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Cephalic Angiotensin Receptors Mediating Drinking to Systemic Angiotensin II[1]

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JOHNSON, A. K., AND J. E. SCHWOB. *Cephalic angiotensin receptors mediating drinking to systemic angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1077–1084, 1975. – In rats implanted with cannulae to allow delivery of solutions to the cerebral ventricular system, pretreatment with 5 μg or 0.5 μg of saralasin acetate (Sar[1] Ala[8] Angiotensin II), an angiotensin II competitive analog, significantly attenuated drinking to subcutaneous (SC) injections of 500 μg of angiotensin II. However, pretreatment with either SC (5 μg or 20 μg) or with intravenous (5 μg) saralasin had no effect on drinking to SC angiotensin II (500 μg). Intracranial (IC) injections of 5 μg of saralasin had no effect on drinking in response to SC injections of 0.8 cc of a 10 percent NaCl solution and did not attenuate ingestion of a milk solution in a dessert test. On the basis of the specificity and the greater efficacy shown by IC saralasin in attenuating drinking to systemically applied angiotensin II, it was concluded that circulating angiotensin II reaches brain periventricular receptors which mediate drinking.

Angiotensin II Angiotensin dipsogenic receptor Angiotensin-induced thirst Cellular thirst Saralasin

THE significance of the renal renin-angiotensin system as a hormonal mediator of drinking has been established by Fitzsimons and colleagues [13]. Manipulations which release renin from the kidney produce drinking [17, 22, 25, 28], and the systemic administration of renin, angiotensin I, or angiotensin II induces water consumption [9, 12, 14, 15]. The interaction of angiotensin with a hypothetical dipsogenic receptor is assumed to be the initiating process which leads to an activation of neural thirst mechanisms in the central nervous system and a concomitant mobilization of drinking behaviors. Because of the primacy of the receptor processes involved in the hormonal induction of drinking, the localization of the angiotensin dipsogenic receptors is a necessary step in achieving an understanding of the mechanisms mediating angiotensin thirst and motivated behavior.

Two hypotheses bearing on both the mechanism of action of angiotensin in the control of drinking and on the general location of angiotensin receptors have been presented by Fitzsimons [12]. The first, a peripheral hypothesis, suggests that the renin-angiotensin system acts by increasing the sensitivity of vascular stretch receptors so that afferent sensory discharge is increased and thereby activates central thirst systems. This mechanism would enable the hormone to exert its dipsogenic action without entering the brain and engaging the various brain-barrier systems. The second, a central hypothesis, states that circulating angiotensin exerts a direct action on receptors immediately associated with the brain. At the present time, available experimental evidence does not allow discrimination between the two hypotheses.

The finding that animals drink to direct intracranial (IC) injections of angiotensin II [1,8] and that the threshold dose for eliciting drinking intracranially is 2 to 3 orders of magnitude below that necessary to obtain drinking with systemic delivery of the peptide [4, 8, 27] has been interpreted to support the central hypothesis. However, these data may also be interpreted as showing that IC injections of angiotensin which produce drinking are not mimicking the central action of peripherally generated angiotensin but are revealing the existence of a separate and independent hormonal hydrational system in the brain. Also, at the present time there is no definitive evidence that peripheral angiotensin II, per se, circulating in physiological amounts (which can produce drinking, [5]) gains access to the central nervous system.

Because of the paucity of corroborating and converging data on the site of action mediating the dipsogenic effect of angiotensin, a more definitive analysis is warranted. Recent success with competitive analogs of angiotensin in the elucidation of brain receptors involved in IC angiotensin induced drinking [7] recommends these materials as potential tools for the analysis of the site of action of circulating angiotensin. Sar[1] Ala[8] angiotensin II in the acetate form (Saralasin, previously called P-113, Norwich-Pharmacal), when applied centrally, blocks drinking to intracranially administered angiotensin [6,10] and when infused systemically in sufficient doses attenuates drinking to intravenous angiotensin II [10,30]. The basic experimental rationale was formulated to test whether a low IC dose of saralasin acetate was more effective than an equivalent or greater dose of the peripherally administered

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blocker in attenuating the drinking to peripherally administered angiotensin II. That is, if the brain contains the ultimate target tissue (central hypothesis) upon which angiotensin acts to produce thirst, then the intracranially applied blocker would be closer to the receptive tissue and therefore more effective in reducing the drinking response than peripherally administered competitive analog. Conversely, if the critical receptor tissue is located in the periphery then the intracranially delivered blocker should be either less or at least no more effective than systemically applied blocker.

