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Effect of an Angiotensin Antagonist, Sar[1]-Ala[8]-Angiotensin II on Physiological Thirst[1]

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ABRAHAM, S. F., D. A. DENTON, M. J. MCKINLEY AND R. S. WEISINGER. *Effect of an angiotensin antagonist Sar[1]-Ala[8]-angiotensin II on physiological thirst.* PHARMAC. BIOCHEM. BEHAV. 4(3) 243–247, 1976. – Initially it was shown that infusion of Sar[1]-Ala[8]-angiotensin II (P113) into the third ventricle (50–100 μg/ml at 1.1 ml/hr) effectively abolished the large water intake induced 1–2 min after beginning an intracarotid infusion of angiotensin II at 800 ng/min which causes an unphysiologically high concentration of angiotensin II in cerebral arterial blood. Infusion of P113 (50–100 μg/ml at 1.1 ml/hr) into the third brain ventricle for 20 min prior to and during presentation of water to sheep after 48 hr water deprivation did not reduce water intake. Water intake associated with rapid food intake or carotid artery infusion of hypertonic NaCl was similarly unaffected by intraventricular administration of P113. While high concentrations of angiotensin II are dipsogenic in sheep, these results cast doubt on a contributory role for angiotensin II in thirst caused by water depletion or rapid food intake in the sheep.

Angiotensin II P113 Thirst Physiological role

ADMINISTRATION of angiotensin II into the bloodstream or directly into the brain rapidly induces water drinking in several species [2, 3, 9, 13, 23]. Nephrectomy causes attenuation or abolition of drinking in response to some hypovolaemic situations [8, 11, 15] and it has been proposed that changes in angiotensin II blood level may partly mediate hypovolaemic thirst, although to what extent was not estimated [11]. While it can be shown that angiotensin II is a potent dipsogenic agent in sheep, findings from this laboratory indicated that if exogenous angiotensin II is infused systemically, the blood concentrations required to stimulate drinking in sheep are sudden changes to supraphysiological levels [2].

Since the Sar[1]-Ala[8]-angiotensin II derivative (P113) effectively antagonises the pressor response to angiotensin II [20], and the dipsogenic action of intraventricularly administered angiotensin II [9], this compound (P113) may provide a tool for studying the significance of endogenously generated angiotensin II in physiological thirst mechanisms.

The aim of the present experiments was to test for a role of angiotensin II in 2 physiological situations commonly associated with water drinking in sheep and in which some degree of hypovolaemia and increased renin levels may occur, namely water deprivation and rapid eating. The Sar[1]-Ala[8]-angiotensin II derivative (P113), at doses which inhibit angiotensin II water drinking, has been administered into the third ventricle before and during these 2 situations and its effect on water intake measured.

METHOD

Animals

Four merino ewes, aged 2–4 years, were used. Approximately 1 month before experiments, these animals were surgically prepared with 2 bilateral carotid artery skin loops in the neck [10] and a cannula was permanently implanted in the third ventricle [2]. These sheep were housed in metabolism cages which allowed separate collection of urine and faeces. Unless required otherwise by experimental protocol, animals were fed daily 0.9 kg oaten-lucerne chaff at 1600 hr and were allowed water ad lib.

Determination of Intraventricular Dose of P113 Needed to Antagonise the Dipsogenic Action of a Carotid Artery Infusion of Val[5]-Angiotensin II

Confident undisturbed animals were prepared on the day of the experiment with a polyethylene cannula in a carotid artery, and the contralateral carotid was occluded by pneumatic cuff to allow distribution of infusate from the cannula to both sides of the brain [4,5]. In 9 control experiments, artificial sheep CSF [19] was infused into the third ventricle at 1.1 ml/hr for 40 min with an initial

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loading injection of 0.2 ml. Twenty min after the commencement of the CSF infusion, val^5-angiotensin II (Hypertensin, CIBA) was infused into the carotid artery at 800 ng/min (2 μg/ml in 0.9% NaCl at 0.4 ml/min). Water was available on the side of the cage, and after 20 min of angiotensin II infusion, the volume of water drunk was measured. In 11 other experiments the procedure was identical to the control experiments, except that Sar^1-Ala^8-angiotensin II (P113, Saralasin Acetate, Norwich Pharmacal Co.) was dissolved in the artificial CSF infused into the third ventricle. Concentrations of P113 used ranged from 1–100 μg/ml. On the day following P113 infusions which antagonised water drinking, an additional control experiment was performed to test for reversibility of effects observed.

