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Effect of (±) dihydroxy ephedrine and (±) dihydroxy pseudoephedrine on adrenergic transmission in mesenteric arteries

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Summary

1. The effect of (±) dihydroxy ephedrine (DHE), (±) dihydroxy pseudoephedrine (DHPE) and isoprenaline on the vasoconstrictor responses of the perfused mesenteric arteries of the rat produced by stimulation of their periarterial sympathetic fibres, and in the responses to injected noradrenaline, has been studied.

2. DHE and DHPE inhibited the responses to nerve stimulation and increased the responses to injected noradrenaline.

3. Isoprenaline slightly decreased the basal perfusion pressure and reduced the responses to both nerve stimulation and injected noradrenaline. Since these effects of isoprenaline were abolished by propranolol, they are presumably mediated by β-receptors.

4. The inhibitory effect of DHE and DHPE on the responses to nerve stimulation was not abolished by propranolol.

5. The results suggest that DHE and DHPE impair adrenergic transmission by partially replacing the adrenergic neurotransmitter, thereby acting as a false neurotransmitter.

Introduction

Dihydroxy ephedrine (α-methyladrenaline) which has been detected as a metabolite of α-methyldopa (Muscholl, 1966b) produced a fall of blood pressure (Schaumann, 1931) presumably due to stimulation of vascular β-receptors (Ahlquist, 1948). (±) Dihydroxy ephedrine (DHE) and its diastereomer, (±) dihydroxy pseudoephedrine (DHPE), also produced a transitory fall in blood pressure of the anaesthetized cat and pithed rat which was abolished by a β-adrenoceptor blocking agent, pronethalol (Muscholl & Sprenger, 1966). Large doses of DHE stimulate α-adrenoceptors and raise blood pressure, especially after blockade of the β-adrenoceptors (Muscholl & Sprenger, 1966). Earlier experiments on the perfused mesenteric arteries of the rat (Malik & Muscholl, 1969) demonstrated that DHE increased the perfusion pressure whereas DHPE was ineffective in altering the basal pressure. Infusion of DHE and DHPE in small concentrations, which had no effect on basal perfusion pressure, reduced the vasoconstrictor response produced by stimulation of periarterial nerves.

The present study was undertaken to determine whether the inhibitory action of DHE and DHPE on the vasoconstrictor responses of mesenteric arteries to nerve stimulation is mediated through stimulation of β-receptors or by some other mechanism such as by false transmitter function (Day & Rand, 1963). In order to elucidate the inhibitory action of these substances on the responses to nerve stimulation, the actions of isoprenaline and propranolol have also been examined.

Methods

Female albino rats, weighing 250–300 g, were anaesthetized with ether. The superior mesenteric artery was cannulated and isolated with its small resistance vessels according to McGregor (1965). The cannulated main artery was perfused with Tyrode's solution gassed with 95% O_2+5% CO_2 by means of a Harvard peristaltic pump (Model 1210) at a constant rate of 25 ml/min as described earlier (Malik & Ling, 1969). The temperature of the perfusion fluid was maintained at 22° C. In a few experiments the temperature was 37° C as will be described. At low temperatures the response to nerve stimulation remained uniform for a much longer period. The perfusion pressure was recorded manometrically on a kymograph using a frontal writing lever. The pressure in the cannula before its placement in the artery at a flow of 25 ml/min was 60 mmHg (1 mmHg≡1·333 mbar). During perfusion of the vessels at the same rate, the pressure was 85 mmHg. Thus, the basal pressure in these experiments was 25 mmHg. Since the mesenteric vessels were cut along the intestinal wall, this pressure represents the resistance of mesenteric arterioles.

The periarterial nerves were stimulated by placing a bipolar electrode on the artery, using biphasic rectangular pulses (20 V ; 1 ms ; 7 Hz) for periods of 20–25 s at 4 min intervals with a Grass stimulator (Model S4). Noradrenaline in a volume not exceeding 0·05 ml was injected directly into the cannula leading to the superior mesenteric artery.

All other drugs were added to Tyrode's solution in a reservoir identical to that containing the control solution. The perfusion solutions were changed by clamping the tube from one reservoir and simultaneously opening that from the other.

Drugs

(−) Noradrenaline bitartrate monohydrate (K & K Laboratories), (±) dihydroxy ephedrine hydrochloride, (±) dihydroxy pseudoephedrine hydrochloride and (±) isoprenaline hydrochloride were generously provided by C. H. Boehringer Sohn Ingelheim. Propranolol was kindly donated by Ayerst Laboratories (New York).

