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SOME CENTRAL MECHANISMS OF THIRST IN THE DOG

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SUMMARY

1. Measurements of water intake were made on a population of trained conscious dogs of both sexes prepared with chronic third ventricle cannulae.

2. Injection of 100 ng angiotensin II into the third ventricle lead to a prompt stimulation of drinking, the mean water intake over a 5 min period being 503 ± 89 ml. (n = 6) compared with controls. This dipsogenic effect of angiotensin II was abolished by prior central administration of 10 μg saralasin acetate or 100 ng atropine.

3. Injection of 1 μg carbachol into the third ventricle produced a small, variable increase in drinking.

4. Injection of 0·2 ml. 5% NaCl into the third ventricle stimulated drinking, a response that was not affected by prior administration of 10 μg saralasin acetate or 100 ng atropine.

5. Following a 24 hr period of water deprivation there was an increase in plasma osmolality and plasma-renin activity. The drinking following this period of water deprivation was not affected by prior control administration of either 10 μg saralasin acetate or 100 ng atropine.

6. In two acute dogs, intracarotid infusion of ^{125}I angiotensin II was not followed by significant appearance of radioactivity in the third ventricle or cisterna magna c.s.f.

7. The relevance of these results to the control of water intake is discussed.

INTRODUCTION

Fitzsimons (1969) has suggested that peripherally generated angiotensin II may participate in normal thirst mechanisms because, for example, the increased drinking following caval ligation in the rat is abolished by nephrectomy and may be restored by intravenous infusion of angiotensin II (Fitzsimons & Simons, 1969). In the dog, thoracic caval constriction and renal hypertension lead to increased drinking,

apparently as a consequence of the increase in the activity of the renin-angiotensin system (Ramsay, Rolls & Wood, 1975; Rolls & Ramsay, 1975.)

In the rat, injections of small quantities of angiotensin into the brain increase water intake (Epstein, Fitzsimons & Rolls, 1970). In the dog, preliminary experiments have shown that administration of low doses of angiotensin into the third ventricle and into carotid loops stimulate drinking, whereas similar doses given intravenously do not. Such observations lead to the conclusion that angiotensin reacts with receptors in the brain tissue in order to stimulate drinking. As angiotensin II is an octapeptide, negatively charged at physiological pH, it would only penetrate the blood–brain barrier with difficulty. This raises the possibility, therefore, that the angiotensin II receptors may be outside the blood–brain barrier and there is evidence which suggests that in the rat the receptors are in the subfornical organ (Simpson & Routtenberg, 1973).

The results reported in this paper support the idea that angiotensin II does not penetrate the blood–brain barrier in the dog, and that central nervous system receptors must therefore lie on the vascular side of the barrier. Cholinergic stimulation of the hypothalamus, e.g. with carbachol, causes copious drinking in the rat (Grossman, 1960). This also appears to be true in the dog, and suggests that a neural pathway which transfers information from the angiotensin receptors to hypothalamic cells (Witt, Keller, Batsel & Lynch, 1952), contains a cholinergic link. This thesis is examined. The effects on drinking of injections of hypertonic saline into the third ventricle and of 24 hr water deprivation is considered in relation to these putative central pathways.

METHODS

All the experiments were done on mongrel dogs of either sex weighing from 11 to 16 kg. The animals were maintained on a fixed routine, being exercised in the morning and fed a fixed amount of dry commercial dog food at 2.00 p.m. each day. Apart from this, they remained in their kennels in a room kept at a temperature of approximately 24° C.

Acute experiments. These were designed to determine if angiotensin II crosses the blood–brain barrier and appears in the cerebrospinal fluid (c.s.f.). Two dogs were anaesthetized with sodium pentobarbitone (30 mg/kg I.V.). Polyethylene cannulae were inserted into a carotid artery and a femoral vein. A 20 S.W.G. stainless steel needle was placed either into the third ventricle using a stereotaxic technique or into the cisterna magna.

Angiotensin II (1-asp,5-ileu-angiotensin II, Schwartz/Mann, New York) was labelled with ^{125}I, using a method described by Stockigt, Collins & Biglieri (1971). The labelled angiotensin was infused through the carotid artery for 15 min and samples of c.s.f. were collected from the third ventricle or from the cisterna magna. Blood samples were taken at the same time from the femoral vein.

Chronic experiments. A third ventricle cannula was placed in each dog aseptically during anaesthesia by a method described previously (Reid & Ramsay, 1975). As these cannulae remained patent for some weeks, the animals were used as their own controls. All observations were made in the morning between 10.00 and 12.00 to ensure minimum interference with the normal routine of the animals.

Angiotensin II. Previous experience had shown that a dose of 100 ng angiotensin II injected into the third ventricle elicited a reproducible drinking response, and this dose was therefore employed in the present studies.

