Pharmacology Biochemistry & Behavior, Vol. 8, pp. 409–415. Printed in the U.S.A.

Dissociation of Responses to Extracellular Thirst Stimuli Following Zona Incerta Lesions

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(Received 9 January 1978)

WALSH, L. L. AND S. P. GROSSMAN. *Dissociation of responses to extracellular thirst stimuli following zona incerta lesions.* PHARMAC. BIOCHEM. BEHAV. 8(4) 409–415, 1978. – In male albino rats, bilateral lesions in the anterior zona incerta which decrease ad lib and food-deprivation water intake and osmotic thirst but leave hypovolemic thirst intact, severely impaired or abolished drinking in response to systemic injections of isoproterenol or central administration of angiotensin II. Water intake following water deprivation was reduced by one-fourth. Reasons for the dissociation of responses to hypovolemia, water deprivation, isoproterenol and angiotensin were suggested.

Zona incerta lesions Extracellular thirst Angiotensin Isoproterenol Water deprivation Water intake regulation Drinking

RATS with electrolytic lesions in the anterior zona incerta (ZI) are hypodipsic when allowed ad lib access to dry food and water, and adipsic or nearly so when food deprived [38]. In addition, ZI animals show a marked reduction in water intake following intraperitoneal administration of hypertonic saline [39], a procedure which causes a depletion of intracellular fluid stores, but essentially normal increases in drinking in response to extracellular hypovolemia produced by subcutaneous injections of polyethylene glycol (PG) [39] or Formalin [40].

Depletion of the extracellular fluid compartment can activate drinking control systems in at least two ways: (1) through alteration of input from stretch receptors in the low pressure side of the circulation, and/or (2) through reflexive activation of the renin-angiotensin system of the kidney [13], probably via subsequent stimulation of central angiotensin receptors. Drinking responses to the administration of a hyperoncotic colloid like PG are unaffected by nephrectomy [12] and only slightly reduced by infusions of antiserum to angiotensin II [1], suggesting that this type of treatment produces extracellular thirst stimuli independent of the second route described above. The intact drinking response of the ZI animal to PG or Formalin does not, then, guarantee that it can respond normally to the endocrine or kidney component of extracellular thirst.

The present experiment examined the water intake of rats with ZI lesions to three other extracellular thirst stimuli: systemic injections of the beta-adrenergic agonist, isoproterenol, central administration of angiotensin II, and 24 hr water deprivation. Isoproterenol-induced drinking, in contrast to that stimulated by PG, is abolished by nephrectomy [18] and is severely attenuated by infusions of angiotensin antiserum [1], suggesting it is dependent on the kidney's renin-angiotensin system. Central microinjections of angiotensin II have been shown to produce short-latency drinking responses [34]. Thirst following water deprivation is not thought to be kidney-dependent and may, in fact, be a response to decreases in both extracellular and intracellular fluids [14].

METHOD

Animals and Surgery

Thirty-eight male albino rats (Holtzman, Madison, WI) weighing 350–450 g at the time of surgery were housed individually in an air-conditioned colony with a 12 hr light–12 hr dark cycle. For the isoproterenol and water deprivation tests 13 animals received bilateral electrolytic lesions under Nembutal anesthesia by passing a 1.5 mA anodal direct current for 10 sec through a No. 3 stainless steel insect pin that was insulated except at the flattened tip. The electrode was stereotaxically positioned according to the following coordinates from the de Groot atlas of the rat brain [16]: AP = 5.4; L = 1.7; H = −1.6. Ten additional animals, serving as operated controls, underwent identical surgical procedures except the electrode was not lowered into the brain. For the angiotensin tests, 10 animals received bilateral ZI lesions according to the procedures outlined above and five animals served as unoperated controls. The rats with ZI lesions were then subjected to a standard series of drinking tests (described below). Five lesion animals whose behavior was most characteristic of rats with symmetrical ZI damage, and the five unoperated controls, were then implanted, unilaterally, with cannulae aimed at the lateral ventricles (de Groot coordinates:

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[2] We thank A. Plumber of Ciba Pharmaceuticals, Summit, NJ, for the generous supply of Hypertensin Ciba.

[3] Support for this research was provided by USPHS grant MH 10130 to S. P. Grossman.

