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# The effects of etilefrine on blood vessels in the rat tail

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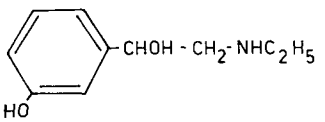
Etilefrine was found to constrict blood vessels in the rat tail through a mechanism which was partly dependent on the sympathetic nerves present in these vessels. The response to the drug was enhanced by pretreatment with noradrenaline and cocaine, and totally abolished by the α-receptor antagonist phentolamine. When compared with several other sympathomimetic agents which were tested on the vessel, etilefrine appeared to have a low order of vasoconstrictor activity. These findings would seem to have considerable relevance to the clinical situation where an attempt has been made to use etilefrine in the treatment of patients with orthostatic hypotension.

Etilefrine (I) is a sympathomimetic agent which has been used in the treatment of patients with orthostatic hypotension. The beneficial effect of drug therapy in these cases has been attributed to the similar action etilefrine has to noradrenaline (Miller, Wiener & Bloomfield, 1973). Mellander (1966) demonstrated that etilefrine had 'a local constrictor effect which was produced by α-receptor engagement' and also a 'distant' dilator effect which was mediated via the sympathetic nervous system. He further showed that etilefrine caused a pattern of response which was quantitatively similar to that of noradrenaline.

I 


An important consideration in the selection of a sympathomimetic agent to treat orthostatic hypotension is the ability the drug has to exert a direct constrictor effect on vascular smooth muscle. Parks, Sandison & others (1961), in reviewing the drug therapy of postural hypotension, showed that in patients with autonomic degeneration the indirectly acting sympathomimetics, ephedrine and methylamphetamine, had no constrictor effect on denervated blood vessels. With etilefrine, therefore, it is important to know to what extent the drug relies on the sympathetic nerves for its constrictor action. In the present study an attempt has been made to determine the mechanism of action of etilefrine on a sympathetically innervated vascular bed, i.e. a perfused rat tail segment, and to compare the effects of the drug with other sympathomimetics on the same preparation.

The isolated perfused rat tail is a vascular resistance model which is easy to set up and is sensitive to vasoactive substances with reproducible dose response curves (Wade & Beilin, 1970). The ventral caudal artery of the rat has a rich sympathetic nerve plexus at the medial-adventitial border (Hodge & Robinson, 1972) and constricts readily to noradrenaline, adrenaline, 5-HT, vasopressin and sympathetic nerve stimulation (Nicholas, 1969; Wade & Beilin, 1970). However, Wade & Beilin (1970) found that during prolonged perfusion the isolated tail became oedematous, causing a consequent rise in resting perfusion pressure. To overcome this problem, in our study isolated rat tail segments were used.

## METHODS

Male Sprague-Dawley rats (250–350 g) were stunned and exsanguinated. The tail was then severed from the trunk and its proximal end divided into two 3 cm segments. The ventral artery was identified and a fine polythene cannula inserted into its proximal end. The cannulated segments were placed in a warming chamber at 37° and perfused at a constant rate (4–5 ml $min^{-1}$) with Krebs bicarbonate solution containing EDTA (4·5 μg $ml^{-1}$). Furchgott (1955) has reported that concentrations of EDTA of this order of magnitude neither inhibit nor potentiate the effects of adrenaline and related compounds on vascular smooth muscle.

The artery was allowed to equilibrate for 30 min before drugs were administered and the recorded

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resting perfusion pressure was approximately 30 mm Hg. Drugs were always added to the perfusion fluid and administered intraluminally to the vessel until a maximum response was obtained. The concentrations of drug are therefore expressed per ml of perfusion fluid.

The following series of drug applications were carried out:

(1) Increasing doses of adrenaline, noradrenaline, phenylephrine, metaraminol and etilefrine were applied to the artery and dose-response curves constructed in a manner described by de la Lande, Glover & Head (1967). The relative constrictor activity of the drugs was determined by constructing a mean dose-response curve for each agent and from it calculating the appropriate dose producing pressure responses of 50, 100, 150 and 200 mm Hg. A mean was then obtained from the four available measurements and the shift to the right of the adrenaline curve expressed as a ratio of 1 : mean shift (i.e. a reciprocal was obtained).

(2) Etilefrine and noradrenaline were administered to the tail artery segments from rats which had been pre-treated with guanethidine (25 mg $kg^{-1}$ intraperitoneally on alternate days for a period of 7 weeks).

(3) The effects of etilefrine (1 $\mu$g $ml^{-1}$) and noradrenaline (25 ng $ml^{-1}$) infusions were examined on tail segments from rats which had been pre-treated with reserpine. The latter was injected intraperitoneally at a dose of 2·5 mg $kg^{-1}$, 24 h before the experiment.

(4) The effect of the $\alpha$-receptor blocking drug phentolamine on the vasoconstrictor response of etilefrine, phenylephrine, noradrenaline and adrenaline was examined. The doses of these agents were selected so as to give equivalent vasoconstriction on the rat tail segments and the phentolamine (500 ng $ml^{-1}$) was administered for 10 min before and also during the testing of these drugs.

