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The effects of angiotensin I and angiotensin II on the isolated tracheal muscle of the cat*

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The effects of Asp[1]-β-amide-Val[5]-angiotensin II (A II) and Asp[1]-Ile[5]-angiotensin I (A I) have been studied on the isolated continuously superfused cat tracheal muscle contracted by 5-hydroxytryptamine (5-HT). Both peptides have been shown to induce dose-dependent relaxation on this muscle. Similar effects have been obtained with synthetic bradykinin, prostaglandin E_2 (PGE_2), noradrenaline and histamine. The effects of bradykinin, A I and A II have been shown to be inhibited by aspirin but not by propranolol, metiamide, SC 19220 or a specific, competitive antagonist of A II. The relaxing effect of A I is not due to the conversion of decapeptide to octapeptide A II. The possible mechanism of the relaxing effects of A I and A II on the cat isolated tracheal muscle is discussed.

Some naturally occuring polypeptides, lipids and amines cause relaxation on the isolated tracheal muscle. Catecholamines are known to induce a relaxation on the cat isolated tracheal ring through the stimulation of β-adrenoceptors. Similar relaxation has been observed with synthetic bradykinin but this effect could not be blocked by β-adrenoceptor blockers (Türker & Kiran, 1965). On the contrary, bradykinin has been shown to produce a contraction on the guinea-pig isolated tracheal muscle (Collier, Holgate & others, 1960). On the other hand, prostaglandins, especially PGE_1 and PGE_2 all relax tracheal muscle (see review of Bergström, Carlson & Weeks, 1968; Türker & Khairallah, 1969). In addition, histamine has been shown to cause a relaxation on the cat tracheal muscle (Maengwyn-Davies, 1968).

The effects of A I and A II on the isolated tracheal muscle have not been studied in detail. We have previously shown that A II has no effect on the isolated tracheal rings of guinea-pigs, cats, dogs, rats and rabbits when the peptide comes in contact with the muscle at resting condition (Türker & Kiran, 1964). We have recently found that when the cat isolated tracheal muscle is contracted by 5-HT and acetylcholine, both peptides caused a relaxation. The present study deals with the mechanism of the relaxing action of A I and A II on the cat isolated tracheal muscle and to compare them with the other agonists which have similar effects.

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MATERIALS AND METHODS

Tracheae were obtained from mongrel, adult cats anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). Several rings from whole trachea were prepared according to Akcasu (1959). The tracheal rings were superfused separately by a multichannel peristaltic pump (Harvard) prepared in a manner similar to the 'blood-bathed organ technique' (Vane, 1964). The superfusion fluid was warmed (37°) and aerated (5% CO_2 in O_2) Krebs–Henseleit solution. The muscles were subjected to 2–3 g passive tension and the isometric contractions were recorded on a 4 channel Grass polygraph (Model 79 D) by means of force-displacement transducers (Grass FT .03). The superfusion flow was 5 to 8 ml min^{-1} and was kept constant throughout the experiments. The muscles were initially allowed to superfuse for 30 to 60 min with normal Krebs solution until a steady base-line in resting condition was established. Isometric contractions of the muscle were induced by 5-HT or in some experiments by acetylcholine added to the superfusion medium at 8 to 10 ng ml^{-1}. The agonists which induce relaxation on the cat tracheal muscle were injected through a rubber tubing segment placed in the channels used for superfusion. The system was regulated so that the injected amount of agonists could come in contact with the muscles in 1 min. So the final concentration of the agonists was estimated by dividing the injected amount of drugs by constant superfusion inflow per min. The volume of the injected agonists was kept constant between 0·1 to 0·2 ml. The antagonists were added to the superfusion medium at different concentrations.

The relaxation induced by different agonists was expressed as % of maximum response considering as maximum the zero base-line before producing the contraction by 5-HT. The dose-response curves were determined for bradykinin, A I and A II before and after additions of aspirin, SQ 20881 (nonapeptide: Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) and (*NN*-dimethyl) Gly^1-Ile^5-Ile^8-angiotensin II (DMGIA II) to the superfusion medium.

