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The vascular changes after ephedrine tachyphylaxis

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The changes elicited after ephedrine tachyphylaxis in the dog femoral and rabbit aortic strips isolated from the untreated and ephedrine pre-treated animals have been studied. In untreated strips, ephedrine exhibited dose-dependent contractions which were blocked by phenoxybenzamine. These contractile responses to ephedrine were reduced after pre-treatment with ephedrine *in vivo*. In rabbit aortic strips previously contracted with noradrenaline or KCl, ephedrine induced dose-dependent relaxations at high concentrations, which were not affected by propranolol. These relaxation responses were likewise diminished after ephedrine. Dose-related contractile responses to noradrenaline were potentiated at low concentrations and depressed at high concentrations after ephedrine whereas those to adrenaline were inhibited over the entire agonist range. Responses to KCl were not affected. These reductions in the responses to noradrenaline and adrenaline after treatment with ephedrine *in vivo* were inhibited by increased calcium^{2+} concentration. From the results, it can be presumed that the observed changes in vascular responsiveness may be partially involved in the development of ephedrine tachyphylaxis.

Because ephedrine exhibits a substantial effect in reserpine-treated animals (Krogsgaard, 1956), it has been proposed that it owes part of its peripheral action to a direct effect on the adrenergic receptors. It is known also to have an indirect effect, i.e. releasing endogenous catecholamines. Consequently, rapidly repeated doses become less effective and tachyphylaxis develops to its peripheral actions probably as a result of the depletion of noradrenaline stores in the adrenergic nerve terminals (Goodman & Gilman, 1970). However the mechanisms involved in ephedrine tachyphylaxis, remain unresolved. We have investigated the changes elicited after the development of ephedrine tachyphylaxis in isolated blood vessels and studied the possible mechanisms involved in the tachyphylaxis.

METHODS

Mongrel dogs, 8 to 14 kg, and albino rabbits, 2·3 to 2·7 kg, of either sex were used. The dogs were anaesthetized with pentobarbitone sodium (35 mg kg^{-1}, i.p.) and the rabbits with urethane (1 g kg^{-1}, i.p.). Pretreatment with ephedrine was by successive intravenous injections of ephedrine into the femoral vein at 30 min intervals of single doses of 1 ,3, 5, 6 and 7 mg kg^{-1} repeated 5 times to give a total of 50 mg kg^{-1}.

The animals were killed by bleeding from the common carotid arteries 30 min after the last pretreatment and the relevant tissue removed and cut spirally into strips (2 cm long and 3 mm wide) which were suspended in a 20 ml muscle chamber containing Krebs-bicarbonate solution maintained at 37° and gassed with 5% CO_2 in oxygen. The strips were attached to an isometric force transducer (Nihonkohden Kogyo Co., Tokyo, Japan) and muscle tension was recorded on a polygraph. A resting tension

of 4 g was applied to the strips. The rabbit aortic strips were allowed to equilibrate for 120 min and the dog femoral strips for 180 min before experimentation. During this period the resting tension was maintained at 4 g. The Krebs-bicarbonate solution used contained NaCl 117·7, KCl 4·7, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 24·4 and dextrose 10 mM together with sodium bisulphite 0·24 mM as catecholamine antioxidant. To investigate the pH influence of calcium ion concentration on responses to drugs, a Krebs-bicarbonate solution was prepared, in which the amount of calcium^{2+} was twice that normally used.

The following drugs were used: sodium pentobarbitone (Nembutal, Abbott), (−)-noradrenaline bitartrate monohydrate (Sigma Chemical), (−)-adrenaline bitartrate (Sigma Chemical), (±)-ephedrine hydrochloride (Dainippon Pharmaceutical) and propranolol hydrochloride (Inderal, ICI). Noradrenaline, adrenaline and dopamine was dissolved in 0·01N HCl solution, and ephedrine in distilled water. These stock solutions were kept frozen and used within a week. Working solutions of desired concentration for experimental use were freshly prepared by diluting the stock solution with Krebs-bicarbonate solution before each experiment. The maximal volume of drug solution added to the muscle chamber was 0·2 ml or less with the exception of KCl which was added in volume of 0·1–1·0 ml. All concentrations in the text refer to final concentrations of free base in the muscle chamber and are expressed in terms of molarity.

To study quantitatively the effect of drugs, the technique of the cumulative dose response curve was used. In general, the responses required 7–10 min to reach maximal contractile response to ephedrine; 5 min for maximal relaxation, and 5–7 min for development of contractile responses to noradrenaline or adrenaline; 20 min for responses to KCl. To obtain relaxation responses to ephedrine, the strips were initially contracted by administration of noradrenaline $1{\cdot}25 \times 10^{-4}$ M for 10 min or of KCl 5×10^{-2} M for 20 min before addition of ephedrine. Propranolol 10^{-5} M was applied 5 min before noradrenaline or KCl.