Effect of Intraventricular P113 on Water Intake after 48 Hr Water Deprivation

Animals were deprived of water for 48 hr. In 8 control experiments in which no ventricular infusion was made, the volume of water drunk during the first 20 min after presentation of water following the 48 hr water deprivation was measured. In another 8 experiments following 48 hr water deprivation, P113 in artificial CSF at 50 μg/ml in 3 animals and 100 μg/ml in another animal was infused into the third ventricle at 1.1 ml/hr for 40 min following an initial loading dose of 0.2 ml. These doses had previously been found to effectively antagonise the dipsogenic action of angiotensin II infused into the carotid artery. Twenty min after commencing the P113 infusion, water was presented to the animals and the volume drunk in 20 min measured. Bodyweight was determined before and after water deprivation.

Effect of Intraventricular P113 on Water Intake Associated with Feeding

On 3 experimental days where no ventricular infusion was made, the volume of water drunk and weight of food eaten was measured hourly for 5 hr. On the third experimental day, P113 in artificial sheep CSF was infused into the third ventricle at 1.1 ml/hr for 5 hr, after a loading dose of 0.2 ml. Concentration of P113 was 50 μg/ml in 3 of the animals and 100 μg/ml in the other. Twenty minutes after commencement of P113 administration, food was presented at the usual time and water intake measured hourly for 5 hr. One month later the experiments were repeated on the same 4 animals.

Effect of Intraventricular P113 on Water Intake Caused by Carotid Artery Infusion of Hypertonic NaCl

Using a similar procedure to protocol (i) but using 4M NaCl rather than angiotensin II (800 ng/min) the effect of infusion of P113 (50 μg/ml in 3 animals, 100 μg/ml in the other) into the third ventricle on thirst caused by carotid artery infusion of 4M NaCl at 0.4 ml/min was tested.

RESULTS

Effect of Ventricular Infusion of P113 on Thirst Caused by Carotid Artery Infusion of Angiotensin

In control experiments the volume of water drunk in response to a carotid artery infusion of angiotensin II (800 ng/min) ranged from 450–1600 ml. Mean water intake was 970 ± 242 ml (Mean ± SEM) n = 5. In 3 animals tested twice, P113 infusion into the third ventricle at concentration of 50 μg/ml completely abolished this response to intracarotid angiotensin II (Fig. 1). In the fourth animal, P113 (50 μg/ml) was ineffective in reducing water intake, but at 100 μg/ml reduced water intake to 0 and 50 ml in the 2 tests undertaken. These effective antagonistic concentrations of P113 (50 μg/ml in three sheep and 100 μg/ml in the other) were used in future experiments. Concentrations of 1 and 2 μg/ml had little effect on water intake caused by carotid artery infusion of angiotensin II.

