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Potentiation of the effects of noradrenaline and of sympathetic stimulation of the perfused rat caudal artery by angiotensin

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The isolated perfused caudal artery of the rat develops tachyphyllaxis rapidly to the direct constrictor action of angiotensin but not towards the potentiation of responses to submaximally effective periarterial stimulation or to noradrenaline. These potentiations persist in the presence of maximally effective concentrations of cocaine, are unaffected by increased sodium concentration and are enhanced by raised concentrations of calcium. Thus, angiotensin potentiates the effects of sympathetic nervous activity by an influence on the role of calcium in the contractile process. Hydrochlorothiazide did not affect arterial tone, the responses of the artery, or the potentiation to noradrenaline caused by angiotensin.

The reduction in the pressor effect of injected noradrenaline which is caused in rats by the oral administration of hydrochlorothiazide precedes both the antihypertensive and the diuretic effects of this drug by 1 h, and the cardiovascular actions of hydrochlorothiazide are abolished by nephrectomy (Lockett & Nicholas, 1968). It is possible that these cardiovascular actions of hydrochlorothiazide are mediated by the renin-angiotensin system since the interaction of angiotensin with contractile responses of smooth muscle after sympathetic stimulation are well known. Angiotensin greatly increases the contraction of the vas deferens in response to stimulation of the hypogastric nerves (Benelli, Della Bella & Gandini, 1964) and this effect was attributed to increase by angiotensin of the quantity of noradrenaline released from the terminals of the postganglionic sympathetic fibres per nerve impulse. Support for this interpretation was provided by Zimmerman & Gomez, 1965; and Zimmerman & Gisslen, 1968) who worked on the responses of the cutaneous and renal vascular beds to sympathetic stimulation. Moreover, angiotensin has been shown to release catecholamines from the adrenal medulla (Renson, Barac & Bacq, 1959; Feldberg & Lewis, 1963) and to block the uptake of noradrenaline by blood vessels (Palaič & Khairallah, 1967). The present purpose has been to examine the potentiation by angiotensin of the response of the isolated caudal artery of the rat (Nicholas, 1969) to sympathetic stimulation and to exclude the possibility that hydrochlorothiazide influences this potentiation by a direct effect on the artery itself.

EXPERIMENTAL

Methods

The method used for the isolation and perfusion of the caudal arteries of the rat has been described (Nicholas, 1969). Periarterial stimulation, previously shown to activate solely postganglionic sympathetic neurons, was by platinum electrodes

placed closely adjacent to and on either side of the proximal 1 cm length of the preparation. Rectangular pulses, 1 ms in duration were delivered 2 to 10/s for 3 s, each min at 15 V from a Grass stimulator (S4K). Krebs bicarbonate solution (Umbreit, Burris & Stauffer, 1964), saturated with 5% carbon dioxide in oxygen, was used both as bath medium and perfusate. Depolarization was effected by doubling the concentration of KCl in this fluid. All drugs except hydrochlorothiazide were dissolved in Krebs bicarbonate solution. Hydrochlorothiazide was dissolved in 0·5 M NaOH and was then adjusted to pH 9·0 by addition of N HCl. Administration was either by close arterial injection into the perfusion fluid immediately before its entry into the preparation, or by solution in the perfusion fluid or by addition to the bath fluid (15 ml) surrounding the artery.

Hydrochlorothiazide was received as a gift from Merck, Sharp & Dohme. (—)-noradrenaline (Winthrop Laboratories), angiotensin II val^5 asp.-β-amide (Ciba Laboratories), Vasopressin (Parke Davis & Co. Ltd.) and cocaine hydrochloride (Macfarlane Smith Ltd.) were obtained commercially.

RESULTS

Arteries initially responded to single injections of 1 to 2 ng angiotensin II by constriction and the effects of submaximal periarterial stimulation were potentiated by the drug, Fig. 1 (upper). Whereas tachyphyllaxis developed rapidly to the

