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SYSTEMIC ANGIOTENSIN-INDUCED DRINKING IN THE DOG: A PHYSIOLOGICAL PHENOMENON

BY J. T. FITZSIMONS, J. KUCHARCZYK AND GEORGINA RICHARDS

From the Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG

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SUMMARY

1. Intravenous infusion of the individual components of the renin-angiotensin system caused drinking in dogs in water balance.

2. Angiotensin II was the most potent and rapidly acting peptide inducing drinking. The minimum effective rate of infusion was between 8·3 and 16·6 × 10^{-12} mole kg^{-1} min^{-1} which yielded blood levels of angiotensin II that fell well within physiological limits for the dog and were mildly pressor. Angiotensin I and synthetic renin substrate caused less drinking than angiotensin II, and angiotensin III was the least effective dipsogen.

3. Renin caused significant drinking when infused I.V. at a rate of 0·5 u. min^{-1} for 15 min. Drinking was slower in onset and continued for longer than after other components of the renin-angiotensin system.

4. Within the dose range 1875–15,000 × 10^{-12} mole of angiotensin II the amount of water drunk depended more on the rate of infusion than on the duration of the infusion.

5. During an I.V. infusion of angiotensin II lasting 2 hr, the rate of drinking was greatest during the first 15 min. After this it declined progressively.

6. A delay of 1 hr after the start of an intravenous infusion of angiotensin II before access to water was allowed, did not significantly reduce the amount of water drunk. Nor did infusion of isotonic saline for 105 min reduce drinking in response to a subsequent infusion of angiotensin II. However, a preload of dilute milk approximately equal in volume to the amount of water normally drunk in response to I.V. angiotensin II significantly reduced drinking. Therefore the dog stopped drinking during long-term infusions of angiotensin II owing to the action of satiety mechanisms and not to tachyphylaxis or fatigue.

7. Intracarotid infusion of angiotensin II, angiotensin I, synthetic renin substrate and angiotensin III, at 40 × 10^{-12} mole min^{-1} also caused drinking. Intakes of water were similar to the intakes after I.V. infusion at six times the arterial rate, except that angiotensin I was relatively less effective by intracarotid infusion than by I.V. infusion.

8. Renin, infused at 0·5 u. min^{-1} for 15 min, was much less effective by intracarotid infusion than by intravenous.

9. These results are compatible with a role for circulating angiotensin II in the thirst of hypovolaemia or moderate extracellular dehydration.

INTRODUCTION

In the preceding paper (Fitzsimons & Kucharczyk, 1978) it was shown that the dog drinks vigorously and normally in response to intracranial injections of angiotensin II (AII). Other components of the renin-angiotensin system were less effective. Water intakes for each component tested were dose-dependent and drinking occurred in the absence of peripheral pressor changes, although the arterial pressure rose during the act of drinking. Having established the intracranial responsiveness to AII, in this paper we describe the responsiveness to I.V. and intracarotid infusions of components of the renin-angiotensin system. The relationship between AII-induced drinking and haemodynamic changes and plasma AII levels was also investigated. These experiments were necessary in order to establish whether the phenomenon of drinking induced by intracranial AII is physiological or not, because the way in which AII normally reaches the brain is through the circulation. Drinking in response to intracranial AII has no physiological validity on its own because (1) the route of administration is abnormal, (2) the tissue around the cannula or injector is abnormal, (3) the concentrations injected are too high, (4) the temporal injection pattern does not imitate the normal sequence of stimulation, and (5) an unknown proportion of sensitive tissue is affected. The best evidence that AII may participate in normal thirst must be provided by systemic infusion experiments.

METHODS

Animals. Seven mongrel bitches and four mongrel dogs weighing between 9·5 and 23·0 kg were used in the experiments. Care and housing were as described in the preceding paper.

Surgical procedures. Nine dogs were prepared with unilateral or bilateral exteriorized common carotid arteries enclosed within skin loops.

At the start of each intravenous infusion experiment, a catheter (PP 10 or PP 25 polyethylene tubing) was inserted into the saphenous or cephalic vein through a wide-bore syringe needle (o.d. 1·4 mm) after infiltrating the surrounding skin with a local anaesthetic.

Carotid infusions were made via polyethylene tubing (PP 10 or PP 25) pushed a few cm into the carotid artery through a syringe needle (o.d. 1·4 mm) inserted into the vessel under local anaesthesia.

Blood pressure was recorded in the femoral artery of the conscious dog using the same techniques described in the preceding paper. The femoral artery was cannulated according to the method of Seldinger (1953).

Solutions for infusion. Val^5-AII amide (Hypertensin, Ciba) or Ile^5-AII (Bachem), angiotensin I (AI) (Schwartz-Mann), des-Asp^1-AII (AIII) (Bachem) and synthetic renin substrate (SRS) (Bachem) were dissolved in 0·9 % NaCl to give solutions varying in concentration between 1 and 1000×10^{-12} M. Lyophilized hog renin (Nutritional Biochemicals) was dissolved in 0·9 % NaCl to give solutions varying in concentration between 0·125 and 2·0 u./ml.

Experimental procedures. Half an hour before the start of each experiment the dog was brought to the experimental room and placed on a Pavlov table, to which it was well accustomed, with drinking water available. The appropriate cannulae were inserted. The dog was allowed 15–30 min to settle down after the intravenous or intracarotid cannulation.

