The relaxing effects of angiotensin II and angiotensin III on canine isolated contracted tracheal muscle

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We have recently shown that angiotensin I and angiotensin II (A II) have equal relaxing activities on the cat isolated tracheal muscle contracted by 5-HT (Türker & Ercan, 1976). Acetylsalicylic acid (ASA), a potent prostaglandin (PG) synthetase inhibitor (Vane, 1971), reduces the relaxing effects of both peptides but not as effectively as it inhibits bradykinin. It has recently been reported that bradykinin causes a relaxation mediated through the release of PGs on the contracted airways smooth muscle (Chand & Eyre, 1977). It is also well known that the E series of PGs produce relaxation on the contracted tracheal muscles of the dog (Türker & Khairallah, 1969) and cat (Türker & Ercan, 1976). Thus it is highly possible that the relaxing effects of angiotensin peptides are mediated through the release of PGs from the tissue. On the other hand, evidence has been presented that the natural fragment of A II (Des-aspartic acid)[1]—angiotensin II, so-called angiotensin III (A III) causes a greater release of PGs than A II in the mesenteric circulation (Blumberg, Denny & others, 1976) and rat stomach fundus (Ercan & Türker, 1977).

In a recent study in this laboratory, A III and A II were found to induce a relaxation on the dog isolated tracheal muscle contracted by acetylcholine. It therefore seemed of interest to investigate whether PG-production was involved in this relaxant effect of the peptides by the potent PG-synthetase inhibitor, ASA. An attempt was also made to compare the relaxing potencies of A II and A III on the canine isolated contracted tracheal muscle.

A piece of trachea was extirpated from the pentobarbitone anaesthetized dog and the tissue was prepared in a similar way to that described previously (Türker & Ercan, 1976). The muscle strips were separately mounted in pairs and superfused with warm (37°) oxygenated (5% CO_2 in oxygen) Krebs-Henseleit solution under a resting tension of 3 g. The responses were isotonically recorded on a smoked drum by a frontal writing lever (magnification ×12). Submaximal contraction was elicited by acetylcholine added to the superfusion medium (1 ng ml^{-1}). The dose-response curves of A II and A III were determined and for each peptide ED50 values were calculated. One strip of each pair was treated with ASA (2×10^{-6} M) which did not produce an appreciable change in resting tone. After 15 min, the dose-response curves to A II and A III were determined and the response of the ASA treated muscle was compared with the control. Fig. 1 shows a recorder tracing indicating that both peptides induce a dose-related relaxation in the contracted tracheal muscle. ASA causes an inhibition in the responses to both peptides but it was found to be more pronounced for A III than A II. The calculated ED50 values of both peptides in control and ASA-treated tracheal muscles are shown in Table 1. Similar molar concentrations of both peptides induced equal responses in control experiments. However, in ASA-treated muscle the ED50 value of A III was found to be $1{\cdot}2 \times 10^{-6}$ M while it was $5{\cdot}6 \times 10^{-7}$ M for A II. The calculated ratios of ED50 values in control and ASA-treated tracheal muscles was 50 for A III and 20·7 for A II. It may be suggested from this study that the relaxing effects of A II and A III on the contracted canine tracheal muscle are mediated through the release of PGs from the tissue. Secondly A III appears to have higher agonistic activity in the trachea than A II as has been reported also for the rat stomach fundus (Ercan & Türker, 1977) and mesenteric vascular bed (Blumberg & others, 1976).

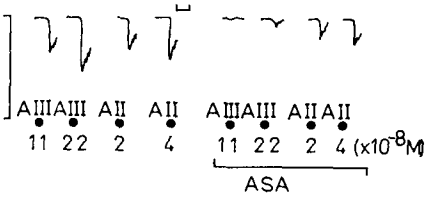

FIG. 1. A recorder tracing from dog isolated superfused tracheal muscle contracted by acetylcholine (1 ng ml^{-1}). The effects of angiotesin III (A III) and angiotensin II (A II) before and after addition of acetylsalicylic acid (ASA; 2×10^{-6} M) to the superfusion medium. Horizontal scale: 5 min; vertical scale: 5 cm.