All drugs were freshly dissolved just before use ; the required concentration for perfusion was obtained by adding 0·5 ml drug solution to 1 l. of Tyrode solution. The final concentrations of these substances refer to the salts.

Results

Effect of (±) dihydroxy ephedrine (DHE) on the responses to sympathetic nerve stimulation and injected noradrenaline (NA)

The infusion of DHE at concentrations of 0·1–0·3 μg/ml for 24 min, which had no effect on the basal perfusion pressure, often increased the initial one

or two responses to periarterial nerve stimulation. This was followed by a gradual inhibition of the responses to below control levels. Perfusion with drug-free Tyrode solution restored the responses to their control height (Fig. 1A). When the infusion of DHE was repeated in the same preparation more than 2–3 times, there was no inhibition of the responses to nerve stimulation, but rather a potentiation of the responses during the infusion of DHE. After stopping the infusion of DHE, the responses were diminished below the initial control levels, followed by gradual increase towards control height. Figure 1B illustrates this effect of DHE when infused a third time in the same preparation. These effects of DHE were consistently observed in six preparations and could also be obtained at 37° C (two experiments).

The infusion of DHE, 0·1–0·3 μg/ml, produced a gradual increase in responses to injected NA (2–3 μg). After stopping the infusion of DHE the responses were restored to their control levels (Fig. 2). This effect of DHE could be repeated as many as five times in the same preparation. It was also observed in those preparations in which the responses of equal magnitude to nerve stimulation were inhibited. The effect of DHE, that is, increased response to injected NA, was observed in four preparations. DHE also increased the response to injected NA at 37° C (two experiments) slightly less than at 22° C.

Effect of (±) dihydroxy pseudoephedrine (DHPE) on the responses to sympathetic nerve stimulation and injected noradrenaline

Infusion of DHPE at concentrations of 0·1–0·3 μg/ml for 20 min inhibited the responses to nerve stimulation. The responses were restored to their control levels when the infusion of DHPE was stopped (Fig. 3). The inhibitory effect of DHPE could be repeated as many as five times in the same preparation (five experiments).

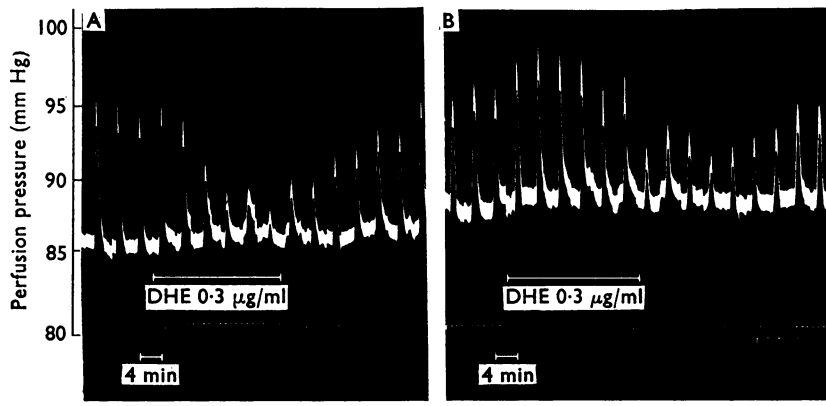

FIG. 1. Effect of (±) dihydroxy ephedrine (DHE) on the perfusion pressure responses of rat mesenteric arteries to sympathetic nerve stimulation. The mesenteric arteries were perfused with Tyrode's solution at a rate of 25 ml/min at 22° C. The periarterial nerves were stimulated using biphasic rectangular pulses (20 V; 1 ms; at 7 Hz) every 4 min for 20 seconds. After recording three control responses, the infusion of DHE, 0·3 μg/ml, gradually reduced the responses to nerve stimulation (A). The responses were restored to their control levels after stopping the infusion of DHE. The repeated infusion of DHE more than twice in the same preparation enhanced the responses to stimulation during infusion of DHE (B). When the infusion of DHE was stopped, the responses decreased for some time below the control heights and then gradually increased towards control levels.

