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Plasma Angiotensin II Levels and Water Intake Following β-Adrenergic Stimulation, Hypovolemia, Cellular Dehydration and Water Deprivation[1]

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ABDELAAL, A. E., P. F. MERCER AND G. J. MOGENSON. *Plasma angiotensin II levels and water intake following β-adrenergic stimulation, hypovolemia, cellular dehydration and water deprivation.* PHARMAC. BIOCHEM. BEHAV. 4(3) 317–321, 1976. — A comparison of the effects of extracellular and intracellular thirst stimuli on plasma levels of angiotensin II was made in rats. The administration of polyethylene glycol and isoproterenol elicited a strong drinking response and resulted in a significant increase in plasma angiotensin II. There was a significant correlation between the volume of water intake and plasma angiotensin II levels following the injection of polyethylene glycol but not following isoproterenol. Drinking was also elicited by the administration of hypertonic saline but there was no increase in plasma angiotensin II. The results suggest that endogenously released angiotensin II contributes to extracellular thirst but not to intracellular thirst.

Angiotensin II Intrajugular chemical injection Polyethylene glycol Isoproterenol Thirst Drinking

A number of procedures that reduce the extracellular fluid volume and activate the renin-angiotensin system [17, 29, 36] have also been reported to elicit drinking [11, 33, 34]. Extracellular thirst stimuli become less effective in eliciting drinking in nephrectomized rats [12, 13, 19, 25]. Since drinking is initiated by the intravenous infusion of angiotensin II [1,16] and by the administration of angiotensin II directly to the preoptic area and other regions of the brain [4, 6, 10, 31] it has been hypothesized that angiotensin II is a chemical mediator, and possibly even a thirst hormone for extracellular thirst [15].

Previous studies suggest that plasma angiotensin II levels should be elevated when drinking is elicited by an extracellular thirst stimulus. The use of a chronic venous cannula for withdrawing blood in freely moving rats [1] makes it possible to investigate this possibility without the complication of angiotensin levels being elevated by stress while obtaining the blood sample. In a preliminary study it was shown that following hemorrhage an increase in plasma angiotensin II levels was associated with the drinking of water [28]. In the present study plasma angiotensin II levels and water intake were determined following the administration of polyethylene glycol, an extracellular thirst stimulus [33], and these values compared to similar measures obtained after the administration of hypertonic saline, an intracellular thirst stimulus [7]. Water intake and plasma angiotensin II levels were also measured following the administration of isoproterenol, a potent stimulus for drinking [24] and for renin release [25], following water deprivation, and following a control injection of isotonic saline.

METHOD

Animals

One hundred seven male Wistar rats weighing 300–400 g at the time of the experiment were used. They were housed in individual wire mesh cages at an environmental temperature of 22 ± 1°C with 12 hr light and 12 hr darkness each day. Unless otherwise indicated Purina rat chow diet and tap water were provided ad lib. Water was provided in glass bottles fitted with standard stainless steel drinking spouts. The intake was measured by weighing the bottle to the nearest 0.5 g and this measurement was equated with volume when expressing the amount of water consumed.

Blood Sampling and Angiotensin II Assay

In order to facilitate obtaining blood samples for the determination of plasma angiotensin II in the conscious, unrestrained animal, a chronic cannula was implanted in the

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right jugular vein of each animal under sodium pentobarbitol anesthesia using the method described previously [28]. After a recovery period of 1 week testing procedures were begun.

Blood samples for measurement of angiotensin II concentration were taken before (control samples) and after the animals were subjected to various thirst stimuli. The jugular cannula was connected to 60 cm length of polyethylene tubing (PE-60) attached to a 1 ml disposable plastic syringe. The first few drops of blood were discarded and the required sample (approximately 1 ml) was withdrawn and transferred immediately into a test tube chilled in ice. Ethylene-diaminetetracetate-Na_2 (EDTA) was used as anticoagulant. Blood samples were spun in a refrigerated centrifuge at 4° C. Aliquots of plasma (0.15 ml) were pipetted into disposable polystyrene tubes (Falcon 2052), sealed with parafilm and stored at −20° C.