The results were statistically evaluated using Student's *t*-test.

The following compounds were used in this study: synthetic bradykinin (BRS-640), Asp^1-β-amide-Val^5-angiotensin II, Asp^1-Ile^5-angiotensin I, (*NN*-dimethyl) Gly^1-Ile^5-Ile^8-angiotensin II, prostaglandin E_2, (−)-noradrenaline bitartrate, histamine dihydrochloride, (−)-propranolol hydrochloride, (1-acetyl-2-(8-chloro-10,11-dihydrobenz-(b,f)(1,4)-oxazepine-10-carbonyl)-hydrazine) (SC19220), metiamide and lysine-aspirin. The concentrations of the substances used were expressed as free base.

RESULTS

Effects of bradykinin, A I and A II on the cat isolated tracheal muscle and inhibition by aspirin

Bradykinin induced a dose-dependent relaxation on the cat isolated tracheal muscle contracted by 5-HT. The minimum concentration of the peptide which induced a relaxation was found to be 10^{-9}M. Similar dose-related inhibitions of the 5-HT-contracted tracheal muscle were obtained with A I and A II. The log dose-response curves of the peptides are shown in Fig. 1. Addition of aspirin to the superfusion medium caused an inhibition in responses induced by the peptides. The curves obtained in the presence of aspirin (10^{-6}M) were shifted to the right remaining parallel to that of controls (Fig. 1). The calculated ED50 values of the peptides before and after aspirin are summarized in Table 1. However, in the same experimental conditions, aspirin (10^{-6}M) failed to inhibit the relaxing effects of PGE_2 noradrenaline and histamine. The calculated results are: 70% ± 6·0% of maximum relaxation for PGE_2 (10 ng ml^{-1}), 60·0 ± 3·0 for noradrenaline (50 ng ml^{-1}) and 70·0 ± 4·0 for histamine (100 ng ml^{-1}). These values were found to be 80·0 ± 7·0 for PGE_2, 55·0 ± 5·0 for noradrenaline and 80·0 ± 8·0 for histamine in the presence of aspirin (mean ± s.e.m. of 10 exp.).

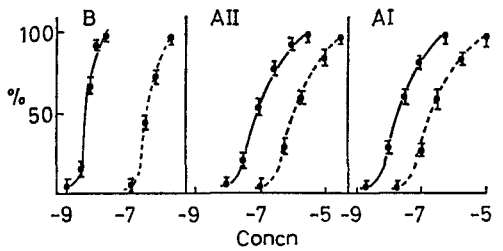

FIG. 1. The dose-response curves of bradykinin (B) angiotensin II (A II) and angiotensin I (A I) on the isolated continuously superfused tracheal muscle of the cat before and after addition of aspirin to the superfusion medium. Each point represents the mean value of 12 experiments Vertical bars indicate s.e.m. x-axis—Log molar concentrations of agonists, y-axis —% of maximum relaxation. ● ——●, control, ● - - -● in presence of aspirin (10^{-6}M).

Table 1. *The calculated ED50 values of A I, A II and bradykinin before and after addition of aspirin* (10^{-6}M) *to the superfusion medium.* (Mean ± s.e.m.)

	Before aspirin (M)	After aspirin (M)
BRS-640 (Brady.)	4·8 ± 0·2 × 10^{-9} (8)	5·0 ± 0·2 × 10^{-7} (8)
A I	2·2 ± 0·1 × 10^{-8} (10)	4·4 ± 0·6 × 10^{-7} (9)
A II	4·5 ± 0·7 × 10^{-8} (10)	8·2 ± −0·1 × 10^{-6} (9)

( ) number of experiments.