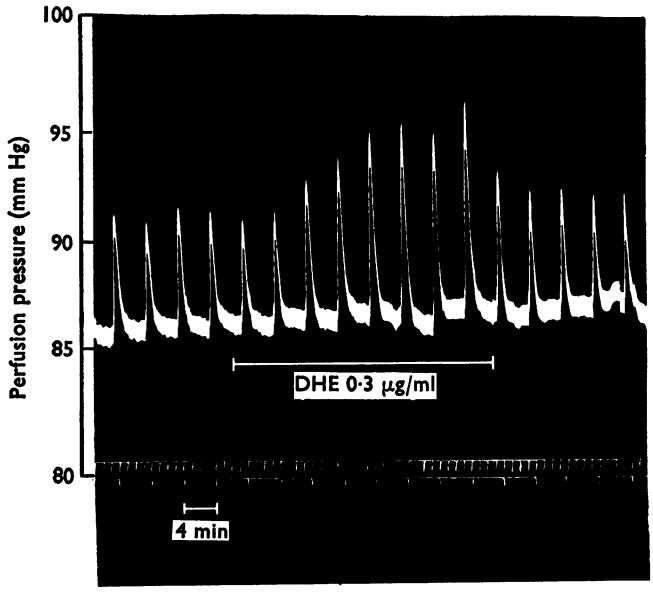

FIG. 2. Effect of (±) dihydroxy ephedrine (DHE) on the responses to injected noradrenaline. The responses to noradrenaline (2 μg) were produced by injecting this amine directly into the arterial cannula every 4 minutes. The infusion of DHE, 0·3 μg/ml, increased the responses to injected noradrenaline which returned to their control heights when the perfusion with drug-free Tyrode solution was resumed.

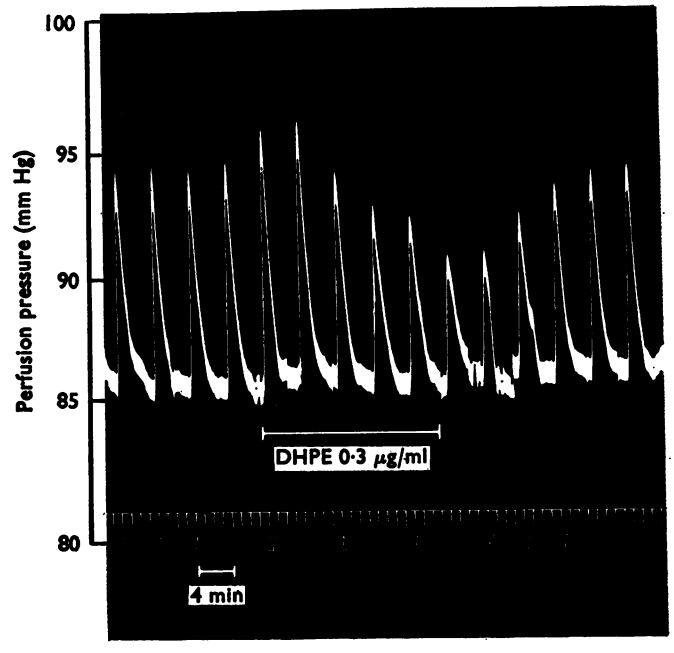

FIG. 3. Effect of (±) dihydroxy pseudoephedrine (DHPE) on the responses to sympathetic nerve stimulation. Recordings as in Fig. 1. DHPE, 0·3 μg/ml, reduced the responses to stimulation. The responses were restored after the infusion of DHPE was stopped.

Infusion of DHPE, 0·1–0·3 μg/ml, enhanced the responses to injected NA. When the infusion of DHPE was changed to perfusion with normal Tyrode solution, the responses returned gradually to their initial control levels. This effect of DHPE is demonstrated in Fig. 4 and could be repeated as many as five times in the same preparation (four experiments).

Effect of isoprenaline on the responses to sympathetic nerve stimulation and injected noradrenaline and their modification by propranolol

Isoprenaline, 0·3 μg/ml, infused for periods of 16–20 min, produced a slight decrease in the basal perfusion pressure and reduced the responses to both sympathetic nerve stimulation (four experiments) and injected NA (five experiments). The effect of isoprenaline on responses to sympathetic nerve stimulation and injected NA is shown in Fig. 5A and 5B, respectively. Propranolol (0·2 μg/ml) produced either no effect or slightly increased the responses to both nerve stimulation and injected NA. When the preparation was perfused with a solution containing propranolol the inhibitory effects of isoprenaline on the responses to nerve stimulation (four experiments) and to injected NA (five experiments) were abolished (Fig. 5A and 5B). Similar results were obtained in three additional experiments at 37° C.

