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Angiotensin II does not inhibit vagally-induced bradycardia or gastric contractions in the anaesthetized ferret

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1 The effects of angiotensin II (AII) and the analogue Sar^1-Leu^8-angiotensin II on the changes in heart rate and gastric pressure induced by vagal stimulation were investigated in the urethane-anaesthetized ferret.

2 AII at high doses (500 ng kg^{-1} i.v.) increased blood pressure and decreased gastric pressure whereas Sar^1-Leu^8-AII at the same dose had a much smaller effect on blood pressure and no effect on gastric pressure.

3 Intravenous injections or infusions of AII or its analogue did not alter the effect of vagal stimulation on heart rate or gastric motility. Our results do not provide support for the proposal that AII modulates parasympathetic activity at peripheral sites (Potter, 1982a, b).

Introduction

The vasoactive peptide angiotensin II (AII) has been shown to exert its pressor effect by a direct action on vascular smooth muscle and by facilitation of noradrenaline release from sympathetic nerve terminals (Starke, 1977; Westfall, 1977). The effects of AII on the peripheral sympathetic nervous system involve both presynaptic facilitation of transmitter release in response to evoked activity in the nerve (Zimmerman & Whitmore, 1967) and increased sensitivity at a postsynaptic site (Day & Moore, 1976). Turker & Kayaalp (1967) have provided evidence that AII may act as a neuromodulator in the gastro-intestinal tract of the cat and in a further publication suggested that facilitation of noradrenaline release was involved (Turker, 1973).

In contrast, little is known about the effects of AII on the peripheral parasympathetic nervous system. Recent short papers by Potter (1982a, b) present evidence for a modulating action of AII on parasympathetic transmission to the heart in the anaesthetized dog. It was also found that AII reduced the amplitude of vagally evoked contractions of the rabbit stomach *in vivo* (Potter 1982a).

In the present study we attempted to repeat and extend the above observations by a quantitative study of the effects of AII on heart rate and gastric motility in ferrets. The ferret has a high resting heart rate (330–440 beats min^{-1}) that is particularly sensitive to vagal stimulation (Andrews *et al.*, 1979). Under urethane anaesthesia the stomach is spontaneously motile and the vagus has potent cholinergic excitatory effects (Andrews & Scratcherd 1980).

Methods

Eight adult male ferrets were fasted overnight and then anaesthetized with urethane (1.5 g kg^{-1} intraperitoneally, 50% w/v in 154 mM NaCl). Rectal temperature was monitored and maintained at 39°C by a homeothermic blanket (Palmer Bioscience). Cannulae were placed in the trachea, external jugular vein and common carotid artery. The stomach was prepared for separate measurement of pressure in the corpus and antrum using the procedure described by Andrews & Scratcherd (1980). A pool was made in the neck and filled with warm (37–39°C) liquid paraffin. The cervical vagi were mobilized so that they could be ligated and cut at the appropriate point in the experiment. The peripheral cut end of the left cervical vagus was stimulated via a pair of platinum electrodes (1 mm diameter, 4 mm electrode separation).

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In two animals the abdominal aorta was cannulated at a point distal to the orgins of the renal arteries and the cannula advanced until its tip lay at the junction of the superior mesenteric artery with the aorta. After surgery the animal was left for at least 45 min before experimentation commenced.

The ECG was monitored from chest leads and recorded on tape (TEAC cassette recorder). Heart rate was measured by two methods: (a) 'on line' by averaging over successive 5 s periods using a D130 Spike Processor (Digitimer) and (b) 'off line' by converting the QRS complexes to TTL pulses and measuring the inter-beat interval using a Cromemco System Three microcomputer. At all stages the ECG trace and spike processor window were carefully monitored using an oscilloscope to confirm accurate gating of the signal. Particular care was taken to ensure that T waves of increased amplitude (that sometimes occurred during vagal stimulation) did not also result in generation of TTL pulses.

Drugs

The following drugs were used: angiotensin II amide (Ciba), Sar^1-Leu^8-angiotensin II (Sigma), atropine sulphate (BDH). All solutions were made up in 154 mM NaCl. Angiotensins were dissolved in sterile 0.9% w/v NaCl solution (Saline) at a stock concentration of 10 μM and stored in siliconized, sterile ampoules at -20°C. Final dilutions were made in plastic tubes during the experiment.