The angiotensin was made up in sterile 0·9 % NaCl so that 0·1 ml. solution contained 100 ng. This was injected via the third ventricle cannula and flushed in with 0·2 ml. saline. The animals were immediately returned to their home kennels and the volume of water consumed in the next 5 min was recorded. In control experiments, the same procedure was followed using 0·9 % NaCl instead of angiotensin.

Hypertonic saline. Preliminary experiments showed that reproducible drinking responses could be elicited by the injection of 0·2 ml. 5 % NaCl into the third ventricle. These experiments showed that 0·1 ml. 5 % NaCl did not always elicit a drinking response, presumably because of dilution of the saline by the c.s.f. in the ventricular system. After the injection, the animal was returned to its home kennel, and drinking monitored for the next 5 min.

24 hour water-deprivation. At approximately 10.30 on the morning of day (1) the dogs were carefully weighed on a spring balance, and a control blood sample collected from a forelimb vein by venepuncture. The animals were then returned to their kennels from which the water bowls had been removed. Precisely 24 hr later (day (2)) the dogs were reweighed and a second blood sample taken. They were then returned to their kennels and the quantity of water consumed over the next 5 min monitored.

Experiments with saralasin acetate. These experiments were designed to test the effect of intraventricular administration of 1-sarc-8-ala-angiotensin II (Saralasin acetate: Norwich Pharmacal Co.) on the drinking responses to angiotensin II, hypertonic saline and following 24 hr water deprivation. As saralasin acetate has some agonist activity, the procedure described previously was employed (Reid & Ramsay, 1975). Saralasin acetate (10 μg) was injected into the third ventricle and the dogs were then left for 2 min without water. In the angiotensin experiments, the test dose of 100 ng angiotensin II was flushed in with a further 10 μg saralasin acetate contained in 0·2 ml. 0·9 % NaCl after the 2 min period. In the hypertonic saline series, the second dose of 10 μg saralasin acetate was given with the 0·2 ml 5 % NaCl. In the water deprivation experiments, the second injection of 10 μg saralasin acetate was given after the 2 min period, and the animal allowed access to water for the next 5 min.

Experiments with carbachol and atropine. Preliminary experiments showed that administration of 100 ng atropine sulphate into the third ventricle had no obvious effects on the animal's behaviour, did not affect heart rate and did not inhibit feeding. There was no stimulatory effect on drinking.

In eight dogs, the effect of intraventricular administration of carbachol in doses ranging from 0·25 to 5 μg on water intake was tested. Doses of 0·25 and 0·5 μg had no effect on drinking. Doses of 2 and 5 μg had widespread systemic effects on the dogs including initiation of vomiting and defaecation. However, an injection of 1 μg carbachol was not followed by these effects. In five of the eight dogs an average of 90 ml. water was drunk over a period of 5 min, the other three drinking nothing. Preliminary intraventricular administration of 100 ng atropine sulphate abolished the rather irregular stimulation of drinking which followed the intraventricular administration of 1 μg carbachol.

The effect of atropine on the drinking responses to angiotensin, hypertonic saline and water deprivation was now studied. The procedure was similar in each case in that 100 μg atropine sulphate in a volume 0·1 ml. was administered into the third ventricle, followed a few seconds later by either 100 ng antiogensin II or 0·2 ml. 5% NaCl and the drinking monitored for 5 min. In the 24 hr water deprivation studies, the effect of atropine on the volume of water consumed in the 5 min test period was measured.

Chemical assays. All blood samples were collected into heparinized syringes.

Plasma–renin activity was measured by a method which utilizes a radio-immuno-assay for angiotensin I (Stockigt *et al.* 1971; Reid, Stockigt, Goldfien & Ganong, 1972). Plasma–renin activity is expressed as ng angiotensin I formed in plasma during a 3 hr incubation (ng/ml. 3 hr).

Plasma sodium and potassium concentrations were measured using an Instrumentation Laboratory Model 143 flame photometer. Plasma osmolality was determined cryoscopically on an Advanced Instruments Model 64-31 Osmometer.

Results are presented as means and S.E. of the mean. Statistical significance was assessed using the paired *t* test.

RESULTS

^{125}I *angiotensin II infusions*

Intracarotid infusion of ^{125}I angiotensin II in two dogs produced plasma radioactivity levels at the end of the infusion periods of 21,546 and 34,360 counts/ml. min. The maximum radioactivity in the c.s.f. of these two dogs was 760 and 235 counts/ml. min respectively. These results indicate that if angiotensin II, rather than its break-down products, enters the c.s.f., it does so at a very slow rate.