Effect of propranolol on the inhibitory action of (±) dihydroxy ephedrine and (±) dihydroxy pseudoephedrine

Propranolol (0·2 μg/ml) failed to prevent the inhibitory action of DHE (0·3 μg/ml) in five experiments and DHPE (0·3 μg/ml) in three experiments. Figure 6 (A and B) illustrates the inability of propranolol to prevent the inhibitory action of DHE and DHPE on responses to sympathetic nerve stimulation. In two experi-

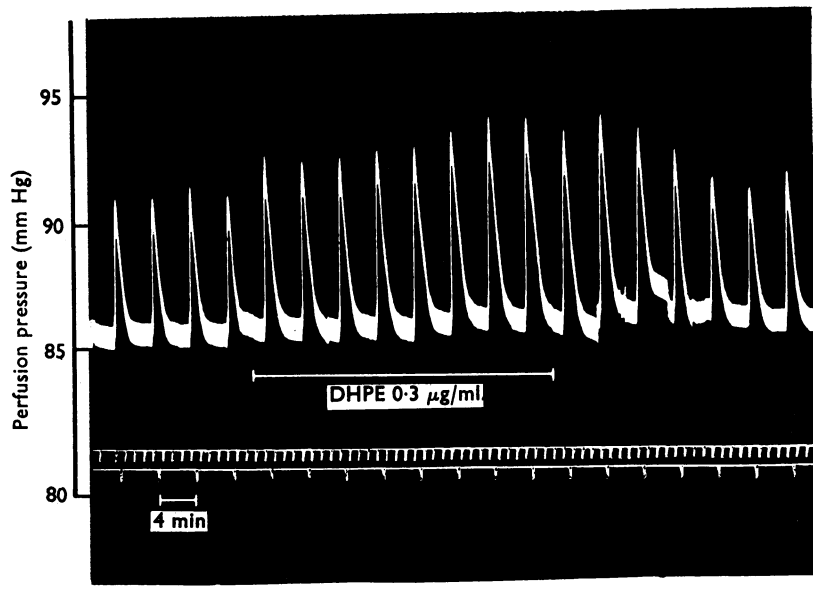

FIG. 4. Effect of (±) dihydroxy pseudoephedrine (DHPE) on responses to injected noradrenaline (2 μg every 4 min). Recordings as in Fig. 2. DHPE, when infused at concentrations of 0·3 μg/ml increased the responses to injected noradrenaline. After stopping the infusion of DHPE, the responses were gradually restored.