Results are expressed as mean $\pm$ s.e.mean.

Results

Bolus intravenous injections of AII (500 ng kg^{-1}) produced similar increases in systolic and diastolic blood pressures in intact and vagotomized animals (intact systolic increase 40.0 $\pm$ 4.0mmHg, diastolic 36.0 $\pm$ 3.0mmHg; vagotomized systolic increase 42.0 $\pm$ 5.0mmHg, diastolic 37.0 $\pm$ 4.0mmHg, $n = 5$ animals). The average blood pressure in the five aminals used in this part of the study was systolic 160 $\pm$ 8mmHg, diastolic 115 $\pm$ 5mmHg and it was not significantly altered by vagotomy. The responses to AII were unaffected by atropine (0.5mg kg^{-1} i.v., $n = 3$ animals). Intra-aortic injections (500 ng kg^{-1}) and intravenous infusions of AII (500 ng kg^{-1} min^{-1}) also produced similar increases in blood pressure. In contrast, the analogue Sar^1-Leu^8-AII (500 ng kg^{-1}) had only a small effect on blood pressure (systolic increase 8.0 $\pm$ 1.5mmHg, diastolic 12.0 $\pm$ 2.3mmHg, $n = 4$ animals). In intact and vagotomized animals the small changes in heart rate that occurred following AII administration were never greater than those produced by the vehicle alone.

The corpus and antrum in the vagally intact animal showed spontaneous rhythmic contractions. In twelve out of thirteen trials in five animals injection of AII (500 ng kg^{-1} i.v.) was followed by a decrease in pressure and suppression of contractions in the corpus (Figure 1a). In the antrum, six out of thirteen injections (in five animals) of AII inhibited spontaneous contractions without a change in pressure (not shown). In three cases, the period of inhibition in the corpus was followed by a period of elevated tone and enhanced contractions. The analogue Sar^1-Leu^8-AII at the same dose was without effect on corpus or antrum in six trials in four animals.

Vagotomy produced an elevation in prevailing corpus pressure as described previously (Andrews & Lawes, 1982), and a marked reduction in the amplitude of contractions in both antrum and corpus. Injections of AII (500 ng kg^{-1}) caused a small reduction in corpus pressure (8 of 10 trials in five animals),

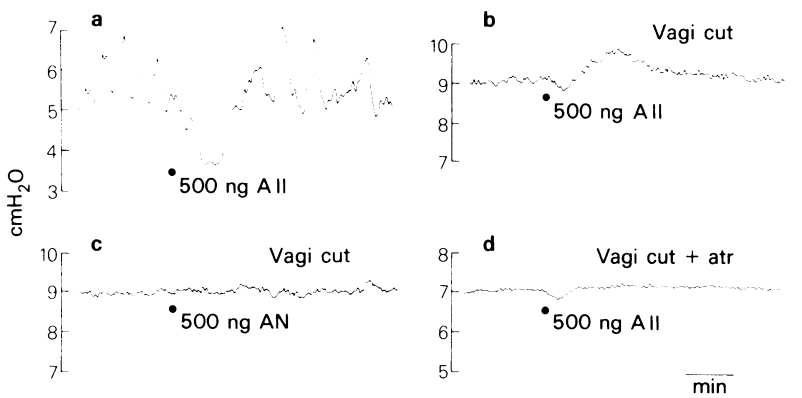

Figure 1 The corpus pressure response to angiotensin II (AII) (500 ng kg^{-1} i.v.) in an intact (a), vagotomized (b, d,), and subsequently atropinized (d) (atr 0.5 mg kg^{-1} i.v.) animal. Also shown is the effect of the Sar^1-Leu^8-AII analogue (AN) (500 ng kg^{-1} i.v.) in the same animal after vagotomy (c).