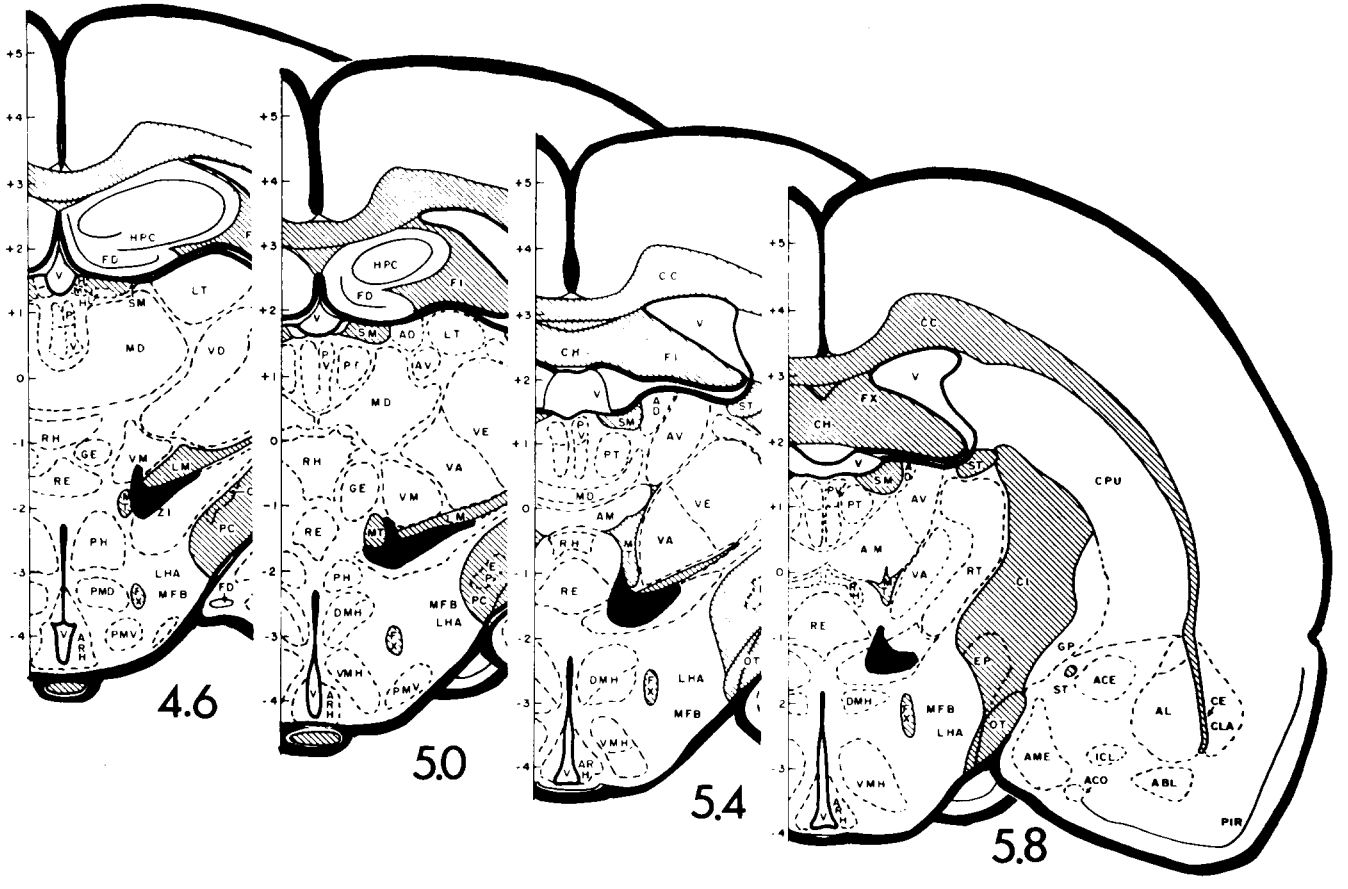

FIG. 1. Diagrammatic representation (after de Groot [16]) of the extent of a typical anterior zona incerta lesion.

AP = 4.8; L = 2.6; H = 3.5) and fixed to their skulls with dental cement. The cannulae were constructed from 23 g (outers) and 30 g (inners) stainless steel tubing. The outer cannulae, and the 30 g solid steel wire styli which occupied them at all times other than test periods, were 11 mm in length; the inner cannulae were 12.5 mm in length. The tips of the outer cannulae were positioned in the tissue immediately above the lateral ventricles. The ventricles were punctured only when the longer inner cannulae were in position during testing.