Angiotensin responses

The injection of 100 ng angiotensin II directly into the third ventricle was followed by a vigorous drinking response lasting up to 4 min. As soon as the injection was made, the stilette replaced in the cannula and the animal returned to its kennel, the whole procedure taking no more than 10 sec, the dog walked to the water bowl and started to drink. No other behavioural response was seen in any of these experiments. Indeed, the response is so reproducible, that we now use it routinely in chronic dogs to test the patency of chronically implanted third ventricle cannulae. Occasionally the dog returned to the water bowl and drank a small amount at 6 and 7 min. All measurements were therefore made over a 5 min period, during which the dogs drank 503 $\pm$ 89 ml. water (Table 1). Injection of 0·1 ml. 0·9% NaCl into the third ventricle did not stimulate drinking.

The prior administration of 10 μg saralasin acetate completely abolished the angiotensin induced drinking. The dogs appeared to show no interest in the water.

Similarly, prior administration of 100 μg atropine sulphate into the third ventricle completely abolished the dipsogenic effect of 100 ng angiotensin II. Two of the six dogs walked over to the water bowl and sniffed at the water, but did not drink and moved away.

TABLE 1. The dipsogenic effect of 100 ng angiotensin II injected into the third ventricle of six dogs, and its modification by saralasin acetate and atropine sulphate. All substances were flushed in with 0·2 ml. 0·9 % NaCl

Treatment	Water drunk in 5 min (ml.)
100 ng angiotensin II in 0·1 ml. 0·9 % NaCl	573 ± 89
0·1 ml. 0·9 % NaCl	0
100 ng angiotensin II in 0·1 ml. 0·9 % NaCl and 10 μg saralasin acetate	0
100 ng angiotensin II in 0·1 ml. 0·9 % NaCl and 100 ng atropine sulphate	0

Means and S.E. of the mean are shown.

In some of the experiments with saralasin acetate and atropine, food was offered at the end of the 5 min test period. The dogs ate this with their normal vigour, so there was no evidence of non-specific reduction in motor activity caused by these drugs.

Hypertonic saline experiments

Preliminary experiments showed that third ventricular administration of 0·2 ml. 5 % NaCl gave a small but reproducible dipsogenic response. In our hands, 0·1 ml. 5% NaCl was not always effective and doses larger than 0·2 ml. produced vomiting and shivering. The dose of 0·2 ml. was therefore used in all experiments.

The injection of 0·2 ml. of 0·9 % NaCl had no effect on drinking, whereas 0·2 ml. 5% NaCl caused a reproducible increase in water intake (Table 2). The response varied from dog to dog, but was similar when tested on different days on individual dogs.

The stimulation of water intake by the hypertonic saline was not affected by prior administration of either saralasin acetate or atropine sulphate.

24 hr water deprivation

In all animals body weight was reduced during the 24 hr period of water deprivation (Table 3). The dogs were not unduly distressed by this procedure and retained their usual friskiness. When presented with water after this period, they drank immediately and stopped, presumably because they were satiated, within 5 min.

TABLE 2. The dipsogenic effect of 0·2 ml. 5% NaCl injected into the third ventricle of four dogs, and its modification by intraventricular salalasin acetate and atropine sulphate

Treatment	Water drunk in 5 min (ml.)
0·2 ml. 0·9% NaCl	0
0·2 ml. 5% NaCl	160 ± 67
0·2 ml. 5% NaCl preceded by 10 μg saralasin acetate	145 ± 47
0·2 ml. 5% NaCl preceded by 100 ng atropine sulphate	163 ± 46

Means and S.E. of the mean are shown.

TABLE 3. Drinking following a period of 24 hr of water deprivation in two groups of five dogs. The saralasin acetate and atropine sulphate were injected via the third ventricle cannula (see text)

Treatment	Weight loss (kg)	Water drunk in 5 min (ml.)
24 hr water deprivation	0·35 ± 0·06	570 ± 146
24 hr water deprivation plus saralasin acetate	0·50 ± 0·16	537 ± 184
24 hr water deprivation	0·63 ± 0·10	534 ± 108
24 hr water deprivation plus atropine	0·66 ± 0·09	522 ± 88

Means and S.E. of the mean are shown.

TABLE 4. Effect of a 24 hr period of water deprivation on plasma-renin activity, plasma osmolality and sodium and potassium concentrations in 8 dogs

Observation	Control	Following 24 hr water deprivation	*P* value for difference
Plasma-renin activity (ng/ml. 3 hr)	1·54 ± 0·21	2·90 ± 0·53	$P < 0·05$
Plasma osmolality (m-osmole/kgH_2O)	299 ± 2·3	304 ± 2.1	$P < 0·001$
Plasma sodium (m-equiv/l.)	142 ± 0·8	148 ± 0·6	$P < 0·001$
Plasma potassium m-equiv/l.	4·1 ± 0·10	4·1 ± 0·06	n.s.