Table 1. *ED50 values of A III and A II* (M) *in control and ASA-treated tracheal muscles (mean ± s.e.m. of 7 experiments).*

	Control[a]	ASA (2×10^{-6}M)[b]	Ratio (b/a)
A III	$2{\cdot}4 \pm 0{\cdot}8 \times 10^{-8}$	$1{\cdot}2 \pm 0{\cdot}4 \times 10^{-6}$	50
A II	$2{\cdot}7 \pm 0{\cdot}4 \times 10^{-8}$	$5{\cdot}6 \pm 0{\cdot}7 \times 10^{-7}$	20·7

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Effect of labetalol and guanethidine on contractile responses to acetylcholine in the rat anococcygeus muscle

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Labetalol is a competitive antagonist at α- and β-adrenoceptors (Brittain & Levy, 1976). It is 6–10 times less potent than phentolamine in blocking α-adrenoceptors, 1·5–3 times less potent than propranolol in blocking β-adrenoceptors, and, hence, 4–8 times more potent at β- than at α-adrenoceptors. This profile of labetalol is unique and has provided an antihypertensive agent which has been successfully used in clinical trials (Prichard & Boakes, 1976). We have investigated the effects of labetalol on contractile responses to acetylcholine in the rat anococcygeus muscle, a tissue which has no cholinergic innervation (Gillespie, 1972).

Mature male Wistar rats killed by a sharp blow at the base of the skull were exsanguinated. Anococcygeus muscles were dissected as described by Gillespie (1972) and mounted under 0·5 g tension in 5 ml organ baths containing a modified Krebs solution at 37°, equilibrated with 5% CO_2 in oxygen. Isometric contractions were recorded with force displacement transducers (Grass, FT03.C) connected to a polygraph (Grass model RPS 7C8A).

When the effects of labetalol, guanethidine, or phentolamine on responses were being examined, these drugs were present in the Krebs solution throughout. For the 6-hydroxydopamine experiments, the isolated muscles were incubated in the presence of 10^{-3} M 6-hydroxydopamine for 3 h and then washed in Krebs solution for 30 min. After this treatment, the accumulation of noradrenaline was inhibited, contractile responses to it were potentiated and responses to tyramine were abolished (Doggrell & Woodruff, unpublished observations).

Responses were calculated as a percentage of the maximum response. Each mean value was determined from at least four separate preparations and is expressed ±s.e.m. The responses, under different conditions, were compared by Student's unpaired *t*-test and considered significantly different whenever $P < 0.05$.

The drugs used were labetalol hydrochloride* (Allen & Hanburys), acetylcholine chloride (BDH Canada, Ltd), phentolamine mesylate* and guanethidine sulphate* (Ciba), and 6-hydroxydopamine hydrobromide (Regis Chemicals Ltd). Compounds indicated with an asterisk were generously donated by the companies.

The modified Krebs solution had the following com-

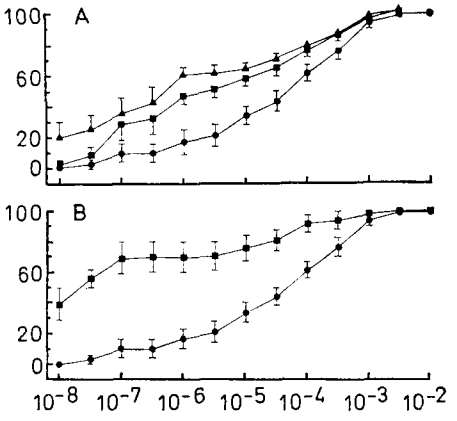

FIG. 1. The effects of labetalol and guanethidine on contractile responses to acetylcholine in the rat anococcygeus muscle. Responses to acetylcholine in normal Krebs solution (●—●). A. Responses to acetylcholine in the presence of 10^{-5} M labetalol (▲—▲) and in the presence of 10^{-6} M labetalol (■—■). B. Responses to acetylcholine in the presence of 6×10^{-6} M guanethidine (■—■). All responses are expressed as a percentage of the maximum response (ordinate). Each value is the mean ±s.e.m. from a minimum of 4 preparations. Abscissa: acetylcholine (M).

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