Procedure

Food and water intake and body weight of all animals were monitored daily for at least one week before surgery as well as postoperatively. Tap water and dry lab chow (Teklad Mouse and Rat Diet, Teklad, Inc., Monmouth, IL) were available ad lib for 6–8 days following surgery. Water intake in the absence of food was then monitored for 2 days. Following this test, food and water were available ad lib for 5–7 days, at which time the animals were given injections of either 4 ml of 1 M saline intraperitoneally or 5 ml of 10% PG dissolved in isotanic saline, subcutaneously (SC). After hypertonic saline injections water intake was monitored every 30 min for the first 4 hr and again 24 hr after the injection. After PG injections water was withheld for 6 hr and then intake was monitored every hour for 2 hr and again 24 hr after the injection. Food was not available during either test. After 5–7 days ad lib access to food and water, the second challenge (hypertonic saline or PG) was administered. After this series of tests, 5 of the 10 ZI animals that were scheduled for angiotensin tests were selected for cannula implantation.

Half of the lesion and control animals in the isoproterenol group received 0.04 mg/kg isoproterenol hydrochloride while the other half received 0.08 mg/kg of the drug. Both doses of the drug were dissolved in isotonic saline so that the injection volume for each animal was 1 ml. The solutions were prepared from a 0.2 mg/ml stock solution (Isuprel, Winthrop Laboratories, New York, NY). Water intake was monitored 1, 2, 3, and 24 hr after the injection; food was not available during the test. After 4–5 days ad lib intake, each animal was again tested for isoproterenol-induced drinking, using the other dose. Five to 7 days later water intake within 1 hr following 24 hr water deprivation was determined. Food was present during the deprivation but not during the 1 hr test. Food was returned after the 1 hr test and food and water intake in the next 23 hr were also measured.

The specified doses of isoproterenol are low compared to those used by other experimenters who have reported optimal drinking with 0.15 or 0.33 mg/kg isoproterenol

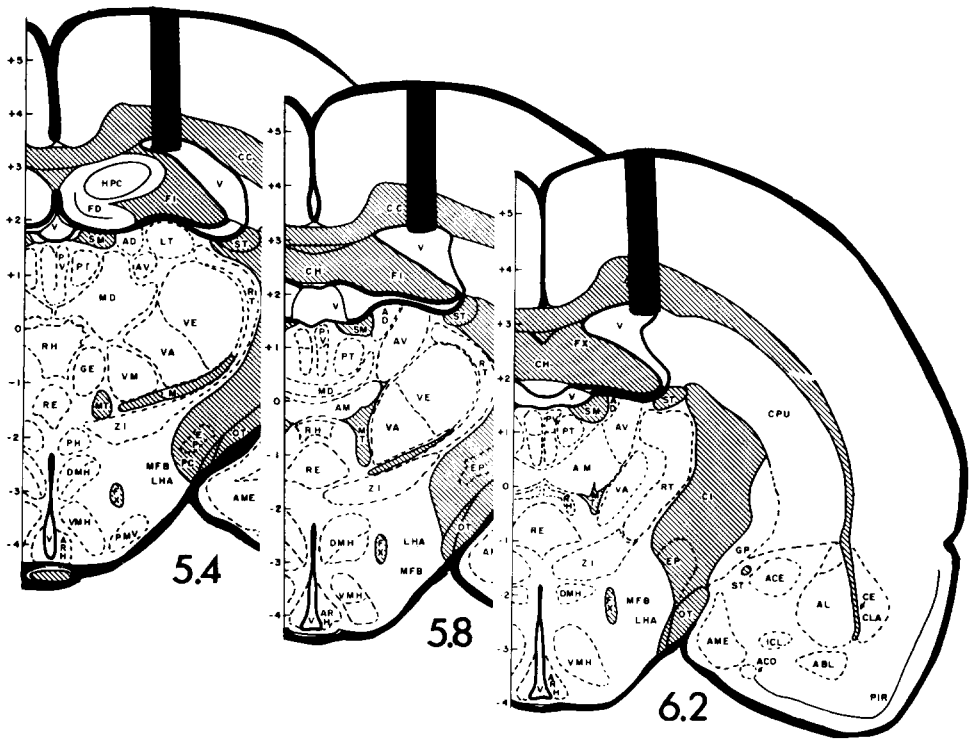

FIG. 2. Diagrammatic representation (after de Groot [16]) of the location of the unilateral cannulae aimed at the lateral ventricle.

