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19. Supported by NIH grant AM 10894, and a grant from the John A. Hartford Foundation.

3 January 1977

Angiotensin: Physiological Role in Water-Deprivation–Induced Thirst of Rats

Abstract. *Cerebroventricular infusion of P-113, the blocking agent of angiotensin II, into rats for 75 minutes prior to their being allowed to drink, significantly attenuated their water intake when they had been deprived of water for 30 hours. However, a similar infusion had no effect on the food intake in rats fasted for 30 hours. The results indicate a physiological role for angiotensin II in the drinking response of rats deprived of water.*

The possible role of the renin-angiotensin system in thirst was first suggested by Fitzsimons (*1*), who showed that drinking was increased in rats subjected to partial aortic constriction and that the response was attenuated by nephrectomy. Later, others demonstrated that both intravenous and intracranial administration of renin or angiotensin II provoke thirst (*2*).

Even though exogenous angiotensin II clearly stimulates thirst, it is not known whether this hormone plays any physiological role in drinking. Some workers have proposed that drinking induced by angiotensin is a pharmacological effect rather than a physiological one (*3*). In previous experiments we found that single intraventricular injections of the blocking agent P-113 (sar^1-ala^8-angiotensin II, where sar is sarcosine and ala is alanine) of angiotensin II attenuated drinking in water-deprived rats (*4*). However, the procedure we used to administer a bolus of P-113 to the animals 1 to 2 minutes prior to the drinking test may have obscured the results. The experiments presented here were designed to test in a more controlled manner the hypothesis that endogenous angiotensin plays a role in thirst.

We used 52 male Sprague-Dawley rats, each weighing 300 to 400 g. They were fed on rat food (Teklad) containing 1 percent NaCl, and water was freely available. The rats were housed individually and exposed to photoperiods of 12 hours of light and 12 hours of darkness. Stereotaxic techniques were used on anesthetized animals to fix a 22-gauge guide tube over the right lateral ventricle, 0.5 to 1 mm anterior to the interventricular foramen (*5*). The end of the guide was 1 to 2 mm above the ventricle. During the 6 to 10 days between surgery and experimentation, the rats were conditioned at least five times to the type of handling to which they would be exposed on the day of the experiment: they were removed from their cages, restrained manually, and weighed; the protective rubber cap above the guide tube was removed and replaced, and then the rats were returned to their cages.

Water intake (from Richter tubes) and weight gain (measured daily for 2 or 3 days prior to experimentation) were used as crude indices of general recovery from surgery and of normal appetitive behavior. No animal was studied which was drinking less than 20 ml per day or was not gaining weight. Animals were grouped so that their water intakes 24 hours prior to the test were similar in the control and experimental groups.

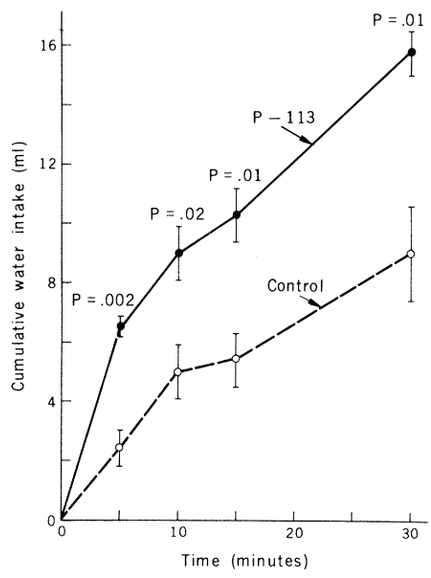

Fig. 1. The results of experiment A, showing the cumulative water intake of rats deprived of water for 30 hours and then given access to water. Infusions into the lateral ventricle (3.3 μl/min) of artificial cerebrospinal fluid with or without P-113 (20 ng/μl) began 30 minutes before the rats were given access to water and continued during the 30-minute test period. Values are means ± standard error (N = 4).

At 0800 hours on the day prior to experiments A and B, water tubes were removed from the cages. The next afternoon each rat was removed from its cage and a 27-gauge needle connected to Teflon tubing was inserted through the guide tube into the right lateral ventricle. The animal was then returned to its cage and the tubing (previously filled with infusate) was connected to a Braun-Melsungen syringe pump set to deliver 3.3 μl per minute. Usually, three control and three experimental animals were infused simultaneously. The infusate was either artificial cerebrospinal fluid (Na^+, 150 mM; Cl^-, 133 mM; K^+, 3.1 mM; Ca^{2+}, 1.2 mM; Mg^{2+}, 1 mM; HCO_3^-, 24.5 mM; phosphate, 0.5 mM; glucose, 50 mg/100 ml) or artificial cerebrospinal fluid containing 20 ng/μl of P-113.