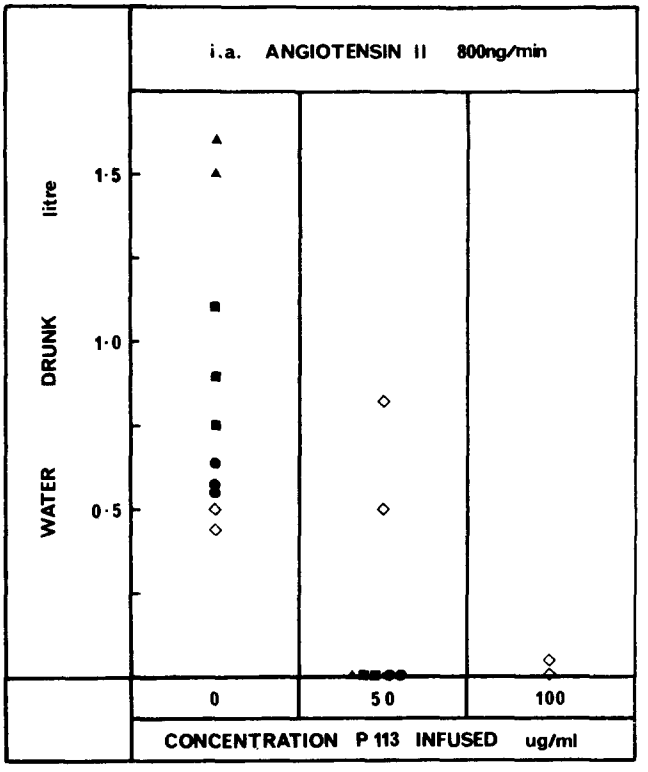

FIG. 1. The volume of water drunk during 20 min in response to intracarotid artery infusion of angiotensin II (800 ng/min) during ventricular infusion (1.1 ml/min) of a normal artificial CSF solution (Control) or P113 at 50 and 100 μg/ml. Gitta ▲, Suzuki ■, Ouida ◇, Winifred ●.

The drinking response to intracarotid infusion of angiotensin II had returned to control levels 24 hr after inhibition by ventricular infusion of P113 (Fig. 2). In addition, no water drinking was observed in response to P113 infusions during the 20 min period of observation prior to commencing angiotensin II into the carotid artery at 800 ng/min.

Effect of Ventricular Infusion of P113 on Water Intake After 48 Hr Water Deprivation

Ventricular infusion of P113 (50 or 100 μg/ml) had no significant effect on water intake caused by water deprivation for 2 days. The volumes of water drunk in the 20 min following 48 hr water deprivation by control and P113 treated animals are shown in Fig. 3. Water intake ranges were 1050–2500 ml in control experiments and 1300–2350 ml in P113 treated experiments, and the mean water intakes for the control and P113 treated animals were

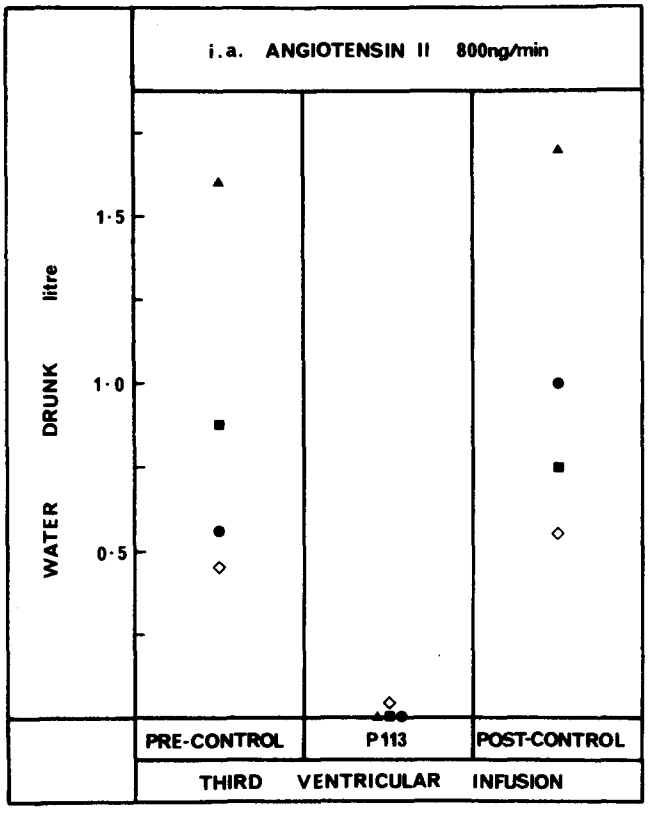

FIG. 2. The volume of water drunk during 20 min in response to intracarotid infusion of angiotensin II (800 ng/min) during ventricular infusion of normal artificial CSF 2 days prior to (pre-control) and the day after (post-control) ventricular administration of P113 to 4 animals at 50 μg/ml (Gitta ▲, Suzuki ■, Winifred ●) or 100 μg/ml (Ouida ◇).