[22,23]. Our lower doses have been shown to produce significant increases in water intake in other laboratories [23]. They were used in the present experiment after pilot studies indicated that both 0.15 and 0.33 mg/kg isoproterenol produced a high rate of mortality in our animals. A dose-response study revealed that 0.04 and 0.08 mg/kg isoproterenol elicited very satisfactory drinking responses (approximately 7.5 and 12.5 ml within 3 hr, respectively) with fewer adverse side effects. However, even these low doses produced noticeable distress in some animals.

Those animals implanted with lateral ventricle cannulae were tested in the following manner. Beginning 5 days after surgery baseline measurements were taken once daily, for 3 days, for a 30 min period in the home cage. Then on at least 2 test days each animal received a 1.0 μl intraventricular injection of angiotensin II (Hypertensin, Ciba) solution (100 ng/μl) delivered in an isoosmotic vehicle at approximately 0.1 μl per sec. The rat was then returned to its home cage and latency of response was recorded as well as food intake 15 and 30 min after injection.

Upon completion of behavioral testing the animals were sacrificed with an overdose of Nembutal and perfused intracardially with isotonic saline and 10% formal saline solution. Frozen sections (50 micra) were cut through the area of the lesions and cannula tracts, were mounted on slides and stained with cresyl violet.

RESULTS

Histological examination revealed that 9 of the 13 animals receiving electrolytic lesions in preparation for the isoproterenol and water deprivation tests had bilateral lesions located in the anterior dorsomedial ZI. The lesions were similar to but somewhat smaller than those described in previous reports [38,39] and spared the ventral and lateral portions of the ZI in many instances. The animals with these small ZI lesions did not, however, differ behaviorally from those with larger ZI lesions. A diagrammatic representation of the extent of a typical small ZI lesion is shown in Fig. 1.

Histological examination of the 5 animals with combined bilateral ZI lesions and lateral ventricle cannulae revealed accurate placement of both lesions and cannulae. The cannulae of the control animals were indistinguishable in placement from those of the ZI animals. The position of the cannula tract of one animal in this group is shown in Fig. 2.

As has been previously noted [38, 39, 40, 41], bilateral lesions in the anterior ZI reduced daily water intake ($\bar{X}$ preop = 50.9 ml per day, $\bar{X}$ postop = 36.3 ml ($p<0.01$). During 24 hr food deprivation the control animals drank an average of 27.9 ml per day while those with ZI lesions consumed only 5.8 ml ($p<0.01$). Rats with ZI damage showed a marked reduction in response to injections of hypertonic saline ($\bar{X}$ controls = 20.4 ml in 4 hr, $\bar{X}$ ZI = 4.7 ml ($p<0.02$) and their intake did not increase significantly within the next 20 hr ($\bar{X}$ = 5.9 ml). In contrast, all rats showed a significant increase in intake following injections of PG ($\bar{X}$ controls = 6.0 ml, $\bar{X}$ ZI = 4.2 (N.S.)). Unlike the 24 hr intake following saline injections, the 24 hr intake of rats with ZI lesions after PG was reliably greater than their 24 hr water intake during food deprivation ($\bar{X}$ baseline = 5.8 ml, $\bar{X}$ PG = 25.3 ml ($p<0.01$).

The response to other types of extracellular thirst stimuli was not, however, similarly intact. Rats with bilateral ZI lesions consumed little water in the 3 hr after either dose (.04 or .08 mg/kg) of isoproterenol ($\bar{X}$ low dose = 2.4 ml, $\bar{X}$ high dose = 3.5 ml). This intake was significantly lower ($p<0.01$) than that of the operated control