Means and S.E. of the mean are shown.

Table 4 shows the effect of 24 hr water deprivation on some body fluid variables. There were significant increases in plasma osmolality, and plasma sodium concentration, but no change in plasma potassium concentration. There was a significant increase in plasma renin activity which nearly doubled over the 24 hr period.

In the first group of five dogs (Table 4), the amount of water consumed was not affected by intraventricular injection of saralasin acetate. Following 24 hr water deprivation in control experiments, the dogs drank 570 ± 146 ml. and following treatment with saralasin 537 ± 184 ml.

The drinking response to 24 hr water deprivation was also unaffected by atropine when this was tested in a second group of five dogs.

DISCUSSION

The experiments with labelled angiotensin II indicate that this peptide does not cross the blood–brain barrier. The absence of angiotensin II activity in both the third ventricle and cisterna magna c.s.f., even after 15 min of intracarotid infusion, makes it extremely unlikely that peripherally generated angiotensin II can exert its effects on the C.N.S. via the major pools of c.s.f. This is particularly emphasized by the finding that dogs will start to drink approximately 5 min after the start of an intravenous infusion of angiotensin II at a rate of 40 ng kg^{-1} min^{-1} (D. J. Ramsay, unpublished). If angiotensin has to pass through the c.s.f. in order to bring about its effects, it should certainly be present there well within 15 min. It is arguable that local penetration of angiotensin into C.N.S. sites might occur at points where the blood–brain barrier is deficient, and the subfornical organ, one of the circumventricular organs, is a likely candidate (Simpson & Routtenberg, 1973).

The finding that injection of angiotensin into the third ventricle stimulates copious drinking in the dog is similar to that reported in other species (e.g. Epstein *et al.* 1970). Presumably angiotensin administered in this way can reach receptors in the C.N.S. which are likely to be similar to these involved in the drinking response to peripherally generated angiotensin. The finding that the drinking is abolished by saralasin acetate is in keeping with the well-known effects of this competitive angiotensin antagonist.

The effects of atropine on drinking induced by central administration of angiotensin II in the rat are controversial (Severs & Daniel-Severs, 1973). Much of the discrepancy in the literature could be due to variations in dose and method of administration of the two agents. In our experiments in the dog, central administration of 100 ng atropine completely abolished the dipsogenic effects of 100 ng angiotensin II. This appeared to be specific in that the same dose of atropine had no effect on drinking due to hypertonic saline or to water deprivation. These findings support the hypothesis that the pathway between the angiotensin receptors and the hypothalamic 'thirst centre' contains a cholinergic link. It is interesting to note that lesions of the subfornical organ in the rat lead to a marked

reduction in angiotensin and carbachol-induced drinking (Simpson & Routtenberg, 1973).

The stimulation of water intake following 0·2 ml. 5% NaCl in the third ventricle of the dog confirms the observations of Andersson (1953) that hypertonic saline introduced in the region of the hypothalamus in the goat causes drinking, whereas 0·9 % NaCl has no effect. In our experiments in the dog, this drinking response to hypertonic saline was not affected by either atropine or saralasin acetate. It is tempting to conclude that there is neither a cholinergic link, nor a mechanism which depends on angiotensin, in the pathway between the perception of the osmotic stimulus presented by the hypertonic saline and drinking.

The rapid satiation of the fluid deficit resulting from a period of water deprivation in the dog shown in our experiments is well known (Adolph, 1950). The 24 hr period of dehydration brought about significant increases in plasma-renin activity and plasma osmolality. As the intake of water following 24 hr deprivation was not depressed by central administration of atropine or saralasin acetate in doses which completely blocked the dipsogenic effect of a relatively large amount of angiotensin II, it would seem unlikely that increased angiotensin levels form an essential part of the mechanism. The plasma osmolality rose by 3·4 % (10 m-osmole/kg) during water deprivation in our experiments. Wolf (1950) has calculated from experiments involving intravenous infusion of hypertonic saline that the percentage decrease in cellular water content brought about by increased extracellular fluid osmolality necessary to stimulate thirst is $2{\cdot}15 \pm 0{\cdot}64\%$ in the dog. This is similar to the increase of 1–2 % in extracellular fluid osmolality found by Verney (1947) to increase antidiuretic hormone secretion in the dog. The increase in 3·4% in plasma osmolality in our dogs would therefore be sufficient to stimulate drinking. It is tempting to draw the conclusion from these experiments that the drinking following 24 hr water deprivation may take place in the absence of the participation of the renin–angiotensin system, and is strongly influenced by the raised plasma osmolality brought about by dehydration.

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