GENERAL METHOD

Animals

Male albino rats (Sprague-Dawley derived, University of Iowa, Department of Psychology Animal Colony), weighing between 300–450 g at the time of the beginning of the experiment, were individually housed in hanging cages (18 cm high, 18 cm wide, and 24 cm long) constructed of hardware cloth (3/4 in. mesh) and sheet metal. Teklad laboratory pellets were always available on the floor except during drinking tests and tap water was presented ad lib from metal drinking spouts which entered the front of the cage near the floor. Ambient lighting was programmed to turn on at 0800 hr and off at 2000 hr and the colony temperature was maintained at approximately 22°C by air conditioning.

Surgery

Intracranial cannulation. Prior to surgery animals were treated with atropine sulfate (0.2 mg/rat) and anesthetized with Equithesin (3.0 cc/kg) (Jenson-Salzburg Laboratories). The head was shaved, the animal was mounted in a stereotaxic instrument, and the scalp was incised. After removing the periosteum, the exposed skull was leveled using bregma and lambda as referents and 4 holes were drilled, one into each of the frontal and parietal bones, to receive stainless steel jewelers screws. A 23 ga guide cannula system, similar to that described by Epstein *et al.* [8], was then stereotaxically implanted through a small hole in the skull. Previous work has shown that drinking following IC injection of low doses of angiotensin is the result of action of the hormone on a periventricular receptor site [3, 18, 21]. The cannula placement, 8.7 mm anterior to the intra-aural line, 1.3 mm to the left of the saggital suture and 6.6 mm ventral from the surface of the skull, was used in order to ensure optimal and reliable delivery of injections to the ventricular system [21]. The guide cannula was fixed to the skull and jewelers screws with methyl methacrylate cement and the incision was closed. A prophylactic dose of penicillin G (Pfizerpan AS 60,000 units/rat) was administered after surgery. A minimum period of 3 days postoperative recovery was allowed prior to screening tests.

Jugular catheterization. Under ether anesthesia the right jugular was exposed. A catheter consisting of a 10 cm piece of PE-50 tubing with a 45° bevel at one tip was inserted into the jugular so that the tip ended at the entrance to the right atrium (i.e., 32 mm from entry into the vein to the heart in the size of rats employed in this study). The catheter was then tied into the vein and the free end run under the skin emerging through a 7 mm midline incision in the skin at the scruff of the neck. The externalized end was fixed to the skin by a suture. The incision over the exposed jugular was closed and the catheter was filled with sodium heparin (100 units/ml) and closed with an obturator. Catheters were maintained by flushing and filling them with the heparin solution at the conclusion of an intravenous test.

Screening Tests

Intracranial angiotensin II. In order to insure reliable delivery of solutions to the ventricular system, animals were screened by response to an IC injection of angiotensin II. Following recovery from surgery, animals were removed from their home cages and housed (food and water ad lib) in special screening cages (18.5 cm high, 22 cm wide, and 37 cm long with front, sides, bottom, and top constructed from hardware cloth and a sheet-metal back) for at least one night prior to an IC angiotensin II test. When screening was begun, the animal and its food were removed from the cage. The obturator was pulled out of the guide cannula and replaced with a 30 ga stainless steel injector which was connected to a remote 10 μl syringe (Hamilton) by a 1 m piece of PE-10 tubing running through the top of the cage. The entire injection system had previously been filled with a solution of angiotensin II amide (Hypertensin, Ciba), 50 ng/μl in isotonic saline. The injector was designed so that its tip was flush with the tip of the permanent guide cannula in the IC site. The animal was returned to the cage and, after the animal was resting quietly, a 1 μl injection of the angiotensin solution was delivered over 10 sec. The latency to drink and the amount of water consumed in 15 min following the injection were recorded. An IC injection was judged to be positive if the animal began drinking within 2.5 min following the treatment and consumed at lease 1.5 ml within the 15 min test period.