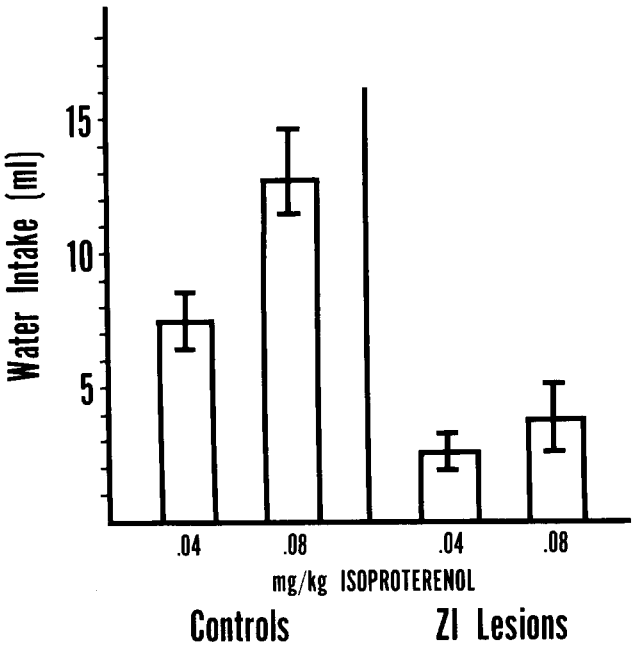

FIG. 3. Mean water intake of control animals (n = 10), and of rats with ZI lesions (n = 9) in the 3 hr following systemic injection of either .04 or .08 mg/kg isoproterenol.

animals ($\bar{X}$ low dose = 7.3 ml, $\bar{X}$ high dose = 12.7 ml). The 0.08 mg/kg dose produced one fatality in the ZI lesion group. Water intake of all animals following injections of isoproterenol is shown in Fig. 3.

Rats with ZI lesions also failed to drink in response to intraventricular administration of angiotensin. Following a 1 μl injection containing 100 ng of angiotensin II, control animals began to drink with a median latency of 20 sec and continued to drink for several minutes. They consumed an average of 12.0 ml of water within 15 min. The drinking responses of these animals were consistent and reproducible and represented a significant ($p<0.01$) increase over their baseline intake ($\bar{X}$ = 1.4 ml) during the 30 min observation period. In contrast to the short-latency and vigorous drinking exhibited by the controls, rats with ZI lesions receiving the same dose of angiotensin rarely approached the water bottle at all (median latency = 9 min) and then took no more than a few licks. Only one ZI animal consumed a measurable quantity of water during any of the tests; it drank 4 ml in 15 min each time it was tested. Average intake in 15 min following angiotensin administration is shown for controls and ZI animals in Fig. 4.

Rats with ZI damage drank substantial quantities of water ($\bar{X}$ = 13.4 ml) in the 1 hr test period following 24 hr of water deprivation although food was removed just prior to this test. This intake is reliably greater than their water intake during a full 24 hr of food deprivation ($\bar{X}$ food deprivation = 5.8 ml, $p<0.001$). Water intake in the 24 hr following water deprivation (food present during the last 23 hr) was also significantly greater than normal ad lib intake for that period of time ($\bar{X}$ baseline = 31.1 ml, $\bar{X}$ test = 57.8 ml, $p<0.001$). The increase in drinking could not be attributed to an increase in food intake following water deprivation ($\bar{X}$ baseline = 33.1 g, $\bar{X}$ test = 31.1 g).

Control animals drank more than rats with ZI lesions both 1 hr ($\bar{X}$ controls = 18.2, $\bar{X}$ ZI = 13.4, $p<0.05$) and