Effects of DMGIA II and SQ 20881 on the responses induced by A I and A II

The higher concentrations of DMGIA II, which is a potent and specific blocker of A II (Türker & Gündogan, 1974), failed to inhibit the relaxation induced by A II and bradykinin. It also did not inhibit the effects of the other agonists tested. SQ 20881 which is a potent inhibitor of angiotensin-converting enzyme (Engle, Schaeffer & others, 1973), also did not cause any change in responses induced by A I, A II and bradykinin as well as other agonists.

Effects of SC 19220, propranolol and metiamide on the relaxing actions of bradykinin, A I, A II, histamine, noradrenaline and PGE_2

SC 19220, which is a competitive inhibitor of prostaglandins (Sanner, 1969), when added to the superfusion medium did not alter the responses induced by PGE_2, A II (Fig. 2) and other agonists used in this study. However, propranolol (1 μg ml^{-1}) almost completely abolished the effect of noradrenaline without altering the effects of the agonists (Fig. 3). On the other hand metiamide (2 μg ml^{-1}) which is a potent blocker of histamine H_2-receptors (Black, Duncan & others, 1973), inhibited the effect of histamine without changing that of other agonists (Fig. 3).

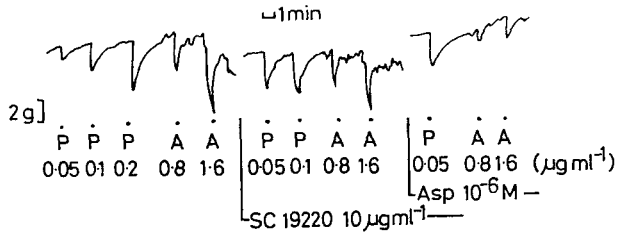

FIG. 2. A recorder tracing from isolated continuously superfused tracheal muscle of the cat. The tension increase is induced by 5-HT (10 ng ml^{-1}). The relaxation induced by PGE_2 (P) and A II (A) is not inhibited by SC 19220 added to the superfusion medium. Further addition of aspirin (Asp) to the medium inhibited the effect of A II but not PGE_2.

Similar findings were obtained with bradykinin, A I and A II when equal contraction was induced by acetylcholine on the isolated tracheal muscle.

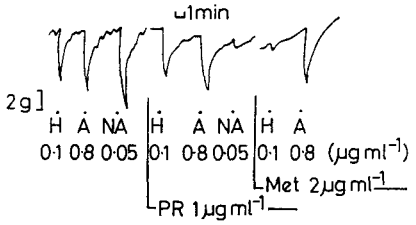

FIG. 3. A recorder tracing from the cat isolated superfused tracheal muscle contracted by 5-HT (10 ng ml^{-1}). The effects of histamine (H), angiotensin II (A) and noradrenaline (NA) before and after addition of propranolol (PR) and metiamide (Met) to the superfusion medium. Propranolol inhibits the effect of noradrenaline without altering the effects of histamine and angiotensin II. Further addition of metiamide to the medium inhibits the effect of histamine without changing the effect of angiotensin II.