Subcutaneous angiotensin II. Animals that were to be used in experiments employing peripheral administration of angiotensin II as the dipsogenic treatment were screened twice (separated by one day) for drinking to a subcutaneous (SC) injection between the scapulae of 500 μg of angiotensin II in 0.67 ml of isotonic saline. Prior pilot dose-response work showed 500 μg to be an optimal SC dose of angiotensin for the elicitation of drinking. The criterion for entry into an experiment was drinking 2 ml or more within 1 hr in response to the second injection.

Subcutaneous hypertonic saline. Animals that were to be used in the experiment employing peripheral administration of hypertonic saline as a dipsogenic treatment were tested for drinking to two SC screening injections (separated by one day) of 0.8 ml of a 10 percent NaCl (Fisher A.C.S.) solution (made in deionized water). The criterion for drinking to the challenge was set at 2 ml or more within 1 hr on the second screening test.

Experimental Tests

Each experiment was designed to evaluate the effect of a primary treatment of blocker (saralasin) on a subsequent drinking response. In experiments where the primary treatment was to be an IC injection, animals were removed from their home cages and injected in a holding cage (same dimensions as the hanging cages previously described) with 2 μl of the appropriate solution at the approximate rate of 0.1 μl/sec. The injection system was like that used for IC angiotensin screening except the PE tubing was generally shorter. The injector remained in place for 2 min after

completion of the injection, then it was removed, the obturator replaced and the animal returned to its home cage. All IC test solutions were either artificial cerebrospinal fluid (ACSF) (Elliot's B solution, National Cancer Institute, NIH; each ml contained NaCl, 7.3 mg; KCl, 0.3 mg; $CaCl_2 \cdot 2H_2O$, 0.2 mg; $MgSO_4 \cdot 7H_2O$, 0.3 mg; $Na_2HPO_4 \cdot 7H_2O$, 0.2 mg; Dextrose (hydrous), 0.8 mg; $NaHCO_3$, 1.9 mg; Phenol Red, 0.1 μg) or saralasin in ACSF.

In experiments where the drinking response was elicited by a peripherally administered thirst challenge the animals were removed from their cages 5 min after receiving the primary treatment and injected subcutaneously between the scapulae with the dipsogenic stimulus or its control and then returned to the home cage. These peripherally injected solutions were (1) isotonic saline (Baxter), (2) angiotensin II amide in isotonic saline, and (3) 10 percent hypertonic saline.

EXPERIMENT 1

The initial experiment examined the effect of intracranial pretreatment with the angiotensin II antagonist, saralasin, on systemically applied angiotensin II.

Method

Fourteen animals were screened for IC drinking to angiotensin II and all responded reliably. The 14 animals were then screened for drinking to SC angiotensin II and 12 met the drinking criterion. These 12 animals were then distributed to receive initially either 1 or 2 blocks of treatment conditions. Block 1 consisted of three conditions: (1) an IC injection of ACSF followed by a SC isotonic saline treatment (0.67 ml); (2) an IC injection of ACSF followed by a SC injection of 500 μg of angiotensin II (0.67 ml); and (3) an IC injection of 0.5 μg of saralasin in ACSF followed by a SC injection of 500 μg of angiotensin II (0.67 ml). Block 2 consisted of 3 treatment conditions with conditions (1) and (2) identical to conditions (1) and (2) in Block 1. Condition (3) in Block 2 was an IC injection of 5 μg of saralasin in ACSF followed by a SC injection of 500 μg of angiotensin II (0.67 ml). The order for the 3 conditions within each block was randomized for each animal. Four animals received Block 1 first and then Block 2. Eight animals went through Block 2 and then received Block 1. Water intakes were recorded 5 min after the primary treatment and 60 min after the dipsogenic treatment.

Results and Discussion

Summarized in Table 1 are the mean water intakes for the 65 min following the primary treatment. There was no difference in water intake 5 min following the primary treatment. One of the animals became ill during the course of testing and was removed from the experiment. A statistical analysis of the treatment conditions in Block 1 showed a significant difference in water intake, $F(2,20) = 8.72$, $p<0.01$. Planned comparisons of the effect of 0.5 μg pretreatment of saralasin as compared with ACSF pretreatment on peripheral angiotensin II showed a significant decrease following application of the blocker, $t(10) = 3.004$, $p<0.02$. An analysis for Block 2 conditions showed a significant difference in water intake across conditions, $F(2,20) = 9.76$, $p<0.01$. The planned comparison of the effect of pretreatment with ACSF or 5 μg of saralasin showed a significant reduction in water intake produced by the competitive analog, $t(10) = 2.744$, $p<0.05$.