During a pretest control period of 15 min, 0·9 % NaCl was infused at a rate of 2·5 ml. min^{-1} in the intravenous experiments and at a rate of 4·0 ml. min^{-1} in the intracarotid series. Following this the infusion of the drug was started at the same volume rate. The amount of water drunk, the latency to drink, and the incidence and size of the individual draughts of water were recorded throughout the experiment.

Measurement of blood AII levels. In experiments on four dogs venous samples of 10 ml. each were collected before the start of an I.V. infusion of AII at a rate of 250×10^{-12} mole min^{-1},

and at 15, 30, 60 and 120 min after the start of the infusion. A final sample was taken 15–20 min after the end of the infusion. The blood was collected from the opposite cephalic or saphenous vein directly into chilled polyethylene test tubes containing 0·5 ml. of a mixture of *o*-phenanthroline (0·025 M) and EDTA (0·125 M) 1:1 v/v. Samples were then centrifuged at 2000 rev/min for 15 min at 4 °C and 1 ml. aliquots of plasma were put onto 100 mg of a hydrogen form of exchange resin (Dowex AG 50W-X2 50 – 100 mesh, Biorad Laboratories). After shaking, centrifugation, and discarding of supernatant, the sample was added to 2 ml. of ammonia/methanol (4:1 v/v). Following further centrifugation the supernatant was transferred to polyethylene LP3 tubes and evaporated to dryness under an airstream. The samples were sent to Dr Fiona Broughton Pipkin of Nottingham University for AII radioimmunoassay.

RESULTS

Effects on drinking and on the circulation of I.V. *infusion of components of the renin-angiotensin system and of renin*

AII was the most potent of the components of the renin-angiotensin system tested (Fig. 1). Infused I.V. at a rate of 250×10^{-12} mole min^{-1} for 15 min, AII caused drinking in each of the ten dogs tested, giving a mean intake of water of $51{\cdot}6 \pm 10{\cdot}7$ (S.E. of mean) ml. after a delay of 4 min 30 sec ± 0 min 20 sec. Compared with the response to infusion of similar volumes of 0·9% NaCl ($1{\cdot}8 \pm 1{\cdot}4$ ml.) when only two dogs out of ten drank, this was a highly significant increase in drinking ($P < 0{\cdot}001$).

All ten dogs infused intravenously with AI at 250×10^{-12} mole min^{-1} responded, drinking a mean volume of $37{\cdot}7 \pm 5{\cdot}5$ ml. after a delay of 7 min 53 sec ± 1 min 03 sec. This was less, but not significantly so, than the response to AII. SRS caused less drinking when infused intravenously at 250×10^{-12} mole min^{-1} for 15 min than AII given at the same rate. Eight dogs out of ten tested with SRS drank. The mean intake for the ten dogs was $13{\cdot}7 \pm 3{\cdot}0$ ml. and the mean delay of the eight that drank was 9 min 39 sec ± 1 min 15 sec. This volume of water drunk was significantly greater than the control value ($P < 0{\cdot}01$) but significantly less than the amounts drunk after AI ($P < 0{\cdot}01$) and AII ($P < 0{\cdot}01$) given at the same rate. In contrast to the brief but vigorous burst of drinking elicited by I.V. infusion of AII, the dogs generally responded in an episodic manner to SRS and AI, drinking water rather slowly over several minutes in several draughts.

AIII was ineffective. Only one dog out of ten tested with AIII infused at 250×10^{-12} mole min^{-1} drank any water, and this dog took only 9·0 ml. At double the rate of infusion of AIII, seven dogs out of ten drank. The mean intake for the ten dogs was $11{\cdot}4 \pm 3{\cdot}7$ ml. water, a trivial but significant increase in intake over the mean control value ($P < 0{\cdot}05$). The latency to the onset of drinking was longer (11 min 6 sec ± 0 min 49 sec) than after the other components given at half the rate of infusion.

Intravenous infusion of renin at 0·05 u. min^{-1} for 15 min caused two dogs out of seven to drink giving a mean intake for the seven dogs of $4{\cdot}6 \pm 3{\cdot}3$ ml. water, an insignificant increase in drinking. When the rate of infusion of renin was increased tenfold, to 0·5 u. min^{-1}, all ten dogs responded, drinking $36{\cdot}6 \pm 6{\cdot}5$ ml. ($P < 0{\cdot}001$) after a mean delay of 12 min 54 sec ± 3 min 48 sec.

The effect of varying the rate of I.V. infusion of a fixed quantity (3750×10^{-12} mole) of AII on drinking was studied in nine dogs. The results, summarized in Table 1, show that the amount of water drunk at a given dose of AII was greater and the

delay to the onset of drinking shorter at high rates of infusion than at low rates. The size of the initial bout of drinking showed the same trend.