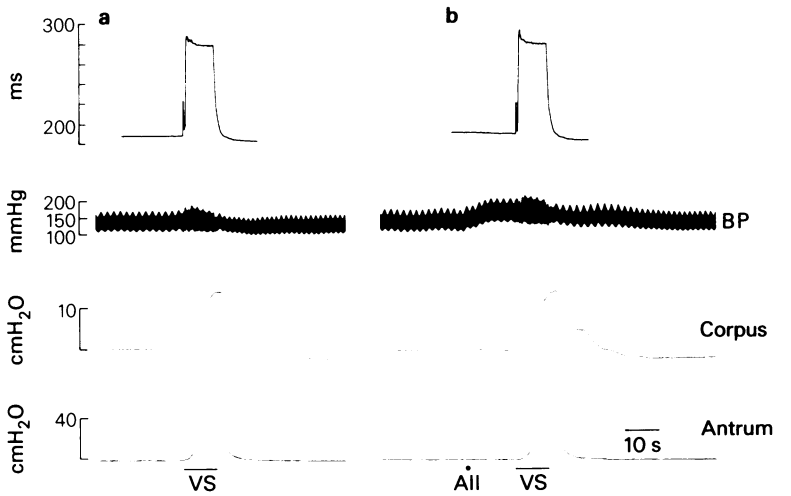

Figure 2 From top to bottom: inter-beat intervals, arterial blood pressure, corpus pressure and, antral pressure. Panel (a) control response to stimulation (4Hz, 20V, 0.5 ms, 10 s) of the peripheral cut end of the left cervical vagus, (b) stimulation in the presence of AII (500 ng kg^{-1} i.v.).

sometimes followed by a period of elevated pressure (3 of 10 trials) (eg. Figure 1b). At the same dose, injections of Sar^1-Leu^8-AII had no effect on corpus or antral pressure (Figure 1c). In only one trial was an AII injection (500 ng kg^{-1} i.v.) followed by a clear inhibition of antral activity. The decrease in pressure in the corpus caused by AII was not affected by atropine (0.5 mg kg^{-1} i.v.) (Figure 1d).

No indication of tachyphylaxis was seen in the above series of experiments with repeated AII injections and each injection was separated by at least 15 min.

Figure 2 shows a typical experiment where the effects of vagal stimulation (4Hz, 20 V, 0.5 ms for 10 s) on heart rate, blood pressure and gastric motility were examined, with and without a preceding bolus injection of AII (500 ng kg^{-1}). The heart rate in the five vagotomized animals used in this part of the study was 362 ± 10 beats min^{-1}. In the absence of AII, vagal stimulation produced a marked bradycardia (Figure 2a, top panel), which was abolished by atropine (0.5 mg kg^{-1} i.v., $n = 5$ animals; results not shown). When the vagus was stimulated after the AII injection and when the effects of AII on blood pressure were at their peak, neither the amplitude nor duration of bradycardia were altered (Figure 2b, top panel). Vagal stimulation during infusion of AII (500 ng kg^{-1} min^{-1} i.v.) also caused a bradycardia similar to control (not shown). This lack of effect of AII on vagal neurotransmission in the heart was consistent in all 5 experiments. In these experiments we also used repeated intermittent stimulation of the vagus (4Hz, 0.5 ms, 20V for 1 s or 3 s at 10 s intervals) similar to that used by Potter (1982a, b). Each episode of stimulation caused an increase in inter-beat interval which returned to control values between stimulations. After obtaining control responses to intermittent stimulation we injected AII (500 ng kg^{-1} i.v.) in 4 trials. The effects of vagal stimulation on the inter-beat interval remained unchanged in each trial.

Also, administration of AII did not modify the contractions of the stomach evoked by vagal stimulation. The stimulation parameters (4Hz, 0.5 ms, 20V, 10 s) were chosen so as to produce a single large contraction, in order to facilitate measurement (Andrews *et al.*, 1980). As shown in Figure 2 (lower traces), the amplitude and duration of the pressure changes recorded in the corpus and antrum in the presence of AII (Figure 2b) were similar to controls (Figure 2a). In each of the five animals the vagus was stimulated three times to provide control measurements and three times in the presence of AII. We did not detect any effect of AII on the responses of either corpus or antrum to vagal stimulation. (Corpus control 14.50 ± 0.75 cm H_2O, AII, 15.25 ± 1.37 cmH_2O; antrum control 27.75 ± 5.3 cmH_2O, AII 26.25 ± 6.25 cmH_2O, $n = 5$ animals). In two animals infusions of AII (500 ng kg^{-1} i.v.), an injection of a higher dose (2 μg kg^{-1} i.v.), or intra-aortic injections of AII (500 ng kg^{-1}; 1 μg kg^{-1}) all failed to influence the vagally evoked contractions.