DISCUSSION

The results indicate that both A I and A II cause a dose-dependent relaxation on the cat isolated trachea when the muscle is contracted by 5-HT or acetylcholine. This observation has not previously been observed by others. However, higher concentrations of A II had no effect on this muscle when it was exposed to the peptide at resting condition (Türker & Kiran, 1964). Several endogenous substances induce similar relaxation on the cat isolated tracheal muscle. We have previously shown that bradykinin produces a relaxation on the 5-HT contracted cat tracheal muscle (Türker & Kiran, 1965). The log dose-response curves of the peptides clearly show that the antagonism between aspirin and bradykinin, A II and A I is competitive since the curves were shifted to the right, but remained parallel to the controls in the presence of aspirin. However, aspirin seems to be a highly specific blocker for bradykinin rather than for A I and A II. This has been based upon the calculated ED50 values of the peptides before and after aspirin. To obtain equal relaxation, in comparison with control, the concentration of bradykinin was increased about 100 times in the presence of aspirin. However, the same dose of aspirin caused about a 10 times increase in the concentrations of A I and A II needed to produce equal relaxation. Moreover, the concentration of bradykinin which induced minimum relaxation was found to be significantly lower than that obtained with A I and A II. The antagonism between bradykinin and aspirin has previously been observed by Collier (1961) and Collier & Shorley (1963). These observations have shown that the bronchoconstrictor effect of bradykinin in the guinea-pig is competitively inhibited by aspirin. In this respect the present results are in agreement with previous observations of these authors and suggest that the relaxing effect of bradykinin on the cat isolated tracheal muscle and the bronchoconstrictor action of the peptide in the guinea-pig may be mediated through the same specific receptors. One of the interesting findings of the present investigation is that the same concentration of aspirin also inhibited the relaxing effect of angiotensin-polypeptides on the same smooth muscle. The question arises whether these two different groups of peptides act on the same receptors and/or whether the effects of the peptides are mediated through the possible release of prostaglandins. It is known that aspirin is a potent inhibitor of prostaglandin biosynthesis (Vane, 1971) besides its blocking effect of bradykinin receptors. The specific competitive inhibitor of PGE_2, SC 19220 (Sanner, 1969), was used by us to provide evidence about the role of the lipids on the relaxing effects of the peptides. PGE_1 and PGE_2 have been shown to cause a relaxation on the isolated tracheal muscle (Bergström & others, 1968; Türker & Khairallah, 1969). SC 19220 at the concentration used, did not block the effects of PGE_2, bradykinin, A I, A II and other agonists. However, this concentration can inhibit the contractile effect of PGE_2 in other smooth muscle preprations (Sanner, 1969). It is difficult to eliminate the possible contribution of endogenous prostaglandins to the relaxing effects of A I and A II in the cat isolated tracheal muscle. On the other

hand, the relaxing effect of A I and A II are not mediated through the β-adrenoceptors since propranolol inhibits the effect of noradrenaline without altering that of A I, A II, bradykinin, PGE_2 and histamine. Histamine-induced relaxation on the cat isolated tracheal muscle (Maengwyn-Davies, 1968) has been shown to be mediated through the stimulation of histamine H_2-receptors, since burimamide, a recently described H_2-receptor blocker (Black, Duncan & others, 1972) competitively inhibits this response (Eyre, 1973). Metiamide, which is another potent H_2-receptor blocker (Black & others, 1973), causes an inhibition on the relaxing effect of histamine without altering that of A I, A II, bradykinin, PGE_2 and noradrenaline according to the results of the present investigation. It is therefore obvious that the effects of A I and A II are not mediated through the stimulation of H_2-receptors.

Both A I and A II have equal agonistic activity on the cat isolated tracheal muscle. DMGIA II which is a potent competitive inhibitor of A II (Türker & Gündogan, 1974) did not inhibit the effects of the peptides on tracheal muscle. The synthetic analogues of A II which competitively inhibit the effect of the parent peptide also inhibit the pharmacological actions of A I on other smooth muscle preparations (Türker, Yamamoto & others, 1971). These findings indicate that angiotensin-induced relaxation on the cat tracheal muscle is not mediated through the specific angiotensin receptors. On the other hand, the effect of A I is not due to the conversion of the decapeptide to the octapeptide A II when it comes in contact with the tracheal muscle. This has been based upon the finding that SQ 20881, a potent inhibitor of converting enzyme (Engel & others, 1973), did not abolish the effect of A I on the cat tracheal muscle.

From the results of the present study it is concluded that both A I and A II have equal relaxing activities on the cat isolated tracheal muscle contracted by 5-HT. This effect is not mediated through the specific receptors of angiotensin. No conversion occurs when A I is superfused over this muscle. This effect is not mediated through the specific β-adrenergic or histamine H_2-receptors. Aspirin competitively inhibits, the relaxing effects of A I and A II but not as effectively as it inhibits bradykinin. It is difficult to interpret whether the inhibitory effect of aspirin against A I and A II is a receptor interaction or mediated through the inhibition of prostaglandin synthesis in the tissue.

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