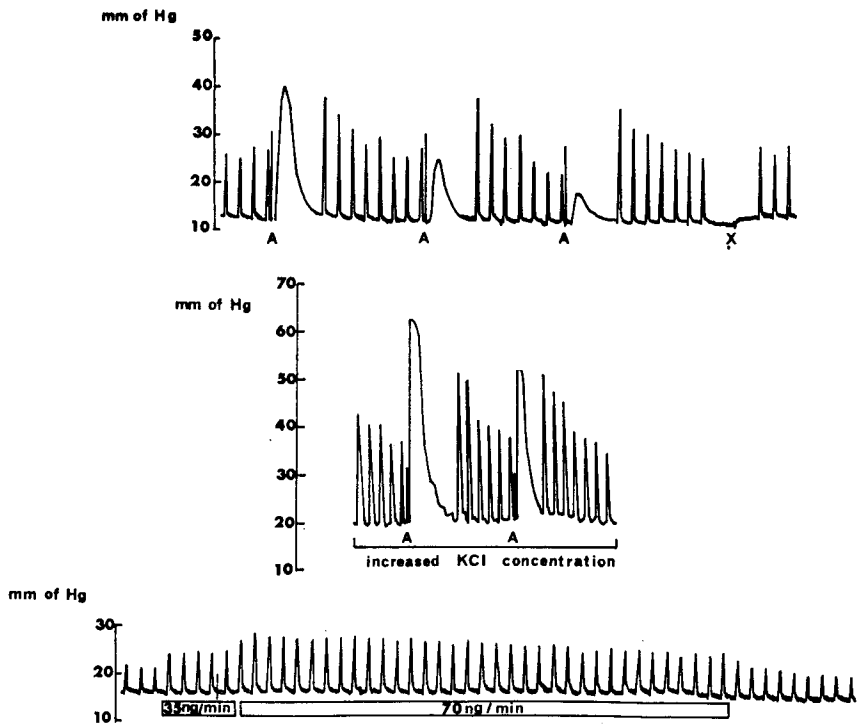

FIG. 1. Responses of perfused rat caudal artery. *Upper:* Tachyphyllaxis develops to 25 ng angiotensin II (A) but not to the potentiation of the response to periarterial stimulation which A causes. X signifies 3 min without stimulation. *Middle:* Doubling the concentration of KCl does not affect the potentiation of the response to stimulation caused by A. *Lower:* Infusions of angiotensin 32 and 70 ng/min cause sustained potentiation of the response to stimulation.

constrictor action of angiotensin, the potentiation persisted unmodified for many hours. Depolarization of the artery did not alter the effects of angiotensin on the preparations, Fig. 1 (middle). Potentiation of the response of the artery to submaxial stimulation could also be produced by infusions of angiotensin in preparations completely tachyphyllactic to the constrictor actions of the drug. Under these conditions the potentiation produced by angiotensin slowly waned; invariably, however, the stimulus response decreased abruptly when the infusion ended. Sometimes the response to periarterial stimulation appeared depressed after a long infusion, Fig. 1 (lower).

The extent of the potentiation of the arterial response to stimulation was directly related to the dose of angiotensin when stimulus rate was kept constant. The degree of the potentiation was also directly related to the frequency of stimulation when the dose of angiotensin was fixed (Fig. 2). Angiotensin still potentiated the responses of the artery to noradrenaline and to stimulation during the maximum action of cocaine (Fig. 3).

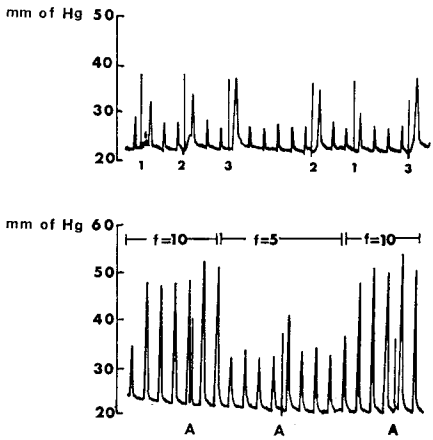

FIG. 2. Responses of a perfused rat caudal artery. *Above:* Potentiation of the response to periarterial stimulation (2/s) caused by (1) 6·25, (2) 12·5 and (3) 25·0 ng angiotensin II, respectively. *Below:* Effect of change in frequency of stimulation on the potentiation caused by 25 ng angiotensin II (A).

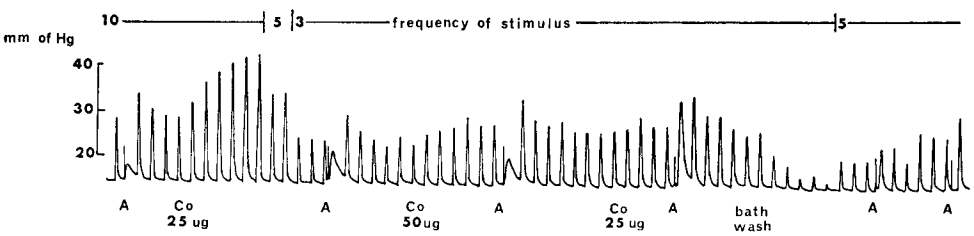

FIG. 3. Responses of a perfused rat caudal artery, showing the effect of 5 ng angiotensin II (A) on the responses to periarterial stimulation during the effect of cocaine (Co) in the bath fluid.