The results indicate that there is a systematic attenuation by intracranially applied saralasin on drinking induced by peripheral administration of angiotensin II.

EXPERIMENT 2

The second experiment tested whether there was a comparable antagonism by saralasin on water intake following SC angiotensin treatment when the peptide inhibitor was administered subcutaneously.

TABLE 1

EFFECT OF INTRACRANIAL SARALASIN ON DRINKING PRODUCED BY SUBCUTANEOUS ANGIOTENSIN

	Treatments			
Primary Treatment + Dipsogenic Treatment	ACSF* (IC) + Isotonic Saline (SC)	ACSF (IC) + 500 μg Angiotensin II	0.5 μg Saralasin + 500 μg Angiotensin II	5.0 μg Saralasin + 500 μg Angiotensin II
Mean Water Intake (mls ± SEM)	0.4 ± 0.21†	4.1 ± 0.87†	2.3 ± 0.92	1.6 ± 0.88
Number of Observations	22	22	11	11
p Value‡	–	–	<0.02	<0.05

*ACSF = artificial cerebrospinal fluid

†Mean intakes for the ACSF + isotonic saline condition and also for the ACSF + 500 μg Angiotensin II condition were statistically not different between treatment blocks so that for tabulation, mean intakes are collapsed over both blocks and the SEM is calculated for the number of observations.

‡Compared with ACSF-Angiotensin II condition within each block of treatments

TABLE 2

EFFECT OF SUBCUTANEOUS SARALASIN ON DRINKING PRODUCED BY SUBCUTANEOUS ANGIOTENSIN

	Treatments			
Primary Treatment + Dipsogenic Treatment	Isotonic Saline (SC) + Isotonic Saline (SC)	Isotonic Saline (SC) + 500 μg Angiotensin II (SC)	5 μg Saralasin (SC) + 500 μg Angiotensin II (SC)	20 μg Saralasin (SC) + 500 μg Angiotensin II (SC)
Mean Water Intake (mls ± SEM)	0.3 ± 0.09*	4.3 ± 0.52*	5.4 ± 0.76	3.7 ± 0.55
Number of Animals	27	27	16	11

*Mean intakes for the Isotonic Saline + Isotonic Saline condition and also for the Isotonic Saline + 500 μg Angiotensin II condition were statistically not different between treatment groups so that mean intakes were collapsed over Blocks for tabulation.

Method

Thirty-five naive rats were screened for drinking to SC angiotensin II. Twenty-seven demonstrated reliable drinking and each of the responsive animals was assigned to 1 of 2 treatment blocks. In this experiment, the primary treatment was a SC injection. Animals were removed from their home cage, injected subcutaneously between the scapulae with 0.1 ml of either isotonic saline or saralasin in isotonic saline, and returned to the home cage for 5 min. After this interval they were removed and injected with either 0.67 ml of isotonic saline or 500 μg of angiotensin II in 0.67 ml of isotonic saline and returned to the home cage.

Sixteen animals received the following conditions in random order: (1) a SC injection of isotonic saline followed by another SC injection of isotonic saline, (2) a SC injection of isotonic saline followed by a SC treatment with angiotensin II, and (3) a SC injection of 5 μg of saralasin followed by a SC injection of angiotensin II. The remaining 11 animals received in random order the same types of treatments except that for condition (3) the primary treatment was a SC injection of 20 μg of saralasin rather than 5 μg. Water intakes were recorded 5 min after the primary treatment and 60 min after the dipsogenic treatment.

Results and Discussion

Shown in Table 2 are the mean intakes for the 65 min following the primary treatment for the conditions across both treatment blocks. There was no difference in water intake 5 min following the primary treatment. Statistical analysis across the 3 conditions for the group of animals which received the 5 μg saralasin treatment showed a significant effect of treatments on water intake, $F(2,30) = 29.58$, $p<0.001$. However, a planned comparison of the effect on angiotensin II induced drinking of primary treatments of SC isotonic saline vs. SC 5 μg saralasin showed no significant differences.

Analysis for the 3 conditions for the group of animals which received the 20 μg saralasin treatment showed a significant effect of treatments on water intake, $F(2,20) = 16.51$, $p<0.001$. Comparison of the effect on angiotensin II drinking by primary treatments of SC isotonic saline vs. SC 20 μg of saralasin revealed no significant difference.