2056 ± 167 ml (Mean ± SEM) n = 8 and 1844 ± 116 ml, n = 8, respectively. There was no significant difference between the water intakes of the 2 groups of animals ($p>0.05$, student t-test). The mean decreases in bodyweight during water deprivation in the control and P113 treated groups were 2.4 ± 0.1 kg and 2.1 ± 0.1 kg respectively.

Effect of Ventricular Infusion of P113 on Water Intake Associated with Feeding

The cumulative water intakes during the first 5 hr of daily access to food, for the 2 days prior to and the day of ventricular infusion of P113 for the 4 animals are shown in Fig. 4. The cumulative water intakes for these 4 animals when the experiments were repeated after 1 month are presented in the same manner in Fig. 5.

The infusion of P113 had no effect upon the water or food intakes of 3 animals during the first 5 hr after feeding (n = 6). Range of water intake in control experiments was 700–1650 ml with mean ± SEM of 1058 ± 89 ml, (n = 12) and range with ventricular infusion of P113 was 700–1800 ml with mean value of 1058 ± 161 (n = 6). There was a highly significant linear relationship between food eaten and water drunk for these 3 animals during control experiments ($p<0.001$) and P113 treated experiments ($p<0.001$). The fourth animal (Winifred) did not drink during the 2 P113 infusions. Water was drunk by this

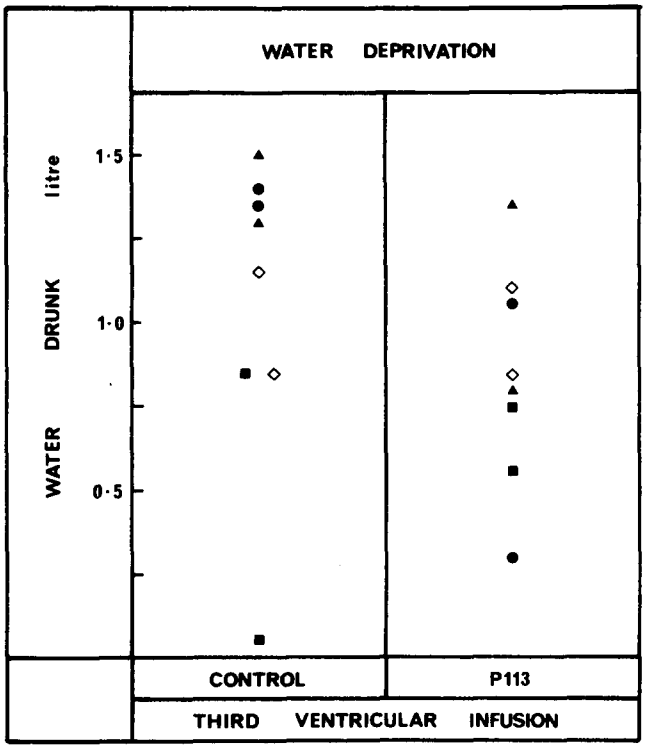

FIG. 3. The volume of water drunk in 20 min following 48 hr water deprivation in control experiments or experiments involving ventricular infusion of P113 (1.1 ml/min) Gitta ▲, Suzuki ■, Winifred ●, at 50 μg/ml and Ouida ◇, at 100 μg/ml.

animal on all control days, 250–950 ml being drunk within 5 hr of presentation of food, with mean of 613 ± 160 (n = 4). In the first series of experiments (i.e. controls and P113 treated) no eating occurred during the observation period. In the second series of control and P113 treated experiments (1 month later) all food was eaten within 2–3 hr of presentation.

Effect of Ventricular Infusion of P113 on 4M NaCl Induced Thirst

In control experiments the volume of water drunk in response to 4M NaCl ranged from 500–1100 ml. The mean volume of water drunk was 775 ± 90 ml (n = 6). During P113 infusion the volume of water drunk in response to 4M NaCl ranged from 300–1250 ml. There was no significant difference between the volume of water drunk when control and P113 experiments were compared by student t-test.