Over the dose range of 1875×10^{-12} to $15{,}000 \times 10^{-12}$ mole AII given I.V., drinking depended more on the rate of infusion of the hormone than on the duration of infusion (Fig. 2). Drinking was significantly correlated with both the rate ($r = 0{\cdot}65$,

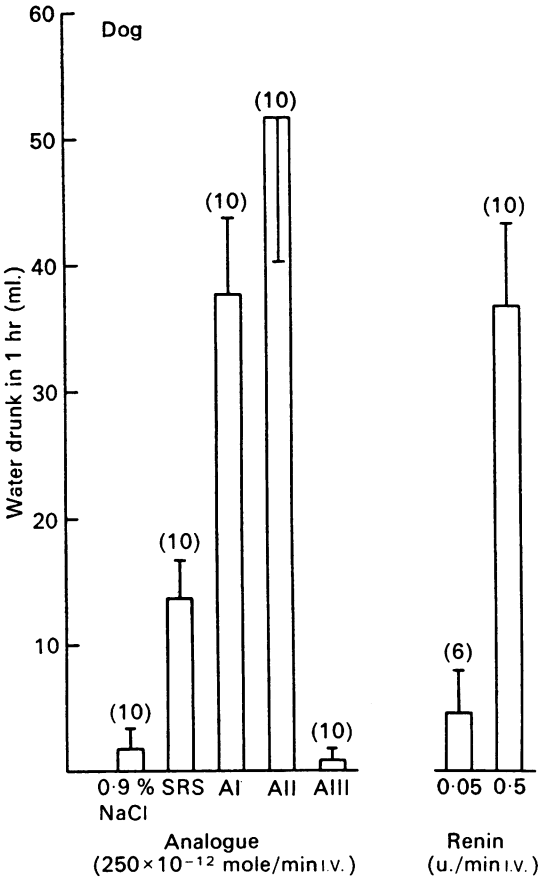

Fig. 1. The mean amounts of water drunk in 1 hr (± S.E. of mean, number of observations in parentheses) in response to a 15 min I.V. infusion of SRS, AI, AII, AIII, all at 250×10^{-12} mole min^{-1}, and renin at 0·05 and 0·5 u. min^{-1}.

d.f. = 20, $P = 0{\cdot}001$) and the duration ($r = 0{\cdot}48$, d.f. = 27, $P < 0{\cdot}02$) of infusion of AII. However, with the durations and rates of infusion used, the dose–response curve was much steeper when the dose was increased by increasing the rate of infusion than when it was increased by extending the duration of infusion.

In order to determine whether AII would continue to exert a dipsogenic action when given I.V. for longer periods of time, drinking was measured in five dogs infused for 2 hr at 250×10^{-12} mole min^{-1}, a rate shown to be effective in the 15 min infusions. When drinking was prevented for the first hour after the start of the infusion of AII, the mean amount of water subsequently drunk during the second hour

TABLE 1. Mean amounts (± S.E. of mean) of water drunk and latencies of drinking by nine dogs given a constant amount of AII (3750×10^{-12} mole) I.V. but varying the rate and duration of infusion.

Rate ($\times 10^{-12}$ mole min^{-1})	Duration (min)	No. of experiments	No. of occasions on which dogs drank	Water drunk* (ml.)	Latency† (min sec)
125	30	8	6	13·0 ± 6·0	16 10 ± 4 30
250	15	9	9	45·1 ± 12·2	4 30 ± 0 20
500	7·5	8	8	41·6 ± 4·5	5 45 ± 0 40
1000	3·75	7	7	92·4 ± 12·6	3 16 ± 0 19

* Mean of all experiments.
† Mean of all occasions on which dogs drank.

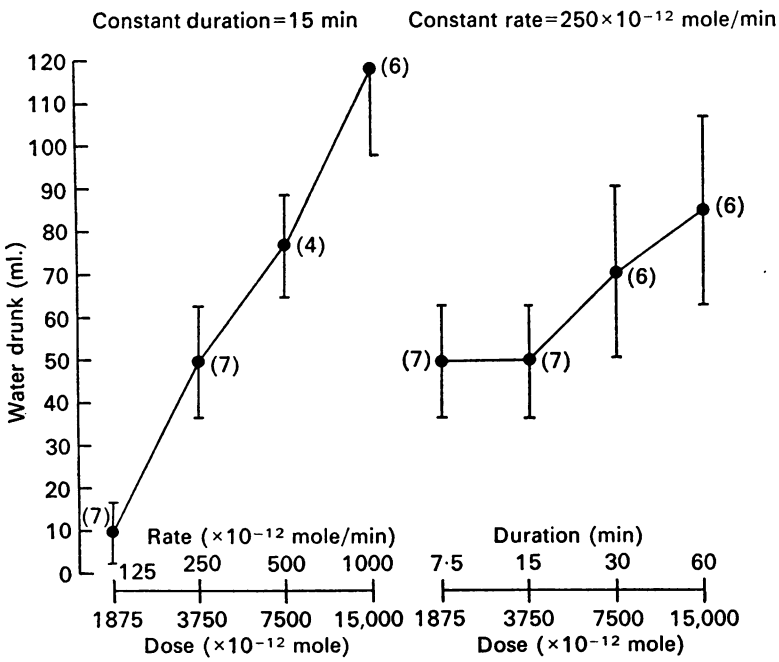

Fig. 2. Water drunk by dogs in response to four different doses of AII infused I.V. On the left the dose was varied by varying the rate of infusion for a fixed duration (15 min) of infusion; on the right by varying the duration of infusion at a fixed rate (250×10^{-12} mole min^{-1}) of infusion. The lowest dose was 1875×10^{-12} mole and the highest $15{,}000 \times 10^{-12}$ mole in each curve. Note that the dose–response curve is steeper when the rate is changed and the duration is constant than when the duration is varied and the rate kept constant.

of infusion was not significantly different from that drunk by the same five dogs allowed immediate access to water (Fig. 3).