After 30 minutes (experiment A) or 75 minutes (experiment B) of infusion, water tubes were returned to the cage (1400 hours); the associated disturbance aroused the rats. Over the next 30 minutes, during which the ventricular infusions continued, we measured water intake at 5-minute intervals to the nearest 0.5 ml. Thereafter, methylene blue (0.2 percent) in artificial cerebrospinal fluid was infused intraventricularly for 10 to 15 minutes. The animals were then killed and perfused intravascularly with saline followed by formalin-saline. The next morning, the brains were sectioned and examined for ventricular staining. Data from any animal not stained in the right lateral, third, and fourth ventricles were excluded from the results.

In experiment C we evaluated the effect of P-113 on another appetitive behavior, food intake. Twenty-four rats were fasted for 30 hours; thereafter they were infused (exactly as in experiment B). After 75 minutes of infusion, water tubes were removed and food was presented to the animals for a 10-minute test period during which the infusion was continued. Thereafter, each rat was killed and treated as in experiments A and B. In addition, the amount of food eaten was determined by emptying the stomach and upper duodenum and drying and weighing the contents.

Figure 1 shows the results for experiment A. It is evident that P-113 amplified

the drinking response to water deprivation; at all time intervals, the rats receiving P-113 drank significantly more water than did the control rats. In experiment B (Fig. 2), the same concentration of P-113 was infused for 75 minutes before the presentation of water. We hoped, by means of the longer infusion, to avoid the agonist action of P-113 and uncover any antagonist effect. Figure 2 shows that P-113 attenuated the drinking responses of all except two of the rats (upper two solid lines, Fig. 2) which appeared to be stimulated to drink. At the end of 30 minutes these two rats drank almost twice the average of control rats ($P = 0.7$). The other experimental rats either drank nothing for the entire experimental period or drank only afer a delay of 10 to 25 minutes. A χ^2 analysis showed that the differences in drinking responses between the two groups were statistically significant for all but the 30-minute time interval; P values for the 5-, 10-, 15-, 20-, and 30-minute periods were, respectively, .002, .002, .005, .02, and .09. This analysis included the two rats in which P-113 appeared to have an agonist action.

These data demonstrate that, during short exposures (30 minutes), P-113 acts as an agonist of the drinking response to water deprivation, whereas it is antagonistic after longer periods; a similar time dependency for the intraventricular or intravenous effect of P-113 on exogenously administered renin or angiotensin has been demonstrated (*6*).

The results from experiment C (fasting) (Table 1) shows that P-113 did not alter the rats' ingestive response to a 30-hour fast, indicating that the depression of the drinking response was not due to some general depression of appetitive behavior. Moreover, a comparison of the eating and 10-minute drinking responses during P-113 infusion shows them to be significantly different, $\chi^2 = 9.6, P < .01$. These data are consistent with three other reports (one on sheep and two on rats) indicating that intraventricular infusions of P-113 sufficient to block the dipsogenic response to intravenously administered angiotensin (*7–9*) or isoproterenol (*10*) have no effect on either the dipsogenic response to hypertonic saline or the intake of food (*7–10*).

While our study was in progress, two other groups reported the failure of intraventricularly administered P-113 to block the drinking response in goats (*11*) and sheep (*9*) deprived of water for 24 or 48 hours. There are several possible explanations for these apparently contradictory results. First, the species are different; although dehydration increases plasma renin activity in both sheep (*12*) and rats (*13*), the magnitude of the response tends to be greater in rats, suggesting that thirst induced by renin-angiotensin might play a greater role in rats. Second, it is evident that the agonist effects of P-113, which vary over the period of infusion, may obscure any antagonist effect. Since the infusions of P-113 in sheep (*9*) and goats (*11*) began 30 minutes prior to the presentation of water, the effect should be compared to our experiment A. We found P-113 under these circumstances to be agonistic rather than antagonistic to the drinking response. Moreover, it would not be surprising if it took more than 30 minutes to reverse some unknown effect on the centrol nervous system of angiotensin II which may have required up to 30 hours for its generation. Differences in the P-113 concentration, the relative infusion rate, the locus of the infusion needle, and the locus of the receptor which responds to angiotensin II during dehydration in these different experiments might play a role in determining whether the concentration of P-113 at the receptor was agonistic or antagonistic at the time of the test. Another unsuccessful attempt to inhibit with intraventricular P-113 the thirst induced by changes in the volume or osmolality of the body fluids is that of Summy-Long and Severs working with rats (*7*). However, these investigators administered a bolus of P-113 only 30 minutes before the drinking test, a period which we find yields agonistic effects.