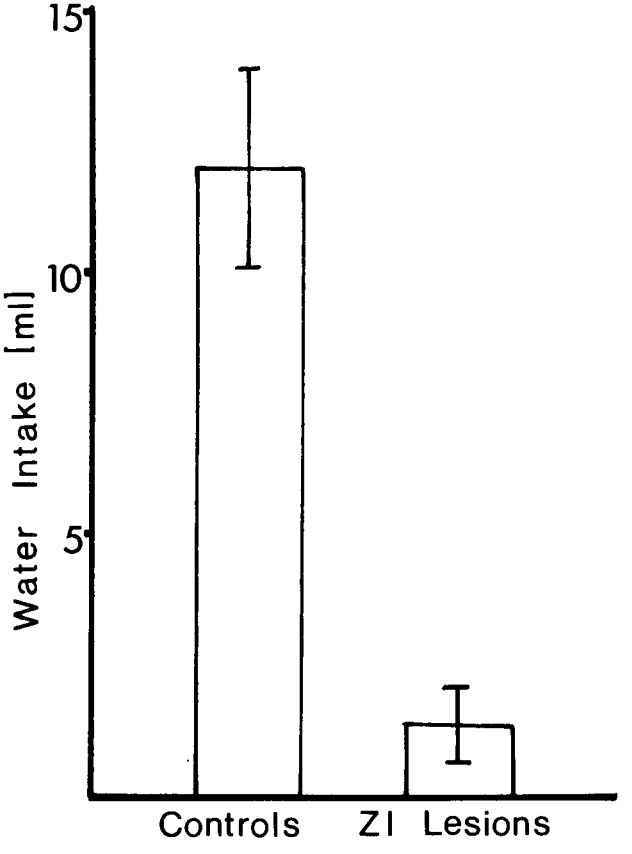

FIG. 4. Mean water intake of control animals (n = 5), and of rats with ZI lesions (n = 5) in the 30 min following a 1.0 l_t intraventricular injection containing 100 ng angiotensin II.

24 hr ($\bar{X}$ controls = 72.0 ml, $\bar{X}$ ZI = 57.8 ml, $p<0.05$) after the return of water. Thus although ZI animals consumed quite substantial quantities of water in the first hr after water deprivation, they drank approximately 5 ml less than control animals. The rise in water intake represented, however, an average increase of 86% of baseline intake for the lesion group and only 51% increase for the control group. The results of the water deprivation test are also shown in Fig. 5.

DISCUSSION

We previously reported that ZI lesions impaired osmotic but not hypovolemic thirst [39]. It now appears that ZI damage precipitates another interesting dissociation in drinking behavior; although ZI animals can respond to the reduced plasma volume that follows PG or Formalin injections or periods of water deprivation with food present, with an increase in drinking, they are unable to respond to two other extracellular thirst stimuli, systemic injections of isoproterenol or intraventricular angiotensin II.

There is some precedent for dissociations among the various extracellular thirst stimuli. Nephrectomy abolishes β-adrenergic thirst but not hypovolemic thirst [12,18] and eliminates, of course, the primary source of the angiotensin substrate, renin. Angiotensin antiserum produces a much more pronounced attenuation of isoproterenol-induced drinking than PG-induced intake [1], and intraventricular

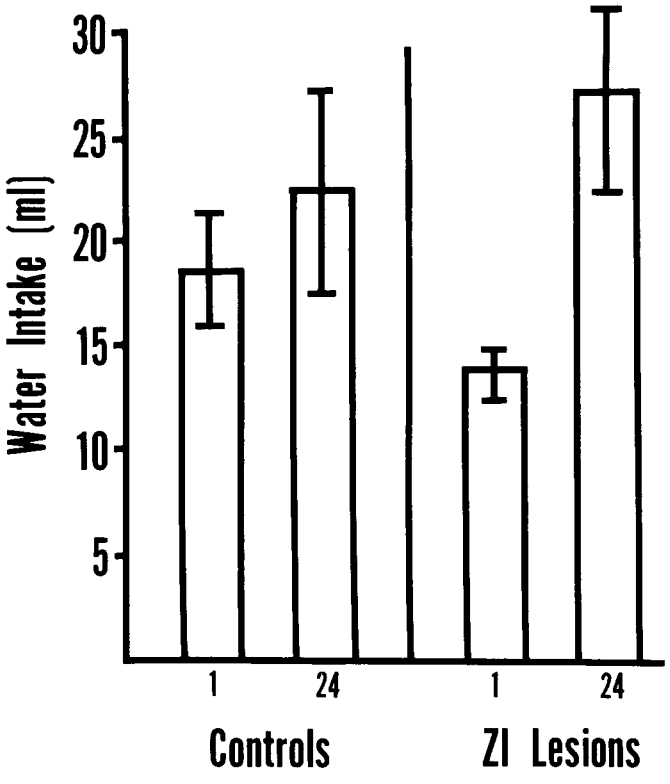

FIG. 5. Mean increase in water intake of control animals (n = 10) and of rats with ZI lesions (n = 9) in 1 hr and 24 hr following 24 hr of water deprivation.

administration of an angiotensin converting enzyme inhibitor or angiotensin receptor antagonist does not affect hypovolemic thirst although it prevents that stimulated by angiotensin [35]. Thus although hypovolemia increases renin release and subsequently angiotensin production [15], this does not appear to be the primary mechanisms of PG dipsogenesis.