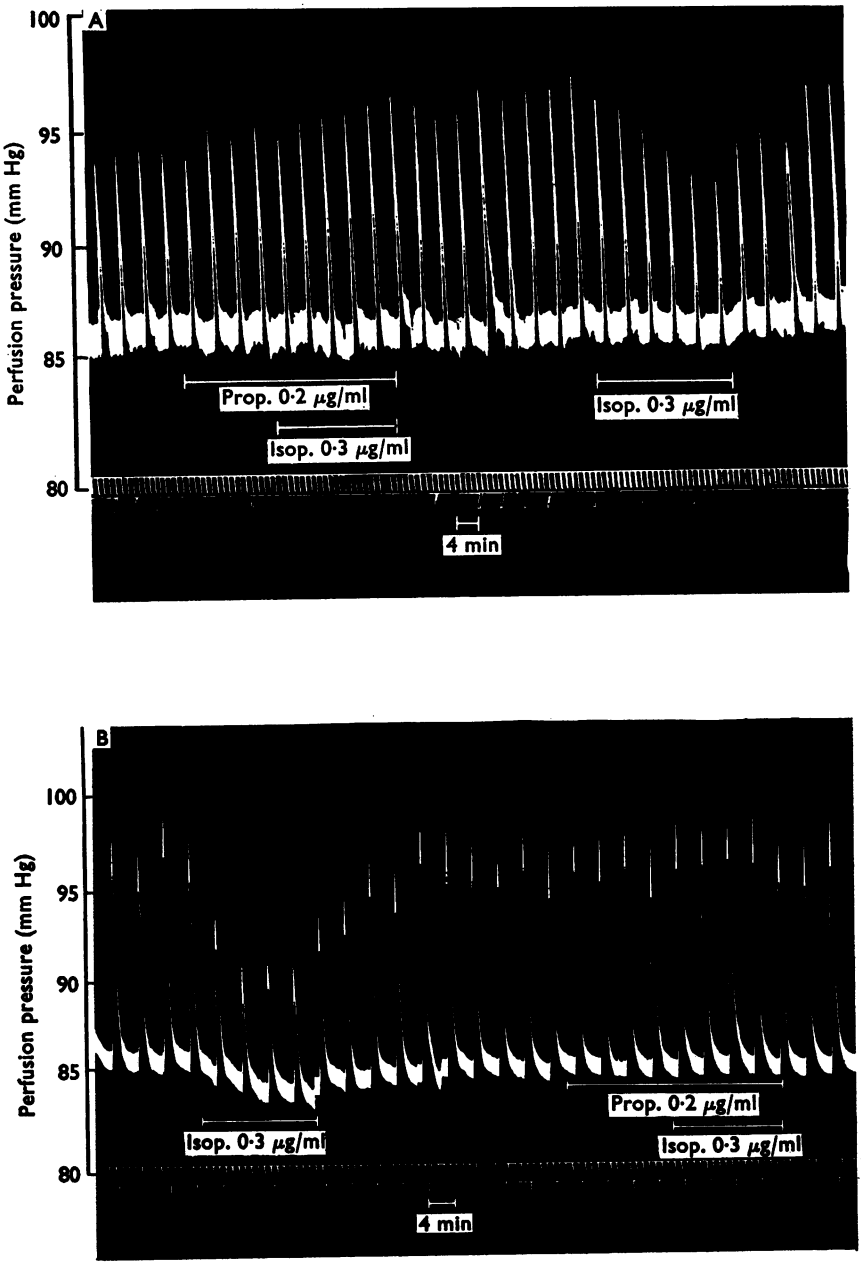

FIG. 5. Reversal by propranolol of the inhibitory action of isoprenaline on responses to sympathetic nerve stimulation (A) and to injected noradrenaline (B). A, Recordings as in Fig. 1. The infusion of isoprenaline (Isop.), 0·3 μg/ml, in the presence of propranolol (Prop.), 0·2 μg/ml, did not alter the responses to nerve stimulation, whereas the infusion of isoprenaline in the absence of propranolol reduced the responses to stimulation. Perfusion with drug-free Tyrode solution restored the responses to their control levels. B, Responses to noradrenaline (3 μg) were obtained by injecting a solution of this amine directly into the arterial cannula. The infusion of isoprenaline (Isop.), 0·3 μg/ml, slightly decreased the basal perfusion pressure and reduced the responses to injected noradrenaline; however, in the presence of propranolol (Prop.), 0·2 μg/ml, this effect of isoprenaline was abolished.

ments at 37° C, DHE and DHPE also inhibited the responses to sympathetic nerve stimulation in the presence of propranolol.

Discussion

Stimulation of the periarterial nerves supplying the mesenteric vessels releases the adrenergic transmitter, thereby producing vasoconstriction. Thus, the effect of nerve stimulation is inhibited by adrenergic blocking agents, guanethidine, bretylium or pretreatment of animals with reserpine. Since the vasoconstrictor effect of periarterial nerve stimulation is unaffected by ganglionic blocking agents, the effect of electrical stimulation is apparently mediated by excitation of postganglionic sympathetic fibres (McGregor, 1965 ; Rogers, Atkinson & Long, 1966). The infusion of DHE and DHPE, 0·1–0·3 μg/ml, which were without effect on basal pressures, produced an increase of the first few responses to nerve stimulation followed by gradual inhibition. This inhibition could be the consequence of diminished release of NA from the sympathetic nerve endings or of the effect of DHE and DHPE on the adrenergic receptor sites. Since the infusion of DHE and DHPE increased the responses to injected NA it appears that the inhibitory effect of DHE and DHPE on the responses to nerve stimulation is due to diminished release of adrenergic transmitter. These results are in agreement with those of Lindmar, Muscholl & Sprenger (1967) who infused DHE and DHPE into rabbits and found that the frequency-response curves of the isolated heart to sympathetic nerve stimulation were shifted to the right, thus indicating sympathetic transmission failure.