Levels of plasma angiotensin II were later determined according to the radioimmunoassay procedure described by Bailie and coworkers [5]. The sensitivity of the assay was estimated to be approximately 4 pg/ml and the variation coefficients for the increments of 6, 25, 50 and 100 picograms of angiotensin II in the incubation mixture were 2.9, 2.7, 5.2 and 6.5% respectively.

Drinking Tests

The procedures for the initiation of drinking described below began between 9:00 a.m. and 10:00 a.m.

Polyethylene Glycol (PG)

Food was removed from the cage and a sample of blood obtained. One hr later PG (20,000 J. T. Baker Chemicals, Phillipsberg, N.J., U. S. A.) prepared in a concentration of 30% W/V in 0.9% NaCl or isotonic saline (0.9% NaCl) was injected subcutaneously and the water bottles removed. The injections of PG and saline were 10 ml/kg body weight, half administered to each side of the lower back. In the first series of animals a second blood sample was collected and water made available 3 hr after the injection. In a second series of animals the blood sample and replacement of water occurred 6 hr after the injection. The experiment was terminated after a total of 8 hr so that for Series 1 water intakes were recorded each hour for 5 hr and for Series 2 each hr for 2 hr.

Isoproterenol

Food was removed from the cage and a sample of blood obtained. One hr later isoproterenol (0.15 mg/kg) or isotonic saline was injected subcutaneously in a volume of 0.6 ml/kg body weight. A second blood sample was obtained either at the onset of drinking or after a period of 15, 30 or 60 min after the injection. Water intake was recorded for 2 hr after the injection.

Hypertonic Saline

Food was removed from the cage and a sample of blood obtained. One hr later hypertonic saline (2.5 M) or isotonic saline in a volume of 5 ml/kg body weight was injected subcutaneously. Water intake was recorded 30 min later and a second blood sample obtained. Water intake was also recorded for the next 2.5 hr. Since the animals drank a considerable amount of water during the 30 min prior to obtaining the second blood sample in a second series of animals the water bottles were removed from the cages for 30 min after the injection.

Water Deprivation

A sample of blood was obtained and 2 hr later water, but not food, was removed. After 24 hr of water deprivation a second blood sample was obtained and water made available. Water intake was recorded each hr for the next 6 hr. For the control procedure two blood samples were obtained 24 hr apart with water available ad lib.

Statistical analysis of the data was performed with t test for correlated samples. Pearson Product Moment correlations between plasma angiotensin II concentration and water intake were also calculated. In all cases, a probability level of less than 5% (i.e. $p<0.05$) was accepted as being statistically significant.

RESULTS

The effects of subcutaneous administration of polyethylene glycol on the plasma levels of angiotensin II and on water intake are shown in Table 1. For Series 1, 3 hr after the administration of polyethylene glycol the plasma angiotensin II had increased 97%. When water was then made available the rats drank 3.1 ± 0.29 ml compared to 0.5 ± 0.12 ml for the control test during the next hr and 10.0 ± 0.58 ml during the 5 hr observation period. There was a significant positive correlation ($r = 0.62$, $p<0.05$) between the angiotensin II levels and the volume of water intake. For the rats in Series 2, in which water was not made available until 6 hr after the administration of polyethylene glycol, the level of plasma angiotensin II had increased 178% and the water intake was 6.9 ± 0.95 ml compared to 1.1 ± 0.08 ml for the control test during the next hour and 7.9 ± 0.95 ml during the 2 hr observation period. There was a low positive correlation between plasma angiotensin II levels and volume of water intake but it was not significant ($r = 0.26$, $p>0.05$). Following control injections of isotonic saline in the same animals there was no change in the levels of plasma angiotensin II (see Table 1).

Plasma angiotensin II levels and water intake following the subcutaneous administration of isoproterenol compared to control injections of isotonic saline are shown in Table 2. A three-fold increase in plasma angiotensin was observed 15 min following isoproterenol and persisted for at least 1 hr. In another series of animals plasma angiotensin II levels, determined at the onset of drinking, 29 ± 5.2 min after administering isoproterenol, increased from 85 ± 8.0 pg/ml to 358 ± 43.3 pg/ml ($n = 14$, $t = 6.21$, $p<0.005$). There was no significant correlation between plasma angiotensin II levels and water intake in this experiment ($r = 0.107$, $p>0.05$) or for those shown in Table 2.