As is evident from Fig. 3 the rate of drinking in response to I.V. infusion of AII declined as the infusion continued, although the circulatory changes lasted throughout the infusion and plasma AII (see later) continued to rise. It was of interest to see whether this was attributable to the animals becoming tired owing to the relatively

long duration of the test or whether other factors were involved. When three dogs were infused I.V. with 0·9% NaCl at 2·5 ml. min^{-1} for 105 min followed by a 15 min infusion of AII at 250×10^{-12} mole min^{-1} the mean amount of water drunk was not significantly different from the mean intake when AII was given at the same rate for 15 min at the end of a 15 min infusion of 0·9% NaCl (Table 2). Fatigue therefore does not account for the decline in rate of drinking.

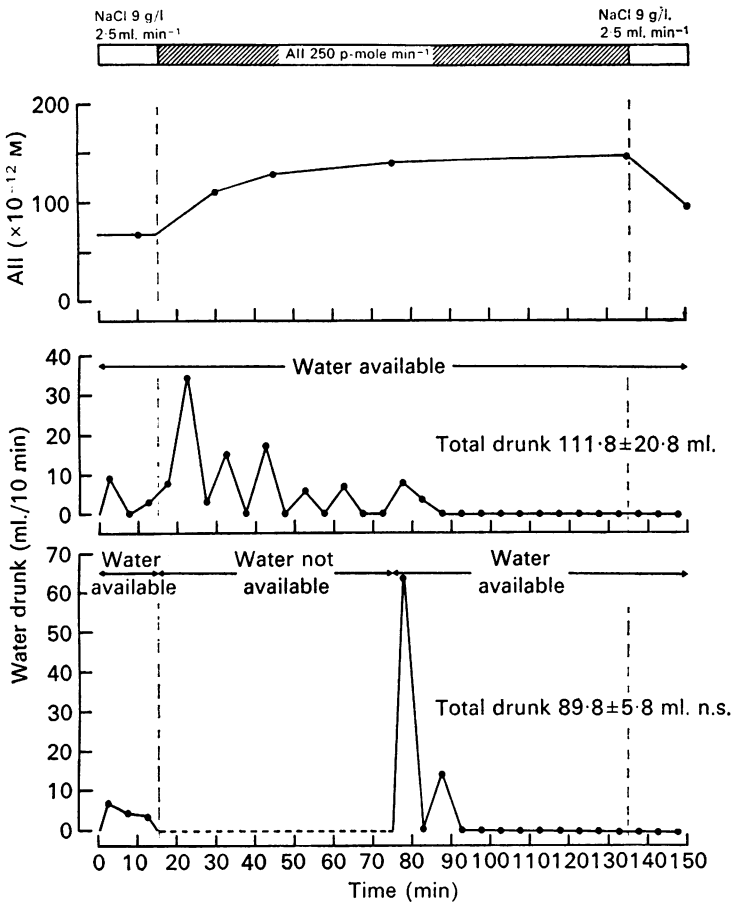

Fig. 3. Rate of water intake in 10 min periods of five dogs infused I.V. with AII at 250×10^{-12} mole min^{-1} for 2 hr with immediate access to water (middle panel) and when access to water was not allowed until 1 hr after the start of the AII infusion (bottom panel). The mean total amounts of water drunk did not differ significantly. Note that in each case the rate of drinking was maximal when water was first made available and then fell off markedly even though the infusion was continued at the same rate. AII levels produced by a similar infusion schedule in other experiments are shown in the upper panel.

The possibility that temporary satiety mechanisms might account for the inhibition of intravenous AII-induced thirst was tested in five dogs by allowing the dog to preload itself by drinking an amount of diluted milk slightly greater than the amount of water that the dog had been shown to drink in a previous test and then

seeing whether it would drink water in response to a similar infusion of AII. Dogs need little encouragement to drink diluted milk. Individual dogs varied markedly in their response to a preload of 125–175 ml. of fluid. One dog showed a slight increase in drinking in response to AII infused I.V. at 250×10^{-12} mole min^{-1} for 2 hr, two dogs showed a substantial reduction in response and in the remaining two dogs drinking was completely abolished throughout the 2 hr infusion by the preload of diluted milk. The mean intake of water fell from the control value of $115{\cdot}0 \pm 19{\cdot}7$ ml.

TABLE 2. Mean ($\pm$ S.E. of mean) amounts of water drunk by three dogs infused I.V. with 0·9 % NaCl at a rate of 2·5 ml. min^{-1} for 15 min followed by a 15 min infusion of AII at 250×10^{-12} mole min^{-1} ('Immediate AII') and by the same three dogs when AII was given at 250×10^{-12} mole min^{-1} for 15 min at the end of a 105 min period of I.V. infusion of 0·9 % NaCl at 2·5 ml. min^{-1} ('Delayed AII')

	Water drunk (ml.)	Latency (min sec ± min sec)
Immediate AII	$33{\cdot}0 \pm 9{\cdot}0$	4 50 ± 0 25
Delayed AII	$28{\cdot}3 \pm 14{\cdot}9$	4 16 ± 1 16

TABLE 3. Haemodynamic changes in four conscious dogs given AII by I.V. infusion for 15 min. Mean values $\pm$ S.E. of mean are shown. Number of experiments in parentheses.