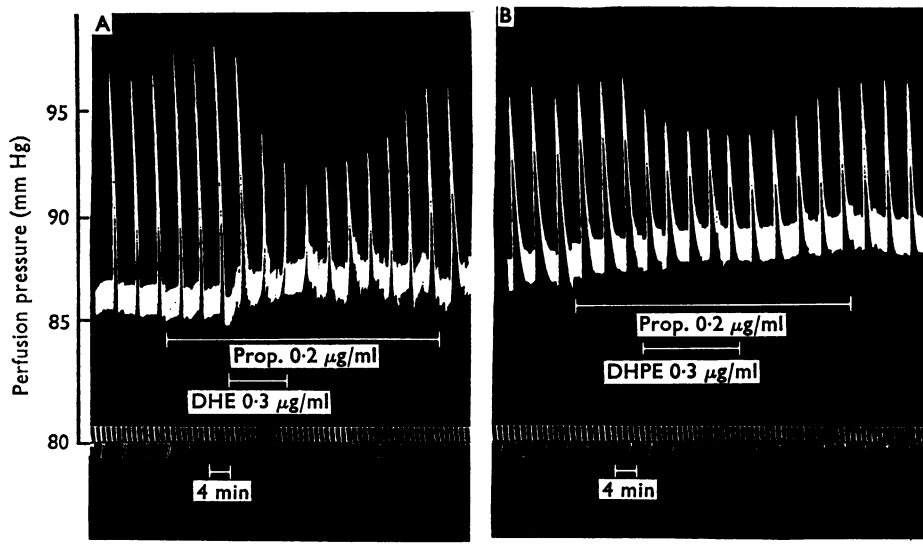

FIG. 6. Effect of propranolol on the inhibitory action of (±) dihydroxy ephedrine and (±) dihydroxy pseudoephedrine on the responses to sympathetic nerve stimulation in two different preparations. Recordings as in Fig. 1. After few control responses propranolol, 0·2 μg/ml, was added to the perfusion solution and further responses to stimulation recorded. The infusion of DHE (A), 0·3 μg/ml, and DHPE (B), 0·3 μg/ml, in the presence of propranolol produced inhibition of responses to nerve stimulation. After stopping the infusion of these agents the responses to stimulation were gradually restored.

The failure of adrenergic transmission in mesenteric blood vessels after DHE and DHPE can be explained by the hypothesis of false transmitter function proposed by Carlsson & Lindqvist (1962) and Day & Rand (1963). Many sympathetic amines are taken up by adrenergic nerves partially displacing the adrenergic transmitter and are subsequently released with NA by nerve stimulation (see reviews by Muscholl, 1966a and Kopin, 1968). DHE and DHPE are also taken up by adrenergic nerves and released by nerve stimulation (Lindmar, Muscholl & Sprenger, 1967). During constant infusion, DHE and DHPE appear to be taken up by the adrenergic nerves in mesenteric blood vessels and are then released by nerve stimulation. The resulting vasoconstrictor response would be related to the proportion and the relative vasoconstrictor potencies of the amines released on stimulation. DHE having one-twelfth the vasoconstrictor activity of NA on the mesenteric vessels (Malik & Muscholl, 1969) when released by the nerve impulse would result in a smaller response. DHPE which has one-twenty-fifth the depressor activity of DHE (Muscholl & Sprenger, 1966) also reduced the responses of the mesenteric arteries to nerve stimulation.

The transitory potentiation (Fig. 1B) of the responses to nerve stimulation during subsequent infusions of DHE may result from increased uptake of DHE with loss of NA from the nerves; a part of which becomes available to the receptor sites. The decline of responses observed after stopping the infusion of DHE (Fig. 1B) is most probably due to enhanced release of DHE which had been taken up by the adrenergic nerve endings during infusion.

The inhibitory action of DHE and DHPE on the responses to nerve stimulation is unlikely to be due to their β-adrenoceptor activity since DHE and DHPE did not reduce the responses to injected NA but rather increased them. The increase in responses to injected NA produced by the infusion of DHE and DHPE seems to be due to inhibition of the NA uptake process similar to that produced by threo-corbadrine (Malik & Muscholl, 1969), dopamine and α-methyl dopamine (Tsai, Langer & Trendelenburg, 1967; Malik & Muscholl, 1969).

The mesenteric blood vessels of rat presumably contain β-receptors since isoprenaline produced a slight decrease in the basal perfusion pressure and reduced the responses both to sympathetic nerve stimulation and injected NA. The interpretation was strengthened by the abolition of these actions of isoprenaline by a β-receptor blocking agent, propranolol. In contrast, Rogers, Atkinson & Long (1966), and McNeill, Barnes, Davis & Hook (1969) could obtain no evidence for the presence of β-receptors in perfused mesenteric vessels of the dog.

In the present experiments, propranolol failed to prevent the inhibitory action of DHE and DHPE on the responses to nerve stimulation which suggests that the inhibition of adrenergic transmission is not due to their β-receptor activity but to their function as false adrenergic transmitters.

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