Contractile responses in excess of the resting 4 g tension are expressed as absolute tension increases (g) and relaxation responses as per cent of the maximal possible relaxation; i.e. relaxation of the contracted muscle to the resting base line tension.

Results were compared statistically using Student's *t*-test ($P < 0{\cdot}01$ —0·05).

RESULTS

Contractile responses to ephedrine

Ephedrine applied to untreated preparations brought about dose-dependent contractions of both the rabbit aortic and dog femoral strips (Fig. 1), and the sensitivity of the strips to ephedrine was greater in the aorta. These contractions were completely inhibited by phenoxybenzamine 10^{-6} M 40 min previously.

The contractile dose-dependent responses to ephedrine were significantly inhibited in the femoral strips isolated from the ephedrine pre-treated dogs when compared with those obtained from the untreated dogs (Fig. 1).

Relaxation responses to ephedrine

In the rabbit aortic strips, noradrenaline $1{\cdot}25 \times 10^{-4}$ M and KCl 5×10^{-2} M caused near maximal contractions with increased tension response plateaux of $2{\cdot}18 \pm 0{\cdot}07$ g ($n = 5$) and $3{\cdot}06 \pm 0{\cdot}33$ g ($n = 5$) respectively.

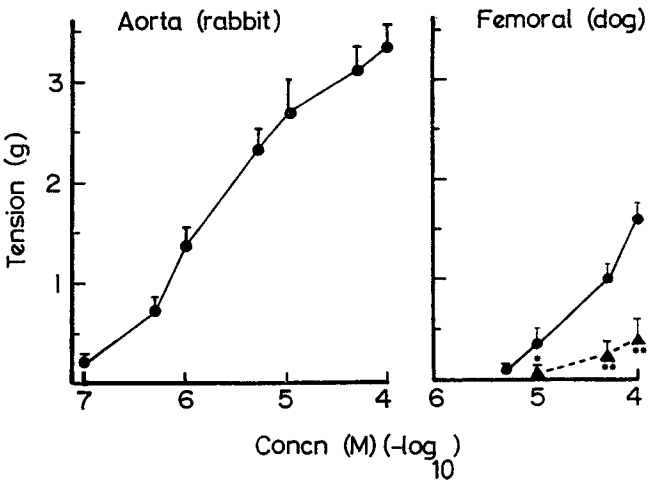

FIG. 1. Cumulative dose-dependent contractile responses to ephedrine in arterial strips isolated from the control untreated and ephedrine-treated animals. Vertical bars represent standard errors. Left panel: rabbit aortic strip, right panel: dog femoral strip.* Significant difference from the untreated ($P < 0.05$), ** $P < 0.01$. ●—● Untreated, n = 7; ▲—▲ ephedrine-treated, n = 7.

In rabbit aorta strip after noradrenaline, ephedrine 10^{-3} M induced a further slight contraction as shown in Fig. 2. Additional application of ephedrine 6×10^{-3} M resulted in an inhibition of the contraction of strips and the further additional application of ephedrine $1{\cdot}6 \times 10^{-2}$ M, 3×10^{-2} M elicited dose-dependent relaxations. When propranolol 10^{-5} M was administered 5 min before noradrenaline, the contractile responses to ephedrine appeared to be decreased and relaxation responses potentiated, but the differences in the responses were not statistically significant.