Infusion of isotonic calcium chloride at 1 ml/min into perfusate entering the artery at 3 ml/min increased the response of the artery to stimulation approximately threefold. When reduction in stimulus frequency during the infusion of calcium chloride

had reduced the response of the artery to match the initial pre-calcium effect, the potentiation caused by angiotensin was much greater than that initially observed. Single injections of 0·1 ml isotonic $CaCl_2$ caused transient and reproducible increases in perfusion pressure which were unaffected by injections of angiotensin. Fig. 4

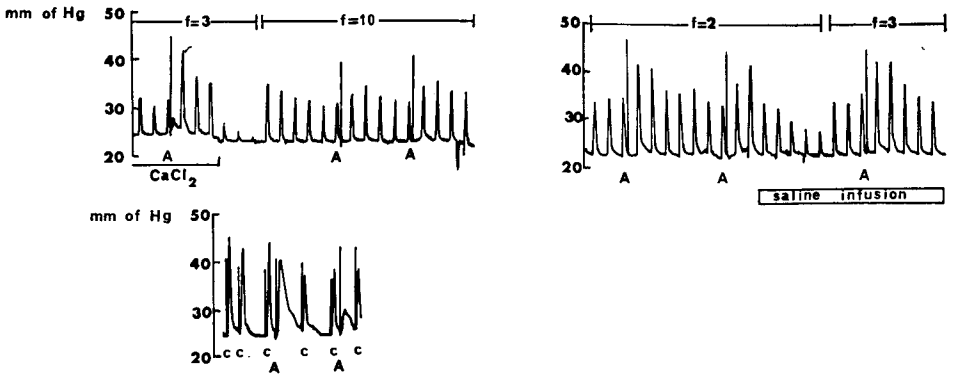

FIG. 4. Responses of a perfused rat caudal artery. *Above left:* The effect of infused isotonic $CaCl_2$, 0·2 ml/min, on the constriction caused by periarterial stimulation and its potentiation by 2·5 ng angiotensin II (A). *Below left:* The effect of injections of 5 ng angiotensin II (A) on the constrictor action of 0·1 ml injected isotonic CaCl(c.) *Above right:* the effect of doubling the NaCl concentration in the perfusate on the response to periarterial stimulation and its potentiation by 5 ng angiotensin II (A).

shows that on cessation of the infusion of calcium chloride, the augmented response to periarterial stimulation rapidly declined, as does the potentiation of the stimulus effect by angiotensin.

The maximum potentiation of the effects of periarterial stimulation attainable by single injections of angiotensin II were approximately 200 to 250% of the original responses. Corresponding figures for minor potentiations caused by noradrenaline and by vasopressin were 27 and 36% respectively.

Doubling the concentration of NaCl in the perfusion and the bath fluid depressed the response of the artery to electrical stimulation, increased the constrictor effect of angiotensin and did not affect the potentiation of the response to stimulation caused by angiotensin (Fig. 5).

Exposure of the artery to hydrochlorothiazide in the bath fluid (35 μg/ml) or in normal or high sodium perfusate (20 μg/ml) for 30 min, or both, did not alter the perfusion pressure. The responses of the artery to electrical stimulation, potentiation of these responses by angiotensin and the constrictor effects of angiotensin remained unchanged.

DISCUSSION

Interaction between angiotensin and sympathetic nervous activity is well established. McCubbin & Page (1963) demonstrated potentiation of pressor responses to tyramine, ephedrine and dimethylphenylpiperazinium by infusions of angiotensin in dogs. Although these substances act indirectly by releasing noradrenaline, the angiotensin did not potentiate the pressor effects of injected noradrenaline. Angiotensin does, however, potentiate the responses of both the vas deferens (Bennelli & others, 1964) and the renal vascular bed (Zimmerman & Gisslen, 1968) to sympathetic

stimulation. By contrast Day & Owen (1968) found that angiotensin inhibited the constrictor response of the perfused central artery of the rabbit ear to sympathetic stimulation. On the other hand, the present work demonstrates that angiotensin invariably potentiates the responses of the perfused caudal artery of the rat both to noradrenaline and to sympathetic stimulation.

Potentiation by angiotensin of the effects of sympathetic stimulation on the perfused caudal artery of the rat cannot be due to an increase in the amount of noradrenaline liberated per nerve impulse since the effects of exogenous noradrenaline are similarly increased. If potentiation by angiotensin of constrictions induced both by endogenous and by exogenous noradrenaline is to be attributed to reduction in uptake into the terminal fibrils, then the uptake component blocked has been shown to be insensitive to inhibition by cocaine. It is unlikely the potentiations are a consequence of any membrane action of angiotensin since these potentiations are demonstrable after depolarization of the vascular smooth muscle. There can be no close linkage between the mechanisms of the vasoconstrictor action of angiotensin and of the potentiation of noradrenaline vasoconstriction by this polypeptide. Whereas a rise in the sodium concentration of the bath fluid increases the effect of angiotensin on smooth muscle (Blair-West, Harding & McKenzie, 1967), potentiation of the action of noradrenaline by angiotensin is independent of sodium concentration. It it possible that angiotensin potentiates the constrictor effects of noradrenaline on vascular smooth muscle by an influence at the site of action of calcium in the contractile mechanism, since infusions of calcium chloride increase the effects of exogenous and endogenous noradrenaline.

It is of interest that hydrochlorothiazide had no effect on the tone of the caudal arterial smooth muscle, on the responses of the artery to sympathetic stimulation and to exogenous noradrenaline or on the potentiation of responses to noradrenaline caused by angiotensin. Hence the antihypertensive action of this diuretic is not attributable to an effect on any interaction between angiotensin and endogenous noradrenaline in vascular smooth muscle.

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