Rate and duration of infusion of AII	Time after start of infusion (min)	Mean arterial blood pressure (mmHg)	Pulse pressure (mmHg)	Heart rate (beats min^{-1})
40×10^{-12} mole min^{-1} for 15 min (1)	Pre-infusion (0·9 % NaCl infused at 2·5 ml. min^{-1})	126	80	120
	0–5	132	82	90
	6–10	125	70	110
	11–15	130	80	110
250×10^{-12} mole min^{-1} for 15 min (3)	Pre-infusion	$130{\cdot}8 \pm 8{\cdot}1$	$71{\cdot}5 \pm 3{\cdot}5$	$104{\cdot}5 \pm 3{\cdot}5$
	0–5	$146{\cdot}5 \pm 7{\cdot}4$	$82{\cdot}3 \pm 2{\cdot}6$	$92{\cdot}2 \pm 1{\cdot}6$
	6–10	$142{\cdot}1 \pm 7{\cdot}2$	$81{\cdot}2 \pm 0{\cdot}7$	$91{\cdot}9 \pm 6{\cdot}2$
	11–15	$140{\cdot}6 \pm 14{\cdot}9$	$78{\cdot}4 \pm 3{\cdot}0$	$92{\cdot}8 \pm 2{\cdot}4$
500×10^{-12} mole min^{-1} for 15 min (1)	Pre-infusion	146	75	115
	0–5	160	78	90
	6–10	145	80	100
	11–15	145	78	115
1000×10^{-12} mole min^{-1} for 15 min (2)	Pre-infusion	87·5	65·0	110·0
	0–5	130·0	79·0	95·0
	6–10	95·0	72·5	112·5
	11–15	87·5	67·5	115·9

for the same five dogs to $38{\cdot}6 \pm 20{\cdot}9$ ml., a significant decrease in drinking ($P < 0{\cdot}05$). It seems probable therefore that the dog stopped drinking during long-term infusion of AII because of inhibition of thirst by temporary satiety mechanisms and not because of tachyphylaxis or fatigue.

I.V. infusion of AII at a rate of 250×10^{-12} mole min^{-1} for 2 hr in four dogs (corresponding to a mean rate of $16{\cdot}6 \times 10^{-12}$ mole kg^{-1} min^{-1}) gave blood levels of AII in the range of $110–244 \times 10^{-12}$ M compared with a mean concentration of $69{\cdot}0 \pm 28{\cdot}9 \times 10^{-12}$ M pre-infusion (Fig. 3). This level of AII in the blood is comparable to values reported in dogs at the end of 3 days of sodium depletion (B. H. Douglas,

personal communication to Trippodo, McCaa & Guyton, 1976) and suggests that the infusion rates used in the present experiments produced drinking responses at blood AII concentrations that are within physiological limits in the dog.

The main effects of intravenous infusion of AII on the circulation were an increase in mean arterial blood pressure and pulse pressure and a decrease in heart rate, which depended on the rate and duration of the infusion (Table 3). The increases in mean arterial blood pressure and pulse pressure and the bradycardia were evident within 1–2 min of the start of the infusion, reached their maximum values within the first 5 min, and generally persisted for the duration of the infusion of AII returning to normal within a few minutes of the end of the infusion (Fig. 4, upper panel).

The standard rate of infusion used in these experiments did not produce tachyphylaxis since circulatory changes lasted as long as the infusion. In one experiment on a 19 kg dog, intravenous infusion of AII at a rate of 250×10^{-12} mole min^{-1} for 2 hr caused the mean arterial blood pressure to rise within two min of the start of the infusion and to remain elevated by 40–50 mmHg above the pre-infusion level of 75 mmHg for the duration of infusion of AII.

Drinking and haemodynamic changes induced by intracarotid infusion of components of the renin-angiotensin system and of renin

When infused into the carotid artery AII was the most potent component of the renin-angiotensin system to cause drinking in the dog (Fig. 5). Given at less than one-sixth the I.V. rate of 250×10^{-12} mole min^{-1}, intracarotid infusion of AII at 40×10^{-12} mole min^{-1} for 15 min produced similar intakes to those produced by I.V. infusion (compare Figs. 1 and 5). The mean intake after intracarotid AII was $43{\cdot}3 \pm 8{\cdot}5$ ml. a highly significant increase ($P < 0{\cdot}001$) over the control. All six dogs responded with a mean latency of 5 min 9 sec ± 1 min 13 sec. AII infused at half the rate, at 20×10^{-12} mole min^{-1} for 15 min, caused drinking in three dogs out of six tested but the mean intake was only $7{\cdot}3 \pm 3{\cdot}4$ ml. which was not significantly greater than the control intake. The mean intake of water after infusion of AI at 40×10^{-12} mole min^{-1} was less than after the standard rate of AII but it was significantly greater than the control intake ($P < 0{\cdot}001$). It is worth noting that in contrast to the comparable effectiveness of AI and AII by I.V. infusion, AI was significantly less effective than AII when both were given by intracarotid infusion ($P < 0{\cdot}05$). SRS infused into a carotid artery at 40×10^{-12} mole min^{-1} for 15 min caused all six dogs to drink giving a small but significant ($P < 0{\cdot}001$) mean intake. AIII, however, infused at the same rate caused only one dog out of five to drink, this animal consuming 48 ml. water. The mean intake of the five dogs was not significantly greater than control values. AIII was therefore the weakest of the components of the renin-angiotensin system tested whether given intravenously or by intracarotid infusion.