Plasma angiotensin II levels and water intake following 24 hr water deprivation appear in Table 3. During the deprivation period body weight dropped by 29 g ($t = 2.16$, $p<0.05$). Water intake during the next hour was 19.0 ± 1.09 ml compared to 1.8 ± 0.40 ml for the controls and during the next 6 hr was 30.3 ± 1.47 ml compared to 3.5 ± 0.56 ml for the controls (in both cases, $p<0.001$). There was a significant correlation between plasma angiotensin II

TABLE 1

THE EFFECTS OF POLYETHYLENE GLYCOL ON PLASMA ANGIOTENSIN II CONCENTRATIONS AND WATER INTAKE*

	N	Body Weight (g)	Water Intake (ml)	Angiotensin II Concentrations (pg/ml) Before Injection	After Injection
Series 1					
30% Polyethylene glycol	10	430 ± 16.7	10.0 ± 0.58§	63 ± 4.4	124 ± 4.6‡
0.9% NaCl	10	446 ± 16.1†	3.3 ± 0.30	75 ± 3.3	70 ± 5.5 (NS)
Series 2					
30% Polyethylene glycol	10	516 ± 7.4	7.9 ± 0.95‡	73 ± 6.5	203 ±16.8‡
0.9% NaCl	10	522 ± 7.5	2.1 ± 0.18	73 ± 5.3	73 ± 5.0 (NS)

*Blood samples were collected and water became available 3 hr (Series 1) and 6 hr (Series 2) after injection of either polyethylene glycol (PG) or 0.9% NaCl (Control). For Series 1 water intake is for 5 hr and for Series 2 for 2 hr.
†Results expressed as mean ± SEM.
‡$p<0.01$.
§$p<0.0001$.

TABLE 2

THE EFFECT OF ISOPROTERENOL ON PLASMA ANGIOTENSIN II CONCENTRATIONS AND WATER INTAKE

Series	N	Body Weight (g)	Water Intake (ml/2 hr)	Angiotensin II Concentrations (pg/ml) Before Injection	After Injection
1 — 15 min after Isoproterenol	8	515± 9.4*	9.2±1.47	69± 6.8	204±32.8†
2 — 30 min after Isoproterenol	9	508±10.3	10.2±1.03	69± 5.7	222±22.5‡
3 — 60 min after Isoproterenol	16	489±17.6	9.4±1.11	76±14.0	224±33.5‡
— 30 min after 0.9% NaCl	9	397± 2.6	3.5±0.27	73± 6.6	66± 3.0 (NS)

*Results expressed as mean ± SEM.
†$p<0.01$.
‡$p<0.001$.

TABLE 3

THE EFFECT OF 24-HOUR WATER DEPRIVATION ON PLASMA ANGIOTENSIN II CONCENTRATIONS AND WATER INTAKE

Treatment	N	Body Weight (g) Before	After	Water Intake (ml/6 hr)	Angiotensin II Concentrations (pg/ml) Before	After
Water Deprivation	22	487±9.6	458±9.3†	30.3±1.47‡	85±2.1	205±8.7‡
Control	9	539±8.7*	536±8.9 (NS)	3.5±0.56	83±3.7	80±4.4 (NS)

*Results expressed as mean ± SEM.
†$p<0.05$. Water deprivation resulted in weight loss of 6%.
‡$p<0.001$.

TABLE 4

THE EFFECT OF HYPERTONIC SALINE ON PLASMA ANGIOTENSIN II CONCENTRATIONS AND WATER INTAKE

	N	Body Weight (g)	Water Intake (ml)	Angiotensin Concentrations (pg/ml) One Hour Before Injection	One Hour After Injection
Series 1					
Hypertonic saline†	7	516±6.3	17.9±2.08‡	67±7.1	76± 4.3 (NS)
0.9% NaCl	9	529±9.6*	3.1±0.46	70±3.5	88±11.5 (NS)
Series 2					
Hypertonic saline	10	329±5.6	14.4±1.18‡	77±2.2	72± 1.6 (NS)
0.9% NaCl	3	320±2.9	1.5±0.76	78±1.5	74± 0.9 (NS)

*Results expressed as mean ± SEM.