After KCl, however, ephedrine caused dose-dependent relaxations (Fig. 2) in the

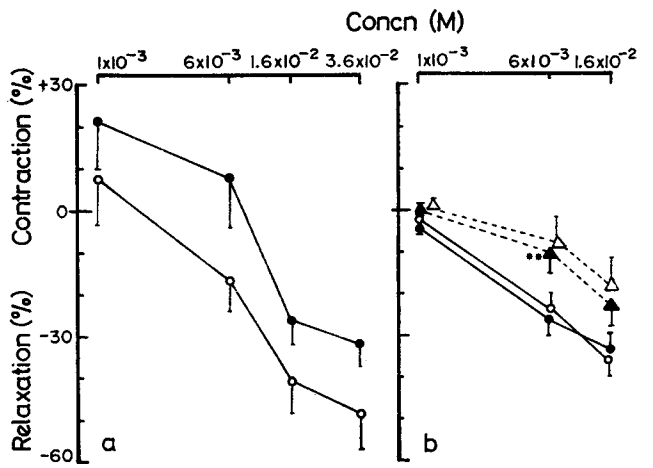

FIG. 2. Cumulative dose-response curve to ephedrine in aortic strips isolated from the control untreated or ephedrine-treated rabbits and contracted previously with noradrenaline $1{\cdot}25 \times 10^{-4}$ or KCl 5×10^{-2} and the influence of propranolol or calcium^{2+} concentration on the responses. ** Significant difference from the control untreated ($P < 0.01$). (a) Contracted with noradrenaline ($1{\cdot}25 \times 10^{-4}$ M); (b) contracted with KCl (5×10^{-2} M); ●—● untreated, n = 5; ○—○ after propranolol (1×10^{-5} M), n = 5; ▲—▲ ephedrine-treated ($1 \times$ Ca), n = 5; △—△ ephedrine-treated ($2 \times$ Ca), n = 5.

rabbit aorta. Propranolol administered 5 min before KCl did not affect the relaxation responses to ephedrine.

In the strips isolated from the ephedrine pre-treated rabbits, however, the inhibitory responses to ephedrine administered after KCl were significantly reduced compared with those obtained from the untreated rabbit (Fig. 2). When increased Ca^{2+} Krebs-bicarbonate solution was used instead of the normal Krebs-bicarbonate solution, the relaxation responses to ephedrine were not altered in the ephedrine pre-treated strips (Fig. 2).

In these series of experiments, additional administration of $NaNO_2$ 5×10^{-3} M produced a further relaxation of 13–20%; after washing, the strips responded to noradrenaline or KCl as previously.

Contractile responses to noradrenaline, adrenaline or KCl

In the dog femoral strips, noradrenaline, adrenaline and KCl produced dose-dependent contractions (Fig. 3). In the strips isolated from the ephedrine pre-treated dogs, the contractile responses to noradrenaline were significantly potentiated at low concentrations and inhibited at high concentrations compared with those obtained from the untreated dogs, whereas those to adrenaline were reduced over the entire concentration range. Those to KCl were unaffected by treatment with ephedrine (Fig. 3).

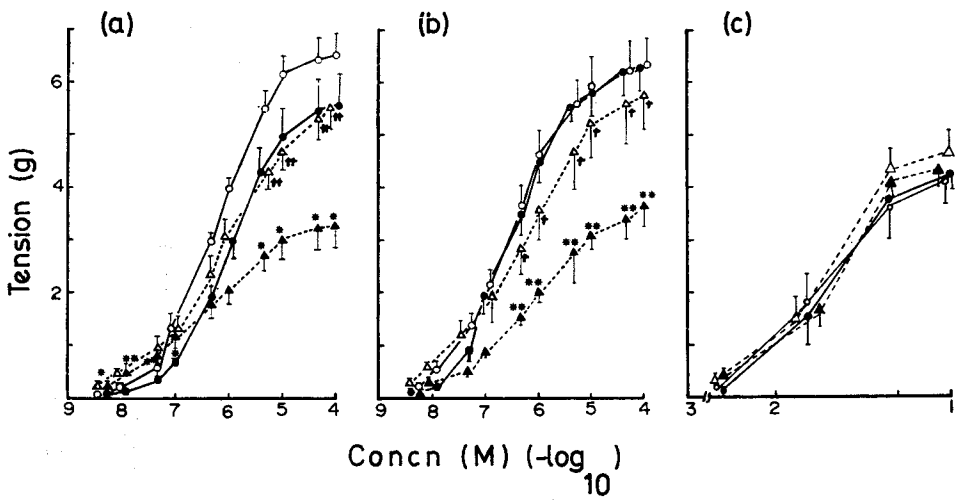

FIG. 3. Cumulative dose-dependent contractile responses to noradrenaline, adrenaline and KCl in femoral strips isolated from the control untreated and ephedrine-treated dog, and the influences of double calcium^{2+} concentration on the responses. Vertical bars represent standard errors. * Significant difference between the untreated and ephedrine-treated strips ($P < 0.05$), ** $P < 0.01$; † significant difference between the normal and twice the normal calcium^{2+} concentration ($P < 0.05$), †† $P < 0.01$. (a) Noradrenaline; (b) adrenaline; (c) KCl; ●—● untreated (1 × Ca), n = 7; ○—○ untreated (2 × Ca), n = 7; ▲—▲ ephedrine-treated (1 × Ca), n = 7; △—△ ephedrine-treated (2 × Ca), n = 7.

The contractile responses to adrenaline or KCl, in the strips isolated from the untreated dogs, were not affected significantly by using the increased Ca^{2+} Krebs-bicarbonate solution while those to noradrenaline and adrenaline, in the strips isolated from the ephedrine pre-treated dogs, were significantly potentiated and the reduced responsiveness abolished. Those to KCl were not altered by increased Ca^{2+} concentration.