DISCUSSION

Intraventricular doses of P113 used in these experiments inhibited water drinking to carotid artery infusion of angiotensin II at 800 ng/min. This carotid artery infusion produces a sudden unphysiologically high concentration of angiotensin II (120–240 ng/100 ml) in cerebral blood [2]. Thus the doses of intraventricular P113 which inhibited thirst in response to intracarotid angiotensin II would have been expected to inhibit the central dipsogenic action of any contemporary physiological level of angiotensin II in circulating blood.

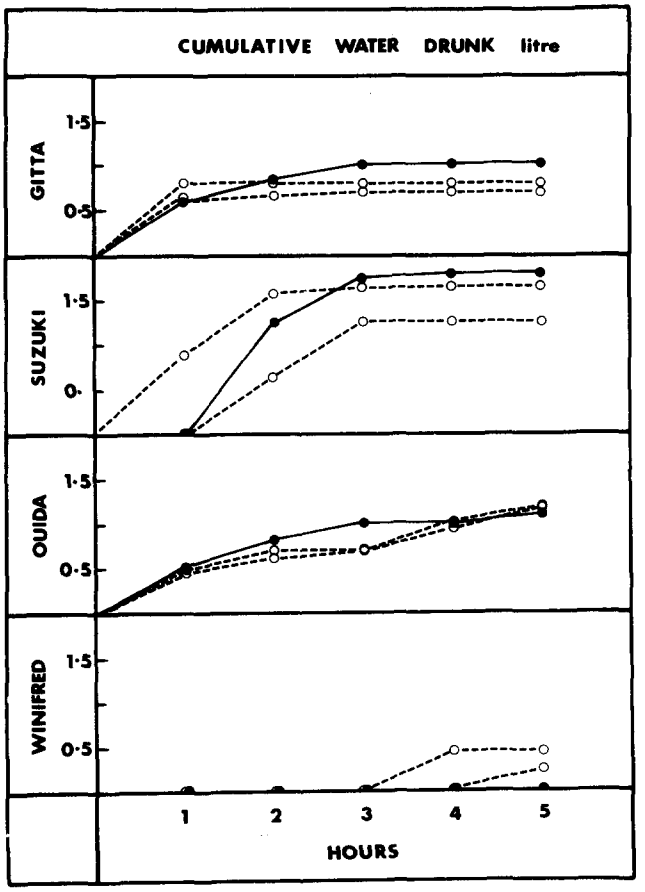

FIG. 4. The cumulative water intake during the first 5 hr of daily access to food, for 2 days prior (broken lines - - - -) and the day of ventricular infusion (1.1 ml/hr) of P113 at 50 μg/ml (Gitta, Suzuki and Winifred) or 100 μg/ml (Ouida). (Solid lines ———).