The results of the present study indicate that saralasin applied peripherally in doses even 40 times greater than that shown to be effective by central administration did not significantly alter the water intake following peripheral angiotensin II.

EXPERIMENT 3

Although the results of Experiments 1 and 2 support the hypothesis of a brain related receptor mediating drinking to peripheral angiotensin II, it is possible that the IC route is more effective in attenuating angiotensin drinking than SC treatment merely because the intracranially applied blocker has more ready access to the systemic circulation. In order to be certain that the relative efficacy of IC injection as compared to SC treatment was not an artifact of the facility of entry into the circulation, the effect of direct vascular injection (intravenous) of blocker on SC angiotensin II was examined.

Method

Seventeen out of 21 animals that were screened for drinking to peripheral (SC) angiotensin responded reliably. The day following the last screening test the positive animals received an intravenous catheter and, after a day of recovery, the animals entered the testing regime. On Day 1 animals received as a primary treatment either 0.2 ml of isotonic saline or 5 μg of saralasin in 0.2 ml of isotonic saline through the intravenous catheter. Both treatments were followed immediately with a 0.2 ml wash of isotonic saline. The animals were then returned to their home cages and 5 min later a dipsogenic treatment of a SC injection of angiotensin II in 0.67 ml of isotonic saline was given. On Day 2 the animals rested, and on Day 3 the animals received the remaining test condition which they had not received on Day 1. Immediately following the conclusion of the test on Day 3 the animals received a test to diagnose the patency of the intravenous catheter. This was carried out

TABLE 3

EFFECT OF INTRAVENOUS SARALASIN ON DRINKING PRODUCED BY SUBCUTANEOUS ANGIOTENSIN

	Treatments		
Primary Treatment + Dipsogenic Treatment	Isotonic Saline (IV) + Isotonic Saline (SC)	Isotonic Saline (IV) + 500 μg Angiotensin II (SC)	5 μg Saralasin (IV) + 500 μg Angiotensin II (SC)
Mean Water Intake (mls ± SEM)	0.2 ± 0.16	2.6 ± 0.76*	3.7 ± 0.85
Number of Animals	15†	17	17

*Although screening data for this group was comparable to that of other experiments, IV catheterization and/or IV injections appear to have attenuated the response to SC angiotensin II.

†All catheters were shown to be patent; however, two animals did not recover from the narcosis induced by the sodium pentobarbital.

by determining if the onset of narcosis following an injection of 40 mg/kg of sodium pentobarbital (60 mg/ml) through the intravenous catheter had a latency of 5 sec or less following completion of the injection. On Day 4 the animals rested and on Day 5 they received a baseline control test consisting of a 0.4 ml injection of isotonic saline followed 5 min later by a 0.67 ml SC injection of isotonic saline. Water intakes were recorded 5 min after the primary treatment and 60 min after the dipsogenic treatment.

Results and Discussion

Shown in Table 3 are the mean intakes for the various treatment conditions. There was no difference in water intake 5 min following the primary treatment. The critical comparison between the condition of intravenous isotonic saline followed by SC angiotensin II and the condition of intravenous saralasin followed by SC angiotensin II was not significant, $t(16) = 1.547$, $p > 0.05$.

These data indicate that the decrease in drinking to SC angiotensin II produced by IC saralasin in Experiment 1 cannot be due to a more effective delivery of blocker to the systemic circulation by IC injections as compared with SC injections.

EXPERIMENT 4

Another potential source of error in interpreting the blocking effects of saralasin in Experiment 1 is that the reduction of drinking seen following the IC injection might actually reflect a generalized behavioral disruption rather than a specific block of angiotensin induced drinking. The following experiment assesses the generality of the effect of IC saralasin on drinking by examining the effect of the analog on cellular thirst.

Method

Eleven animals were implanted with IC cannulae. After recovery from surgery the animals were screened for drinking to IC angiotensin and 10 were found to drink reliably. These 10 then received 2 hypertonic saline screening tests. All 10 animals met the drinking criterion. Following the screening, animals entered a block of 3 treatments of (1) a primary treatment of an IC injection of ACSF followed 5 min later by a dipsogenic treatment of SC isotonic saline (0.8 ml); (2) IC ACSF, followed 5 min later by SC 0.8 ml 10 percent NaCl; and (3) IC 5 μg of saralasin in ACSF followed 5 min later by 0.8 ml 10 percent NaCl. Animals received these treatments in random order with at least one rest day intervening between any 2 tests. Water intakes were recorded 5 min after the primary treatment and 60 min after the dipsogenic treatment.