DISCUSSION

As ephedrine is administered repeatedly and tachyphylaxis develops, the pressor effect becomes less effective and finally there is a depressor effect. Ephedrine is therefore thought to have both vasoconstrictive and vasodilator components in its vascular effects.

We found ephedrine exhibited dose-dependent contractions in both aortic and femoral strips, and dose-dependent relaxations in aortic strips contracted previously with noradrenaline or KCl. The former contraction is due to its α-adrenoceptor stimulating action because the effect is antagonized by phenoxybenzamine, whereas the latter relaxation is not mediated through the β-adrenoceptor stimulating effect because the effect is not significantly affected by propranolol. There is a possibility that ephedrine produces the relaxation by combining with receptors ordinarily occupied by the more potent vasoconstrictor, noradrenaline; in this respect, however, ephedrine caused the relaxation of the aortic strips contracted previously by KCl which acts through a different mode of action on the smooth muscle. It can therefore be presumed that ephedrine-induced relaxation is not due to agonist displacement between ephedrine and noradrenaline from receptor sites. It was further noticed that the contractions occurred with low concentrations of ephedrine whereas the relaxations appeared only with high concentrations. As changes in the blood pressure are integrated expressions of both pressor and depressor components, and with ephedrine the vasoconstrictive component is predominant, the drug would be expected to induce a pressor response in the normal condition but to produce a depressor response after development of tachyphylaxis, probably as a result of reduction in the pressor effect. After repeated treatment with ephedrine *in vivo*, dose-dependent contractile responses to ephedrine in the femoral strip was reduced. Consequently, this reduction in the vascular response may account for development of the blood pressure tachyphylaxis. However, dose-dependent relaxation responses to ephedrine were likewise diminished in the ephedrine pre-treated aortic strips. This result does not therefore elucidate the nature of the tachyphylaxis.

After pre-treatment of femoral strips with ephedrine, dose-dependent contractile responses to noradrenaline were potentiated at low concentrations though those to adrenaline were unaltered. Pressor effects of noradrenaline and adrenaline are reported to be potentiated after ephedrine and this phenomenon was considered to be due to depression of catecholamine uptake (Iversen, 1967; Furukawa, Yamada & Kushiku, 1970). As to tissue uptake of catecholamine, it is well known that the uptake is greater with noradrenaline than with adrenaline (Iversen, 1967). Therefore, the potentiation in the responses to noradrenaline are thought to be based on an inhibition in tissue uptake of exogenous noradrenaline after ephedrine.

The contractile responses to intermediate and high concentrations of noradrenaline and adrenaline were however equally depressed after ephedrine. Patil, Hetey & others (1970) reported that the sensitivity of the rabbit aorta to selective α-adrenergic agonist, phenylephrine, or histamine was equally reduced after treatment with ephedrine, and they considered that selective blockade of the tissue receptors and non-selective depression of the effector organ were involved in the reduction. This also seems possible in the reduction of responses to the amines brought about by ephedrine, though there is so far no evidence to show which mechanism is mainly involved.

The femoral strips isolated from either untreated or ephedrine pre-treated animals

showed no significant differences in responsiveness to KCl. Potassium ion contraction of the vascular smooth muscle has been proposed to be mediated by membrane depolarization. From these results, therefore, ephedrine does not seem able to inhibit the membrane depolarization directly.

The calcium ion is known to be essential for vascular muscle contraction (Bohr, 1964). In the untreated strips, the dose-response curve of adrenaline was not significantly influenced by increased calcium ion concentrations, however, in the ephedrine pretreated strips, the reduced contractile responses to noradrenaline and adrenaline were restored nearly to the normal responses obtained from the untreated strips. These results imply that α-adrenoceptors were not blocked by ephedrine during conditions of increased calcium ion concentrations. On the other hand, the reduction of inhibitory responses to ephedrine after ephedrine tachyphylaxis was not affected by increased calcium ion concentration. Godfraind & Kaba (1971) reported that, in the presence of adrenaline, there was an increase in both ^{45}Ca uptake and efflux. Waugh (1962) has also proposed that adrenergic neurohormones exert their vasoexcitatory contractile effect by a basically non-electrical membrane reaction which primarily triggers an increased permeability and influx of calcium into the vascular myoplasm; the consequent migration of calcium intracellularly activates the myoplasmic events of vascular smooth muscle contraction. Therefore, it might be possible that ephedrine decreases permeability of calcium caused by adrenergic agonist receptor interaction and thus reduces intracellular calcium available to the contractile apparatus when ephedrine is administered repeatedly, thereby inducing a decrease in responsiveness to the catecholamine.

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