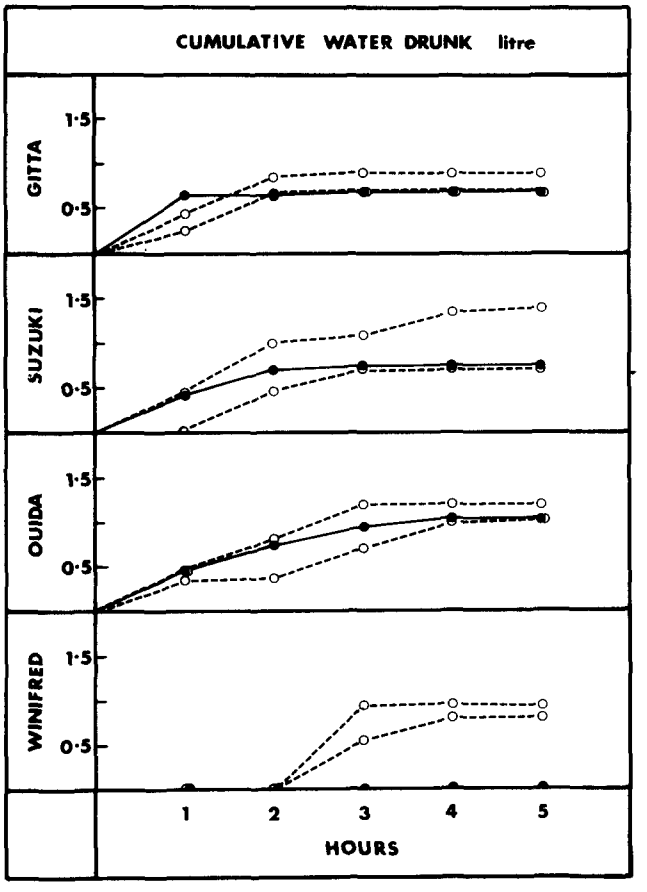

FIG. 5. Repeat experiment of that shown in Fig. 4. The cumulative water intake during the first 5 hr of daily access to food, for 2 days prior (broken lines - - - -) and the day of ventricular infusion (1.1 ml/hr) of P113 at 50 μg/ml (Gitta, Suzuki, and Winifred) or 100 μg/ml (Ouida). (Solid lines ———).

Water deprivation for 48 hr resulted in bodyweight loss of 2 kg, and is probably associated with decreased extracellular fluid volume. Dehydration has been shown to increase plasma renin activity in man [19] and rat [14,22] and water restriction to increase plasma renin concentration in sheep [7]. Since P113 administration failed to significantly change water intake after 48 hr water deprivation, it is unlikely that central actions of angiotensin II of renal origin contribute to thirst resulting from dehydration in sheep. This thirst is probably mediated by changes in body fluid tonicity. The failure of ventricular infusion of P113 to inhibit drinking in response to intracarotid artery infusion of 4M NaCl, at concentrations known to inhibit angiotensin induced thirst, further confirms the distinction between osmotic thirst and angiotensin II induced thirst [8], and also confirms the specificity of P113 as an angiotensin antagonist in that it does not cause nonspecific depression of neural activity involved in thirst. The failure of P113 to influence drinking in response to water deprivation or 4M NaCl can also be regarded as evidence against a hypothesis that angiotensin II is a neural transmitter in elements of the thirst system. One possibility not excluded by the present experiments is that modification of central nervous system activity by blood angiotensin II over 48 hr of water deprivation may not be reversed by ventricular infusion of P113 for 40 min. Such a modification, it has been suggested, may be change in sodium concentration or flux within particular compartments of the CNS [3].

In many species of animals, eating and drinking tend to occur together [21,24]. Simultaneous recordings of eating and drinking in the rat [12,16], showed that at least 70% of the total intake of water occurred just before, during and immediately after meals. The percentage of water drunk in association with feeding in 3 of the 4 sheep would appear to be even greater, possibly because of the dry nature of the diet. The highly positive correlation found between amount of food eaten and water drunk in 3 sheep in the first 5 hr after food presentation is in agreement with findings in the rat [12].

Blair-West and Brook [6] studied the effects of feeding dry food (wheaten-lucerne chaff) on the circulation, renin release and renal function in the sheep. Water was not available during feeding. After commencement of feeding there was a rapid reduction in plasma volume after 5 min which reached maximum after 30–60 min. There was an increase in plasma renin concentration in animals which ate rapidly, but slow feeders did not show this response. Since P113 failed to modify water intake associated with feeding in 3

of the animals it is unlikely that angiotensin II of renal origin is an important factor in this thirst. The fourth animal (Winifred) was inconsistent in its response, and as it did not eat rapidly in the first experiments it is unlikely that plasma renin concentration increased. It is possible that ventricular perfusion per se affected the response in this animal, a factor not ruled out by these experiments.

While these experiments in sheep do not favour an important role for angiotensin II of renal origin in the two physiological thirst states studied, pure hypovolaemia was not produced and it is possible that in another species, the rat, it may be important. It has recently been shown that water intake in response to isoproterenol and to a lesser extent polyethylene glycol is reduced by renin antiserum [1]. The results of Lehr *et al.* [17] using a converting enzyme inhibitor in the rat however, also suggest that other renal factors beside the renin-angiotensin II system may be of importance in extracellular thirst.

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