Results and Discussion

The effect of IC injections of saralasin on drinking to hypertonic saline is shown in Table 4. There was no difference in water intake 5 min following the primary treatment. An IC treatment of ACSF followed by SC 0.8 ml of 10 percent NaCl produced 4.6 ml of water intake, or thirst motivation approximately equivalent to that seen following an IC pretreatment of ACSF followed by SC 500 μg of angiotensin II (Experiment 1) (i.e., 4.1 ml intake). There was a significant effect on water intake across treatments, $F(2,18) = 7.95$, $p < 0.01$. However, the water intake seen following treatment with hypertonic saline was not significantly changed by IC saralasin, $t(9) = 1.709$, $p > 0.5$. These results confirm the findings by Summy-Long and Severs [29] that intraventricular saralasin does not alter the drinking response to cellular dehydration. Consequently, the saralasin blockade in Experiment 1 appears specific to angiotensin induced thirst.

EXPERIMENT 5

The following experiment was designed to determine whether IC saralasin treatment disrupts nonthirst, ingestive behavior. Animals with ad lib access to food and water will consistently consume substantial amounts of a milk solution if daily access is provided [23]. This dessert test allows an assessment of any nonspecific disruptive effect the inhibitor may exert on animals functioning with no apparent fluid or energy imbalance. Also, the consummatory motor patterns for milk drinking are basically the same as those for the ingestion of water.

TABLE 4

EFFECT OF INTRACRANIAL SARALASIN ON DRINKING PRODUCED BY CELLULAR DEHYDRATION

	Treatments		
Primary Treatment + Dipsogenic Treatment	ACSF* (IC) + Isotonic Saline (SC)	ACSF (IC) + 0.8 ml of 10% NaCl (SC)	5 μg Saralasin (IC) + 0.8 ml of 10% NaCl (SC)
Mean Water Intake (mls ± SEM)	1.1 ± 0.28	4.6 ± 0.93	6.4 ± 1.28
Number of Animals	10	10	10

*ACSF = artificial cerebrospinal fluid

Method

Twelve animals were prepared with IC cannulae. All responded reliably to an IC screening to angiotensin II. Following screening, at the same time every day, animals were removed from their home cages (in which food and water was available ad lib) and were placed in a dessert test cage (16.5 cm high, 16 cm wide, and 29.8 cm long, with front, back, and bottom of hardware cloth 3/4 in. mesh, wooden sides, and a metal sliding top). Two drinking spouts similar to those in their home cages entered through the wire mesh near the bottom of the cage. The rats were proffered tap water through one of the spouts and a milk solution through the second consisting of 1 part Bordon's sweetened condensed milk mixed with 2 parts tap water [23] (both milk and water were at ambient temperature). After 1 hr in the dessert cage, animals were returned to their home cage. Animals were exposed to this situation for several days until the daily intake of milk was stable (approximately one week). Then the rats were randomly assigned to receive an IC injection of either ACSF or 5 μg of saralasin in ACSF prior to exposure to the dessert test situation. After completion of the injection and replacement of the obturator, animals were put immediately into the dessert test cage, and allowed to drink for 1 hr at which time intakes of water and milk were recorded. The animals were returned to their home cages. Following the initial IC test injection the rats were allowed to restabilize their intake of the milk for 3 days. Then they received the remaining IC treatment.

Results and Discussion

One animal did not drink any of the milk solution after 2 weeks of exposure and one animal became ill during the course of testing. Both were withdrawn from the experiment. The effect of IC saralasin on the fluid intake of animals is shown in Table 5. Water intake was not significantly altered in the test situation, $t(9) = 0.511$, $p > 0.05$.