Discussion

Using high doses (500 ng kg^{-1}; 2 $\mu g\,kg^{-1}$) of angiotensin II or Sar^1-Leu^8-AII (500 ng kg^{-1}) we have been unable to demonstrate any effect of these peptides on the cardiac or gastric responses to electrical stimulation of the vagus in the ferret. This observation is in contrast to those of Potter (1982a, b) who showed that a bolus dose of AII, 5–10 μg i.v., almost abolished the bradycardia evoked by vagal stimulations (~ 30V, 1.0 ms, 4 shocks, 300 ms apart) in the anaesthetized dog. This author also found that 1 μg of AII injected into the coeliac artery of the anaesthetized rabbit reduced the amplitude of the vagally evoked contractions in the stomach wall.

Although the dog and the ferret are both carnivores, a species difference might be considered as an explanation for the discrepancy in the response and this may be related to the large differences in their heart rates. In both studies the effects of AII were investigated against a decrease in heart rate of about 30% induced by vagal stimulation. It is possible that in the ferret the action of AII on parasympathetic neurotransmission to the heart is weak and therefore antagonism of the potent effects of vagal stimulation may not be detected. However, in the present study the vagal stimulation was frequently well below that required for maximal cardio-inhibition. Further, since the vagus is tonically active in the intact ferret (Andrews *et al.*, 1979) it is difficult to explain why AII did not have some effect on basal heart rate.

It was observed that during vagal stimulation the amplitude of the T wave of the ECG increased markedly and unless the ECG and spike processor output were carefully monitored an erroneous heart rate was displayed due to double gating. Such an effect would be difficult to detect using a conventional tachograph.

Sar^1-Leu^8-AII was also without effect on the bradycardia associated with vagal stimulation. This analogue was used because it has little effect on blood pressure thus allowing the primary responses of the heart and stomach to AII to be separated from possible secondary effects due to the increased blood pressure associated with injection or infusion of AII. Many 8-substituted analogues of AII are antagonists of the parent peptide and are given increased potency *in vivo* by additional substitution of the N-terminal aspartyl residue by sarcosine (Hall *et al.*, 1974). Sar^1-Leu^8-AII was particularly suitable for this study as although it acts as an inhibitor of the pressor action of AII and has little intrinsic agonist activity on non-renal vascular smooth muscle, it retains full presynaptic activity on sympathetic nerve terminals (Peach, 1977). Since neither AII nor Sar^1-Leu^8-AII had any detectable effect on heart rate during vagal stimulation, the possibility that an effect had occurred, but was obscured by a reflex bradycardia associated with increased blood pressure was excluded.

The lack of effect of AII on the contractions of the corpus and antrum induced by vagal stimulation was consistent with its failure in our experiments to reduce bradycardia due to vagal stimulation. Potter (1982a, b) did not investigate the effects of AII on the dog stomach and it is difficult to compare our results directly with those from the rabbit.

In intact and vagotomized ferrets the clearest effect of AII on spontaneous gastric motility was a small decrease in pressure in the corpus. Whilst part of this response is independent of cholinergic activity (since it occurs in the presence of atropine and after vagotomy) some action on spontaneously active cholinergic neurones cannot be discounted. *In vitro* techniques would be more appropriate for a study of this action and also to examine whether AII acts directly on the muscle or via the release of noradrenaline from adrenergic terminals within the gastric wall. In the antrum the effects of AII were more variable but nevertheless an inhibitory action could be demonstrated in the vagally intact animals, indicating possible antagonism of tonic cholinergic activity. The low level of antral activity in the vagotomized animals may have masked any inhibitory effect of AII.

In conclusion we have been unable to demonstrate an effect of AII on the vagally driven responses of the heart, and gastric corpus or antrum. There is some indication that AII can cause inhibition of gastric motility by a mechanism at least partly independent of cholinergic activity. It should be emphasized that the doses required to produce these effects are outside the physiological range for this peptide although the possibility exists that AII may be released locally in high concentrations (e.g. from neurones). It is also possible that high levels of AII occurring in pathological conditions such as malignant hypertension may contribute to disorders of gastric motility.

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