Renin was a less potent dipsogen infused into a carotid artery than into a vein

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Fig. 4. Continuous tracings of arterial blood pressure (B.P.), mean arterial blood pressure (M.A.B.P.) and heart rate (H.R.) during I.V. (top panel) and intracarotid (bottom panel) infusion of AII. The rate of infusion is shown above each record.

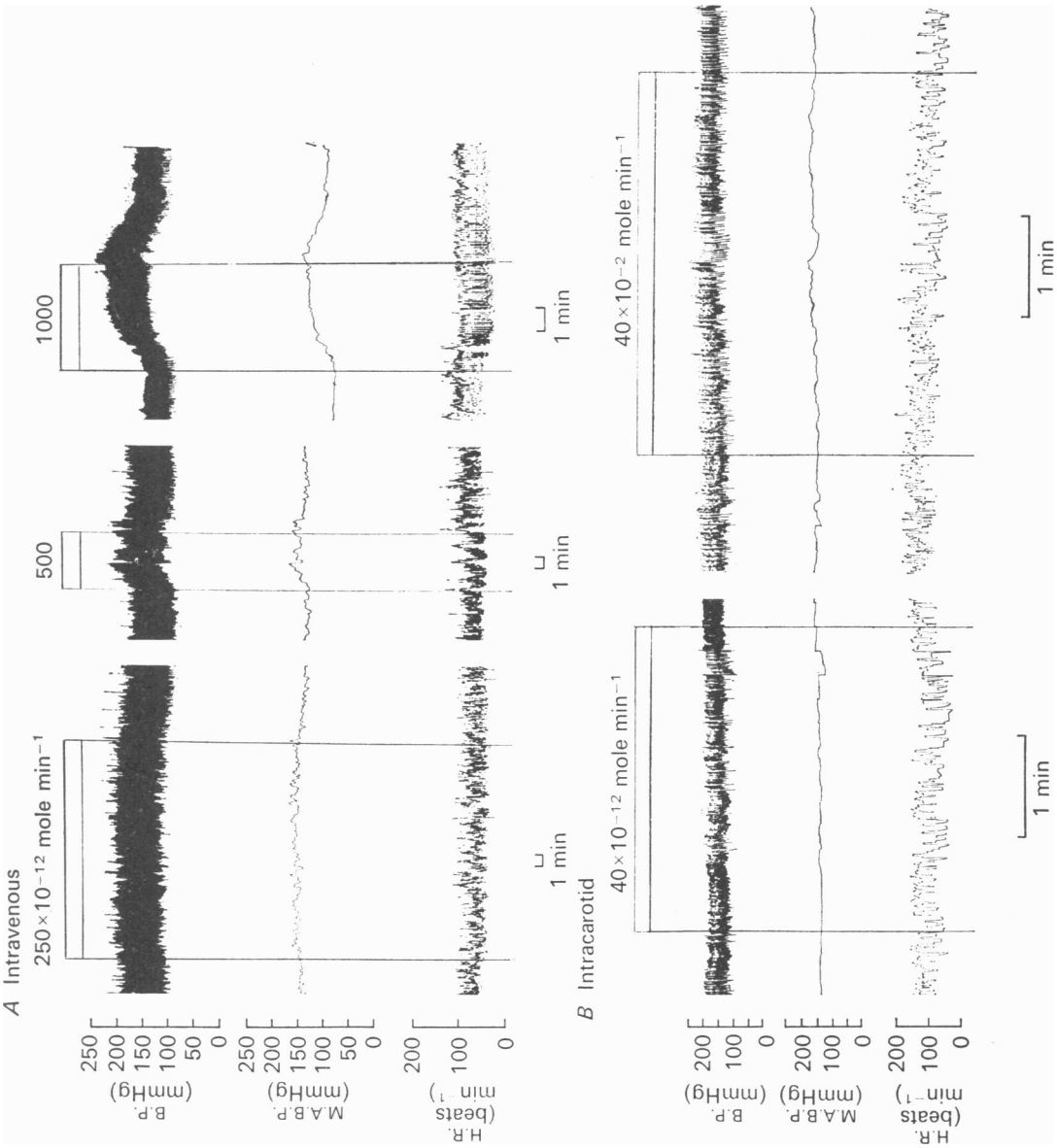

Fig. 4. For legend see facing page.

even when it was given at the same rate of infusion by the two routes. At 0·5 u. min^{-1} for 15 min, intracarotid infusion of renin caused two dogs out of five to drink 13·2 ± 9·2 ml. water after a delay of 7 min 49 sec ± 5 min 19 sec. This was less than the amount drunk in response to I.V. infusion (36·6 ± 6·5 ml. in ten dogs) at the *same* rate, although not significantly so. Renin infused intravenously at the lower rate of