(5) The response to a 1 $\mu$g $ml^{-1}$ infusion of etilefrine was determined before and after a 5 min infusion of each of the following drugs: (a) noradrenaline (25 ng $ml^{-1}$), (b) tyramine (200 $\mu$g $ml^{-1}$), (c) cocaine (10 $\mu$g $ml^{-1}$). In this series etilefrine was also administered to a control artery segment with the same time sequence as that used in (a), (b) and (c) so as to monitor any spontaneous changes in vascular sensitivity which may have occurred during the experiment.

(6) The response to a 1 $\mu$g $ml^{-1}$ infusion of etilefrine was determined in artery segments from rats which had been pretreated with 6-OH dopamine. The 6-OH dopamine was administered in a dose of 100 mg $kg^{-1}$, i.p. on day 1, and on day 2, and the arteries then tested on day 3.

At least five rat-tail artery preparations were used in each series. Mean rates were obtained for all responses and the s.e.m's calculated and included in the figures wherever appropriate.

The drugs used were: etilefrine HCl (Effortil, Boehringer Ingelheim); (−)-noradrenaline bitartrate (Koch-Light); (−)-adrenaline bitartrate (Koch-Light); cocaine hydrochloride (MacFarlan-Smith); phenylephrine HCl (Neo-synephrine, Winthrop); metaraminol bitartrate (Aramine, Merck, Sharp & Dohme); phentolamine mesylate (Regitine, Ciba); tyramine hydrochloride (Koch-Light); guanethidine sulphate (Ismelin, Ciba); 6-hydroxydopamine hydrobromide (Sigma).

The concentrations of these drugs are expressed in ng $ml^{-1}$ or $\mu$g $ml^{-1}$ of the base. All drugs were made up in ascorbic saline stock solutions.

## RESULTS

Fig. 1 shows the dose-response curves to adrenaline, noradrenaline, phenylephrine, metaraminol and

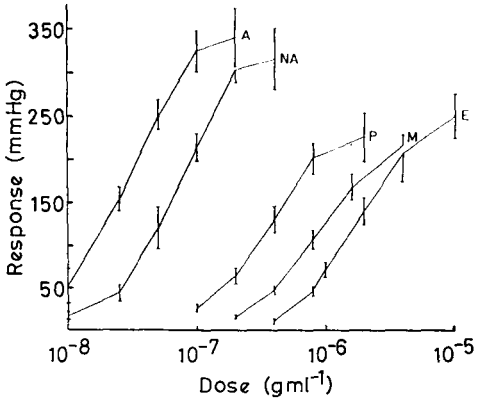


FIG. 1. Comparative dose-response curves for adrenaline (A), noradrenaline (NA), phenylephrine (P), metaraminol (M) and etilefrine (E) on the rat tail artery. Each point represents the mean ± s.e.m. of the results of at least five experiments.

etilefrine as obtained from the rat tail artery segment. Adrenaline was found to be the most active agent on the preparation and etilefrine the least active. The mean ratio of activity of the drugs compared to adrenaline was adrenaline : noradrenaline : phenylephrine : metaraminol : etilefrine, i.e. 1 : 0·38 : 0·06 : 0·02 : 0·01.

The shape of the standard etilefrine response on the rat tail artery is shown in Fig. 2 (a) and com-

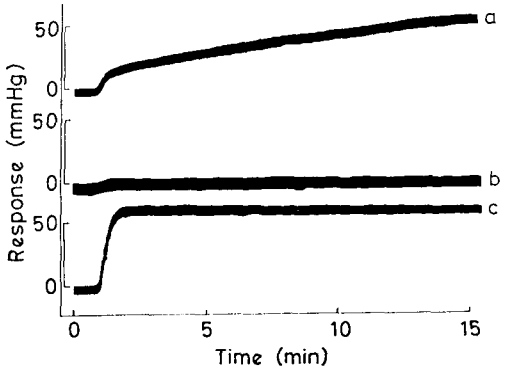


FIG. 2. The actual vascular response (mm Hg) obtained to: (a) a 1 $\mu$g ml$^{-1}$ infusion of etilefrine in an untreated artery; (b) a 1 $\mu$g ml$^{-1}$ infusion of etilefrine in an artery pre-treated with 6-OH dopamine; and (c) a 25 ng ml$^{-1}$ infusion of noradrenaline in an untreated artery.

pared with the response to the drug in a segment pre-treated with 6-OH dopamine, Fig. 2 (b), and the vascular response to noradrenaline, Fig. 2 (c). The etilefrine response demonstrated a prompt initial phase which was followed by a slower rise in pressure, till a maximum was reached in approximately 10 min. In contrast, the noradrenaline response was monophasic, with the maximum constriction being generated within a short time of the drug being applied to the preparation. 6-OH dopamine pre-treatment completely abolished the 'slow-rise' phase of the etilefrine response. The response to noradrenaline in the 6-OH dopamine pre-treated segment was within the normal range.