The dipsogenic response to a subcutaneous injection of isoproterenol in rats can be blocked by an intracranial injection of P-113 (5 μg bolus) (*10*), and the infusion of P-113 (20 ng per kilogram of body weight per minute for 60 minutes) into the third ventricle of unanesthetized dogs also blocks the dipsogenic response to the same drug (*14*). Therefore, it seems that the usual effects of isoproterenol on drinking are mediated by a central action of angiotensin. However, isoproterenol cannot be considered a

Table 1. Effect of intraventricular infusion of P-113 on 10-minute food intake after a 30-hour fast. The data, which are expressed as means ± standard error ($N = 10$), were not significant when examined by the χ^2 test or Student's t-test.

Group	Ratio of animals eating to animals given access to food	Amount eaten (g)*	Latency until eating (seconds)
Controls	10/12	0.231 ± .124	108 ± 46.3
P-113	10/12	0.227 ± .072	114 ± 55.5

*In eight animals fasted for 30 hours and then not allowed to eat, the average weight of the dried stomach contents was 0.026 ± .005, that is, about 10 percent of that measured in animals that ate.

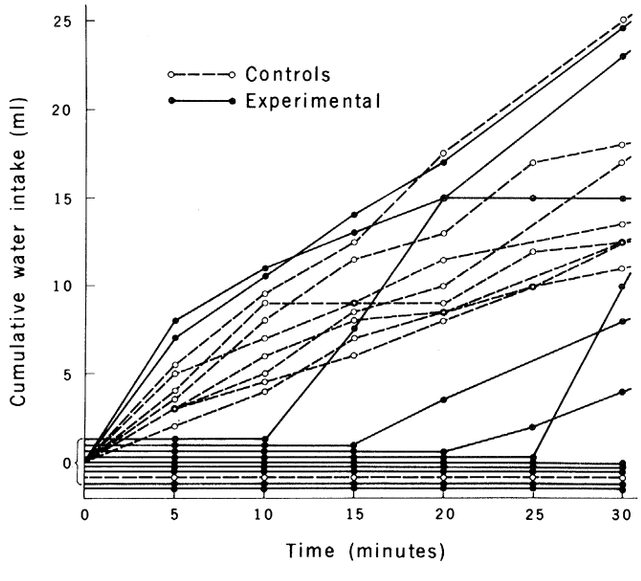

Fig. 2. The results of experiment B, which was similar to experiment A except that the cerebroventricular infusions began 75 minutes before the rats were given access to water. Each line represents the cumulative water intake of one animal infused with artificial cerebrospinal fluid with (experimental) or without (control) P-113. Of nine control animals, all drank immediately except one. Of 11 animals infused with P-113, five did not drink at all in 30 minutes, and four drank only after a delay of 10 or more minutes. The individual data points in this figure are subjected to χ^2 analysis in Table 1.

normal stimulus to thirst, as is the dehydration used in our experiments.

Our experiments do not indicate the source of the endogenous angiotensin blocked by P-113. However, since both angiotensin (*7*) and its analogs (*15*) in plasma can cross portions of the blood-brain barrier and gain access to dipsogenic sites in the brain, we believe that angiotensin from the plasma of the dehydrated rats crossed the blood-brain barrier, and that this was the source of the endogenous angiotensin blocked by the intraventricular P-113. Consistent with this hypothesis are the recent findings in rats that 24-hour dehydration increases the plasma concentration of angiotensin 2½ times and that the individual increments in plasma angiotensin correlate positively with water intake (*16*).

While it is clear that blocking endogenously generated angiotensin attenuates the thirst induced by water deprivation, it is also evident that angiotensin receptors blocked by P-113 cannot be the sole regulators of thirst because most of the rats receiving P-113 did finally begin to drink; thus, other receptor mechanisms are also involved.

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17. We thank J. Hoogland and S. Schock for technical assistance, and Norwich Products, Norwich, N.Y., for providing the P-113. This work was supported by grants from the National Science Foundation (BMS 74-20455), the National Institutes of Health (1R01-NS12825-01), and the Michigan Kidney Foundation.