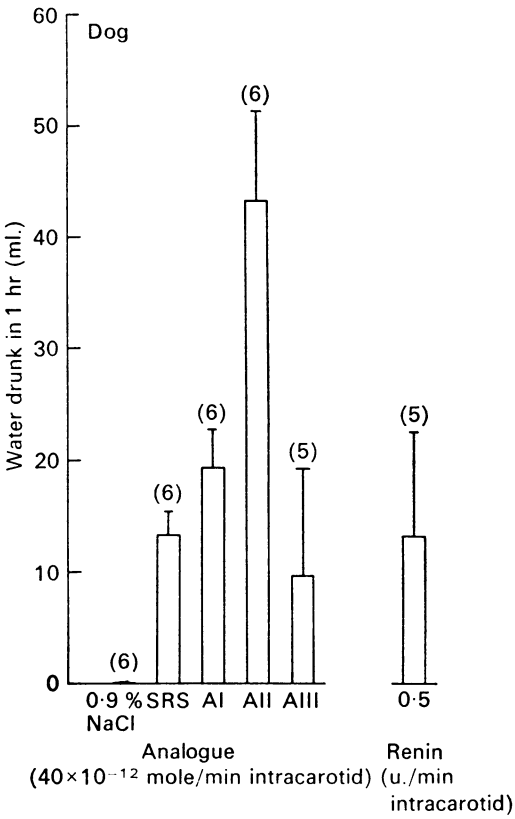

Fig. 5. The mean amounts of water drunk (± S.E. of mean, number of observations in parentheses) in response to 15 min intracarotid infusion of 0·9 % NaCl, SRS, AI, AII, AIII, all at 40 × 10^{-12} mole min^{-1}, and renin at 0·5 u. min^{-1}.

TABLE 4. Haemodynamic changes observed in two dogs given AII by intracarotid infusion at 40 p-mole min^{-1} for 15 min. Mean values

Time after start of infusion (min)	Mean arterial blood pressure (mmHg)	Pulse pressure (mmHg)	Heart rate (beats min^{-1})
Pre-infusion	133·5	75·0	112·5
0–5	134·5	73·5	99·5
6–10	140·0	72·5	90·0
11–15	145·5	75·0	91·0

0·05 u. min^{-1} caused as much drinking as renin infused into the carotid artery at 0·5 u. min^{-1}.

It is evident from Table 4 and Fig. 4*B* (lower panel) that intracarotid infusion of

AII at a rate of 40×10^{-12} mole min^{-1} had only minor effects on the circulation. Mean arterial blood pressure rose slightly and pulse pressure fell very slightly and then rose, but neither of these effects was significant. Heart rate fell by 5–10 beats min^{-1} within the first 5 min of the start of the infusion and remained slightly below the control level throughout the period of the infusion of AII.

DISCUSSION

Intravenous or intracarotid infusion of the individual components of the renin-angiotensin system caused the dog in water balance to drink water. AII was the most effective and rapidly acting peptide both by I.V. and by intracarotid infusion. The minimum effective rate of I.V. infusion at which all dogs drank was between 125 and 250×10^{-12} mole min^{-1} ($8{\cdot}3$–$16{\cdot}6 \times 10^{-12}$ mole kg^{-1} min^{-1}). The minimum effective carotid infusion rate was between 20 and 40×10^{-12} mole min^{-1}) (1–2×10^{-12} mole kg^{-1} min^{-1}). These rates of infusion are extremely low. The carotid infusion rates produced no significant rise in blood pressure. The I.V. infusion rate of 250×10^{-12} mole min^{-1} was mildly pressor and yielded blood levels of AII of up to 244×10^{-12} M, well within the range that has been found in sodium-depleted dogs and similar to values that have been reported in renal hypertension in man (Trippodo *et al.* 1976; Catt, Cain, Coghlan, Zimmet, Cran & Best, 1970).

AI and SRS caused less drinking than AII, and AIII was the least effective dipsogen. There was an interesting difference in effectiveness between I.V. AI and intracarotid AI (compare Figs. 1 and 5). I.V. AI caused almost as much drinking as intravenous AII whereas intracarotid AI caused significantly less drinking than intracarotid AII. Since the angiotensin-sensitive receptor is more responsive to AII than to AI (Fitzsimons & Kucharczyk, 1978), this result could be explained by the fact that when AI is given I.V. it is converted to AII as the blood passes through the lungs before reaching the brain, whereas when AI is infused into a carotid artery there is less time for it to be converted to AII.

A similar kind of explanation could account for the relative lack of effect of renin as a dipsogen when given by intracarotid infusion as compared with I.V. infusion. Renin was infused into the carotid artery at the same rate as it was infused I.V. (instead of at less than one sixth the rate as was the case for the other components), yet despite this, the water intake after carotid infusion was much less than after I.V. infusion. Presumably, renin itself can only cause drinking through generation of AII. Given I.V., there is time for renin to act on substrate and for the AI formed to be converted to AII, but when renin is infused into the carotid artery there is little chance for substantial AII formation before the blood reached the brain.

The effects of intravenous and intracarotid infusion of components of the renin-angiotensin system on drinking behaviour therefore reinforce the conclusion arrived at from the intracranial injection experiments, that the angiotensin-sensitive thirst receptor responds most readily to AII.