Fig. 3 shows the response of tail-artery segments from rats which had been chronically pre-treated with guanethidine. The vasoconstriction produced

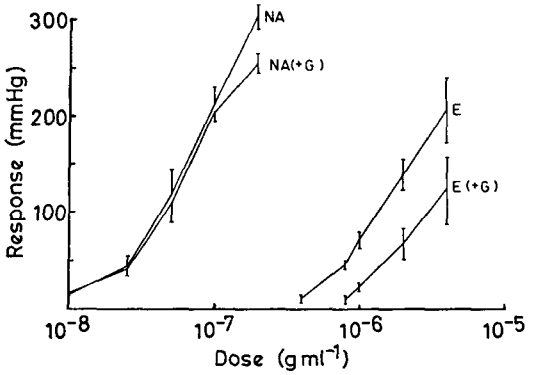


FIG. 3. Comparative dose-response curves to noradrenaline in tail artery segments of untreated rats (NA) and in rats pre-treated with guanethidine (NA + G). Comparative dose-response curves to etilefrine on the same blood vessels are also shown (E and E + G).

by noradrenaline remained unaltered, while the magnitude of the response to etilefrine was significantly reduced. Reserpine pretreatment significantly reduced the vasoconstriction produced by etilefrine on the rat tail artery segment ($P$ <0·005). The vascular response to noradrenaline was unaffected. Phentolamine, the $\alpha$-receptor antagonist, completely abolished the constrictor response to etilefrine, adrenaline, noradrenaline and phenylephrine on the rat tail artery.

Following a 25 ng ml$^{-1}$ infusion of noradrenaline for 5 min, the response to etilefrine was significantly augmented, Fig. 4 (a). Following a 200 $\mu$g ml$^{-1}$ infusion of tyramine for the same time period, the

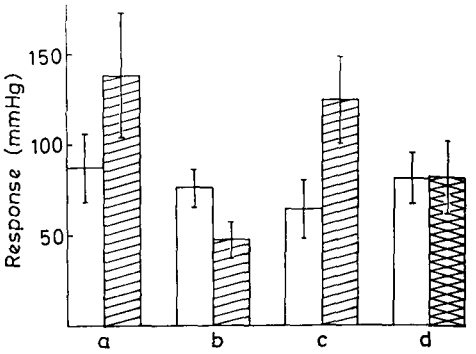


FIG. 4. The open columns represent the mean ± s.e.m. response to 1 $\mu$g ml$^{-1}$ of etilefrine before treatment with noradrenaline, tyramine and cocaine. The hatched columns show the response to 1 $\mu$g ml$^{-1}$ of etilefrine following the infusion of: (a) 25 ng ml$^{-1}$ of noradrenaline; (b) 200 $\mu$g ml$^{-1}$ of tyramine; (c) 10 $\mu$g ml$^{-1}$ of cocaine; (d) the cross-hatched column represents the response to etilefrine given to a control rat tail segment with the same time sequence as the second etilefrine dose in the treated arteries. This was to monitor any spontaneous change in vascular sensitivity with time.

etilefrine response was significantly decreased, Fig. 4 (b). Cocaine (10 $\mu$g ml$^{-1}$) enhanced the etilefrine response on the rat tail segment, Fig. 4 (c). The mean response to etilefrine in the time control remained significantly unchanged from the initial value, Fig. 4 (d). The arteries from the rats pre-treated with 6-OH dopamine showed a significant decrease in their responsiveness to a 1 $\mu$g infusion of etilefrine ($P$ <0·005).

## DISCUSSION

The results of the present investigation indicate that etilefrine possesses a significant indirect sympathomimetic action on the rat tail artery. This statement is based on several findings:

(a) Guanethidine, reserpine and 6-OH dopamine pre-treatment significantly reduced the etilefrine response. These agents are known to cause a depletion of noradrenaline from sympathetic nerve endings and would therefore tend to reduce the response of indirectly acting sympathomimetic drugs.
(b) The prior infusion of tyramine (an indirect sympathomimetic) significantly reduced the magnitude of the etilefrine response. Both these agents are presumably acting on a common pool of releasable noradrenaline and tend to modify the vascular effects caused by each other.
(c) The vascular response to etilefrine which showed a secondary 'slow rise' phase strongly suggests an indirect sympathomimetic component.

Additional supportive evidence for such a component came from the enhancement of the etilefrine response by both noradrenaline and cocaine. The former observation probably represents an augmentation of the releasable neuronal noradrenaline pool by infusion of this agent, while the latter finding suggests a block of re-uptake of noradrenaline released by etilefrine in the presence of cocaine (de la Lande, Frewin & Waterson, 1967). The complete abolition of the etilefrine response which was produced by phentolamine indicates that the vasoconstrictor effects of the drug on the rat tail artery were totally mediated by α-adrenoreceptor stimulation.

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