Intracranial saralasin did not disrupt milk drinking. Rather, the effect of the analog was to produce a slight (17 percent) but statistically reliable increase in milk intake, $t(9) = 4.33$, $p < 0.01$. The reason for the reliable increase in milk intake over the hour test following IC saralasin is unclear. The intracranially injected analog produces core temperature hypothermia (personal communication, John Simpson) and the increased intake of milk may be an energy regulatory response [2]. However, it is unlikely that the blocking effect of IC saralasin on SC angiotensin II is the result of saralasin inducing a competing hunger drive. Data from this laboratory indicate that after 24 hr of food deprivation (water ad lib) animals did not have a significantly altered water intake in a one hr test following 500 μg of SC angiotensin II as compared to their response following ad lib food (for ad lib food condition, mean water intake = 4.7 ± 0.91 ml; for food deprived condition, mean water intake = 7.4 ± 0.743; n = 7).

TABLE 5

EFFECT OF INTRACRANIAL SARALASIN ON MILK AND WATER DRINKING IN A "DESSERT" TEST

	Treatments	
	Artificial Cerebrospinal Fluid (IC)	5 μg Saralasin
Mean Milk Intake (mls ± SEM)	15.1 ± 0.64	17.5 ± 0.91
Mean Water Intake (mls ± SEM)	0.5 ± 0.34	0.8 ± 0.31
Number of Animals	10	10
p Value	–	<0.01

GENERAL DISCUSSION

The present series of studies was designed to examine which of the two current hypotheses [12] of the dipsogenic site of action of systemic angiotensin is tenable. The results of Experiment 1 indicated that IC saralasin, a competitive inhibitor of angiotensin II, effectively reduced the drinking

produced by systemically (SC) applied angiotensin II. When comparable doses of saralasin were applied either subcutaneously or intravenously prior to SC angiotensin treatment, there was no attenuation of the dipsogenic effect. Control experiments examining consummatory responses indicate that IC saralasin does not disrupt consummatory behavior. Taken together these experiments can be interpreted as the first direct experimental evidence showing that peripheral angiotensin II produces thirst by direct action on the brain rather than by action on a peripheral receptor. Furthermore, the results suggest that both blood-borne and cerebrospinal fluid-carried angiotensin exert a dipsogenic action by engaging the same central angiotensin receptors.

The location of central angiotensin sensitive sites has been the topic of considerable research. Early studies [8] concluded that there was a broad band of tissue sites sensitive to angiotensin (e.g., preoptic area, septal region, anterior hypothalamus, etc.). However, further experimental scrutiny of the IC drinking phenomena revealed that, for the activation of drinking mechanisms, the critical aspect of injections into sensitive tissue was that the hormone actually gains access to the cerebrospinal fluid [3, 18, 19, 21] and not the tissue site per se. These findings led to the conclusion that periventricular receptor sites must mediate the dipsogenic response to IC angiotensin II and that the same periventricular sites could possibly be reached by blood-borne angiotensin [18, 19, 20, 21].

Experimental support for periventricular receptors accessible to systemically generated angiotensin comes from the following lines of research: (1) radiometric studies which show that systemically applied angiotensin is excluded from brain parenchyma proper (e.g., anterior hypothalamus, preoptic area, cortex, etc.) [24; Epstein, Johnson and Shrager, unpublished observations], (2) autoradiographic studies in mice [31] and in rats [20,26] which show that following systemic injection of labeled angiotensin II, radioactivity is high in the ventricular spaces, periventricular tissues, the choroid plexuses, and extra blood-brain barrier organs, and (3) radiometric analysis which shows a rapid entry of radioactivity into the cerebrospinal fluid following systemic infusion of labeled angiotensin II [19,20]. The results of Experiment 1, showing that saralasin with access to the ventricular system [21] attenuates drinking to systemically delivered angiotensin II, lend additional support to the hypothesis of a periventricular site of action.

Recent findings have shown that there is a cephalic renin-angiotensin system in which components comparable to those of the renal renin-angiotensin system are synthesized de novo in the brain [11, 16, 32]. The existence of this brain system has prompted many investigators to speculate that this central system may have an important role in thirst. If at some time this proposed function for the central system is experimentally substantiated, it may be necessary to reassess the findings presented here, for they would also be compatible with the more complex hypothesis that there is a peripheral dipsogenic angiotensin receptor with input into a central thirst system and that there is a central angiotensin link that is susceptible to blockade by saralasin. However, in light of our current understanding of the mechanisms of angiotensin thirst, the most parsimonious conclusion to be drawn from the results of the present analysis is that circulating angiotensin II does gain access to periventricular dipsogenic receptors and thereby mobilizes drinking behavior.

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