Kozłowski, Drzewiecki & Zurawski (1972) found that AII infused I.V. at 50×10^{-12} mole min^{-1} (equivalent to 2–3×10^{-12} mole kg^{-1} min^{-1}) did not cause the dog to drink, but that this rate lowered the threshold of thirst induced by infusion of hypertonic saline. Other rates of infusion were not tried. Trippodo *et al.* (1976)

using two higher rates of infusion of AII (13 and 26×10^{-12} mole kg^{-1} min^{-1}) obtained significant increases in daily water intake by four dogs over a 10 day period of infusion (lower rate) and by two dogs over an 8 day period (higher rate). The present experiments in which a range of I.V. and intracarotid infusion rates were used resolve the question whether systemically administered AII alone can induce drinking in the dog, as it does in the rat (Fitzsimons & Simons, 1969; Hsiao, Epstein & Camardo, 1977), by showing that the threshold for thirst induced by I.V. infusion of AII lies between the infusion rates used in these other experiments. The threshold I.V. infusion rate is lower than that found in the rat. Hsiao *et al.* (1977) reported that 50% of rats drank in response to 25×10^{-12} mole kg min^{-1} and that virtually all animals responded to rates of $35–40 \times 10^{-12}$ mole kg^{-1} min^{-1}. In the present experiments an I.V. infusion rate of $8{\cdot}3 \times 10^{-12}$ mole kg^{-1} min^{-1} made half the dogs drink and a carotid infusion rate of 1×10^{-12} mole kg^{-1} min^{-1} made three dogs out of six drink, showing that the dog is considerably more responsive to AII than the rat.

On the other hand, Abraham, Baker, Blaine, Denton & McKinley (1975) concluded that a physiological role for AII in thirst is not proved. They found that I.V. infusion of AII at rates of 83, 333 and 667×10^{-12} mole min^{-1} for 3 days did not increase water intake in the sheep. Indeed at the highest rate of infusion used drinking was actually depressed. The rates of infusion were calculated to increase the arterial concentration of AII to 50, 200 and 400×10^{-12} M, which fall within the range of concentration of 10 to 1000×10^{-12} M that has been observed in the sheep in sodium deficiency and following obstruction of the inferior vena cava. As they obtained no drinking in response to I.V. AII, Abraham and her colleagues infused AII at 100, 400, 800 and 1200×10^{-12} mole min^{-1} for 45 min into one carotid artery with the contralateral carotid artery occluded. There is no basilar artery in the sheep and it was calculated that these infusion rates would produce cerebral blood concentrations ranging from $150–300 \times 10^{-12}$ M at the lowest rate to $200–400 \times 10^{-9}$ M at the highest. At the two highest rates of infusion the sheep drank between 250 and 1150 ml. within 5 min of the commencement of the infusion. At the 400×10^{-12} mole min^{-1} infusion rate, producing concentrations in the range of $700–1400 \times 10^{-12}$ M, i.e. within to just above the range of concentration that has been observed in the sheep, three out of four sheep drank. At the lowest rate one out of the four sheep drank. Despite the reservations of the authors as to the physiological significance of this angiotensin-induced drinking it should be emphasized that on occasions physiological amounts of AII caused the water-replete sheep to drink. Had there been an additional stimulus to thirst operating concurrently then physiological levels of AII would undoubtedly have contributed to drinking behaviour in this species as they do in the rat (Fitzsimons & Simons, 1969).

It is this last point that needs to be emphasized when assessing whether AII-induced drinking is a physiological phenomenon or not. AII-induced drinking is all the more remarkable when it is borne in mind that infusions of AII producing levels of hormone that occur naturally cause animals that are in water balance to drink. In real life such a rise in plasma AII would be associated with hypovolaemia or extracellular dehydration. As well as being responsible for the increased formation of AII, these changes are thirst stimuli in their own right since they cause drinking

in the nephrectomized animal, which lacks renin (Fitzsimons, 1969). Therefore when there is hypovolaemia as well as increased AII the amounts of AII needed to induce drinking would be less; the two thirst stimuli are additive in their effects on drinking.

By varying the rate and duration of I.V. infusion of AII it has also been shown in the present experiments that there is a well-marked dose–response relationship, more evident when the dose of AII was varied by changing the rate of infusion than by changing its duration (Fig. 2). The initial rate of drinking seems to be particularly important in determining this relationship because as the infusion continues the rate of drinking declines (Fig. 3). The initial rate of drinking is closely correlated with the rate of infusion; a high initial infusion rate produces a higher initial burst of drinking than a low infusion rate. But once the initial burst is over the rate of drinking slows down over the next 2 hr, more or less regardless of the rate of infusion. Varying the dose by varying the duration of infusion therefore results in a less significant correlation between water intake and dose of AII.

The decline in the rate of water intake after the initial burst of drinking is certainly caused by the operation of temporary satiety mechanisms (Adolph, 1967). Fatigue is ruled out by the fact that a prolonged infusion of isotonic saline preceding the AII, during which the dog is standing, did not affect the response. Tachyphylaxis is ruled out by the delayed access experiment (Fig. 3). The initial rate of drinking was in fact greater at the end of a 1 hr infusion of AII when the dog had been denied access to water during that hour than when it had been allowed to drink right from the beginning of the infusion. However, a preload of diluted milk slightly greater in volume than the amount of water the animal would have drunk in response to AII, effectively reduced the response to AII.

In conclusion, the experiments described here show that AII infused at rates that produce plasma levels comparable to those that occur in mild sodium depletion or hypovolaemia causes the dog to drink even though there is no concurrent additional stimulus to drink as there would be were there to be increased endogenous AII. The response to AII was dose-dependent, particularly when the dose was varied by changing the rate of infusion, and drinking behaviour was entirely normal. AII itself was the most effective of the components of the renin-angiotensin system tested, by I.V. or by intracarotid infusion, and it seems likely that renin, SRS and AI produced their effects through it; intracarotid infusions of renin and AI were relatively less effective than intravenous infusions, presumably owing to insufficient time for AII formation. Therefore AII is a physiological stimulus to thirst although the present experiments do not determine the extent of its contribution in natural thirst.

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