†The experimental treatment was a subcutaneous injection of 5 ml/Kg of 2.5 M NaCl. The control was a subcutaneous injection of a similar volume of 0.9% NaCl.

‡$p<0.001$.

levels and water intake (for the 6 hr period, r = 0.50, $p<0.05$).

For comparison plasma angiotensin II levels and water intake were measured after the administration of hypertonic saline, an intracellular thirst stimulus, and the results are presented in Table 4. The animals in Series 1 had water available from the time of the injection of hypertonic saline but blood samples for the determination of plasma angiotensin II levels were obtained 30 min later. For Series 2 water was made available 30 min after the injection of hypertonic saline when the blood samples were obtained. The hypertonic saline initiated a significant increase in water intake for both groups ($p<0.01$) but plasma angiotensin levels were not increased ($p>0.05$).

DISCUSSION

Drinking elicited by the administration of polyethylene glycol and isoproterenol was associated with significantly elevated plasma levels of angiotensin II suggesting that the renin-angiotensin system makes a contribution to extracellular thrist. Drinking was also elicited by the administration of hypertonic saline but there was no increase in plasma angiotensin II. It appears from these results that endogenously released angiotensin II contributes to extracellular thirst but not to intracellular thirst.

The correlation between plasma angiotensin II levels and water intake following the injection of polyethylene glycol is lower than that observed by Leenen and Stricker [22] between plasma renin activity and water intake. They made water available immediately after the injection of polyethylene glycol whereas in the present study water was withheld for 3 and 6 hr. A more likely reason for the lower correlation is that our sample size was small. The lower correlation after a 6 hr interval than after a 3 hr interval is to be expected because of a greater contribution of intracellular thirst to water intake and the longer period for the redistribution of water in the body fluid compartments. There was not a significant correlation between plasma angiotensin II levels and water intake following the injection of isoproterenol. This observation agrees with the finding by Leenen and coworkers [23] that plasma renin activity and water intake were also not correlated following the administration of isoproterenol. They suggest that the lack of a correlation may be due to the loss of dipsogenic effects of angiotensin II as a result of water ingested during the test. This proposal would not explain our results for the series in which blood samples for the determination of plasma angiotensin II levels were obtained at the onset of drinking. There is some indication that a part of the drinking following the administration of isoproterenol is attributed to the activation of volume receptors [26]. This could be a factor in accounting for the low correlation between plasma angiotensin II levels and water intake.

Both water intake and plasma angiotensin II levels were increased following 24 hr of water deprivation and the 2 measures were significantly correlated. Although the change in plasma angiotensin II was similar to that observed following the administration of polyethylene glycol or isoproterenol the volume of water intake was considerably greater. Extracellular hypovolemia and the activation of the renin-angiotensin system is only one of the factors contributing to water intake after water deprivation; intracellular thirst mechanisms would also be involved [14]. Presumably a considerable total amount of angiotensin II would be formed during the 24 hr deprivation period but because of its relatively short half-life one would not necessarily expect that this should be reflected in a very high plasma level.

It is of interest to compare these levels of endogenously released angiotensin II with those required to elicit drinking by the exogenous administration of angiotensin II. From the results of Epstein and Simpson [9] it is estimated that it is necessary to raise the level of plasma angiotensin II from 90–100 pg/ml to approximately 250–400 pg/ml in order to induce reliable drinking in the rat. These values are similar to those obtained in the present study.

There is a growing body of evidence, mostly indirect, that the dipsogenic effect of circulating angiotensin II is due to its action on the central nervous system. The injection of a few nanograms or even picograms of angiotensin II into the preoptic region, subfornical organ and other brain sites induces a strong drinking response in the rat and several other species [3, 10, 27, 30, 35]. Drinking induced by the intravenous [8] and subcutaneous [20] administration of angiotensin II was significantly attenuated by

administering a competitive antagonist, 1-Sar, 8-Ala-angiotensin II, into the lateral ventricles. Lesions of the subfornical organ attenuated drinking induced by the intravenous infusion of angiotensin II [2, 9, 32] and by the peripheral injection of renin or isoproterenol [21]. Although the central receptive site for angiotensin II is uncertain and controversial at the present time possibilities are the subfornical organ [31], an intraventricular site [18] and the preoptic region [4].

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