27 October 1976; revised 4 January 1977

Siamese Cats: Abnormal Responses of Retinal Ganglion Cells

Abstract. *Comparison of optic tract recordings in Siamese and ordinary cats reveals that Siamese cats have a significantly lower percentage of Y-cells than of X-cells. In addition, Siamese cats show depressed responses to a contrast-reversal stimulus, a result that supports the lower spatial contrast sensitivity demonstrated behaviorally by these animals. Both physiological findings suggest neurophysiological anomalies in the Siamese retina.*

It has been well documented that some of the retinogeniculate fibers in the Siamese cat's visual pathway are misrouted (*1–4*). Furthermore, the geniculocortical pathway of these animals is also different from that of ordinary cats (*5–7*), and this aberrance may involve more than just the lateral geniculate nucleus (LGN) and the cortex (*8*).

The physiological consequences of abnormal projections in the central visual pathways of Siamese cats include not only suppression of certain inputs (*7*) but also the apparent loss of binocularity in cortical neurons (*6, 7, 9*), in which binocular excitation is routinely demonstrated in ordinary cats (*10*). This latter finding has been used to explain the apparent lack of stereopsis (*11*), as well as the existence of a convergent squint in Siamese cats (*6*).

Siamese cats display depressed spatial contrast sensitivity compared to that in ordinary cats (*12*), a phenomenon which implies that the physiological characteristics of neurons in the visual system of these animals may be different in some respects. Since certain properties of retinal ganglion cells (for example, classification as X- or Y-cells) may be related to visual acuity (*13, 14*), we felt it important to begin our studies at the retinal level.

We have been investigating the responses of the Siamese cat's retinal ganglion cell, and now report observations on 136 optic tract fibers obtained from three Siamese cats and 84 optic tract fibers obtained from three ordinary cats. We have found that (i) the ratio of Y- to X-cells in the Siamese cat is significantly lower than that in ordinary cats; and (ii) the response to contrast in Siamese ganglion cells, as measured electrophysiologically, is depressed.

Conventional techniques were used to record extracellular action potentials from optic tract fibers. A 3° bipartite field whose contrast reversed every 0.5 second was used to classify all of the cells into X- and Y-types (*15*). The same stimulus configuration, electrodes, and recording conditions were used in the experiments on ordinary cats.

Table 1 shows the relative numbers of X- and Y-cells in Siamese and normal cats. Only 8 percent of the Siamese units are classified as Y-type compared with 32 percent of ordinary cat units. This lower percentage of Y-units is not specific to either on- or off-center cells nor to normally routed or misrouted fibers (*16*). Furthermore, more than 90 percent of the receptive fields encountered in these experiments were located no more than 30° from the area centralis for both breeds. After determining the null position for the bipartite field with respect to the recep-

Table 1. Relative frequency of X- and Y-units in the optic tract of Siamese and ordinary cats. Corrected $\chi^2 = 19.38$; $P < .001$.

Type of units	Siamese cat		Ordinary cat	
	N	Percent	*N*	Percent
X-cells	125	92	57	68
On-center	85		43	
Off-center	40		14	
Y-cells	11	8	27	32
On-center	4		9	
Off-center	7		18	
Totals	136	100	84	100

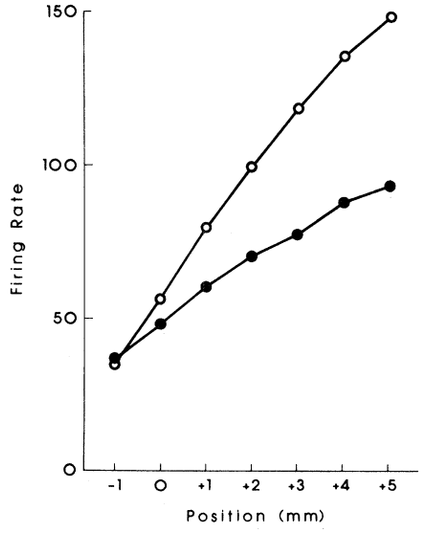

Fig. 1. Responses of optic tract fibers in Siamese (•) and ordinary (○) cats to contrast. Averaged maximum firing rate (impulses per second) to an edge pattern turned on and off in various positions across the receptive field. Position 0 indicates the null position for X-cells and the equal-response position for Y-cells. Contrast of target, 82 percent; background luminance, 0.86 cd/m²; 1 mm = 4.3′ of arc.