rectal caecum while a concentration of 10 μg/ml was ineffective in organs showing alpha effects such as the rabbit uterus or the perfused rabbit ear [see (6) for references]. Takagi, *et al.* (7), using rabbit aortic strips found that pronethalol in a concentration of 1 μg/ml did not block NE-induced contractions while 3 μg/ml did reduce the response. There is some question of the specificity of this latter response which cannot be answered at this time.

The results with ISO on isolated vena caval strips are in sharp contrast to those of Kaiser *et al.* (8) in the intact dog. In their preparation, in which changes in vascular volume were measured, the venoconstrictor effect of ISO was not blocked by phenoxybenzamine but was reduced by pronethalol. This discrepancy may be the result of the different species used or of different sections of the venous system studied.

Summary. Epinephrine (E), norepinephrine (NE), and isoproterenol (ISO), contract isolated strips of rabbit aorta and vena cava. ISO is less potent than either E or NE. In all cases, the contractions are considerably

reduced by phenoxybenzamine and not significantly altered by pronethalol. It is concluded that the contractile response to the three catecholamines is initiated by *alpha*-adrenergic receptors in venous as well as arterial strips.

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