That ZI lesions impair both isoproterenol and angiotensin stimulated drinking may be taken to support the contention that isoproterenol is dipsogenic by virtue of its effects on the renin-angiotensin system. Administration of isoproterenol does increase plasma angiotensin II levels [30] and, as mentioned above, angiotensin antiserum interferes with isoproterenol-induced drinking [1]. The rat with ZI lesions may be unable to respond to angiotensin – endogenous or administered – and therefore does not drink following either isoproterenol or angiotensin administration. Other evidence suggests, however, that β-adrenergic thirst may be independent of the renin-angiotensin system. Falk and his colleagues report that compounds with depressor, renin-releasing properties similar to isoproterenol are not always dipsogenic, indicating that isoproterenol-induced drinking may not be attributable to these actions [11]. Administration of an angiotensin II receptor antagonist attenuates drinking following renin or angiotensin but leaves β-adrenergic thirst intact [35]. Peripheral administration of atropine reduced thirst following angiotensin, but not isoproterenol-stimulated water intake [23]. That isoproterenol may stimulate drinking via some endocrine concomitant of renal blood flow other than renin [11] or that its depressor effects might affect volume receptors [26] have been offered as possible alternative mechanisms of action. The latter hypothesis seems unlikely unless the receptors affected are different from those which presumably mediate the hypovolemic message following PG or Formalin, since ZI animals do respond to these 2 stimuli but not to isoproterenol. Whatever the mechanism of action, the integrity of the ZI is necessary for the normal response to this β-adrenergic agonist and the present data suggest inability to respond to angiotensin may be the most parsimonious explanation.

As discussed in METHOD, the doses of isoproterenol employed in the present experiment are lower than those commonly used [21,22] but have been reported to produce significant increases in water intake [22]. A dose-response study in our laboratory revealed that these doses produced maximal water consumption while minimizing debilitating side effects. Higher doses were associated with much higher mortality rates. Since the injections produced highly consistent and copious water intake in control animals (more than double that typically elicited by PG injections) it seems unlikely that the failure of rats with ZI lesions to respond can be explained on the basis of the potency of the challenge.

Similarly it seems unlikely that the failure of ZI rats to drink following intracranial angiotensin can be attributed to dosage. The dose (100 ng) was the same as that employed to relate angiotensin-induced drinking to the lateral hypothalamus (LH) [21] and is fully 1000 times as large as the lowest effective dose reported by Simpson and Routtenberg [34], using the same route of administration. Control animals responded very promptly and consumed within 15 min about one-third their usual daily intake.

Lateral hypothalamic (LH) lesions (which damaged the ZI) have been reported to abolish angiotensin-stimulated drinking [5]. More recently reductions of drinking following angiotensin, renin or isoproterenol have been observed following the placement of small lesions in the medial LH which do not impinge on the ZI [21]. It seems possible that the disruption of responding to these types of extracellular thirst stimuli by ZI and LH lesions may be attributable to damage to pathways common to both areas. Both the ZI and dorsomedial LH carry ascending projections of the locus coeruleus (the dorsal noradrenergic bundle) [19,25] as well as ascending cholinergic pathways originating in the reticular formation, and cholinergic cell bodies [19,33]. There is also recent evidence for adrenaline-containing neurons with terminals in the perifornical region, extending dorsally into the subthalamus and ventrally to the basal surface of the hypothalamus [17]. Angiotensin-stimulated drinking has been related to cholinergic and catecholaminergic systems (e.g., [7, 15, 33]); ZI and medial LH lesions would be expected to interrupt some of the adrenergic and cholinergic pathways that have been directly implicated in the control of drinking. We have not assessed the effects of ZI damage on cholinergic systems but have demonstrated that ZI lesions which impair drinking behavior are followed by a 50% reduction in forebrain norepinephrine, most probably due to destruction of parts of the dorsal noradrenergic bundle and catecholaminergic periventricular pathways. Forebrain and striatal levels of serotonin and dopamine were unaffected [41].

Numerous studies (e.g., [3,6]) suggest that angiotensin and osmotic receptors [2] reside in the vicinity of the third ventricle, and that these two thirst stimuli may actually interact in their activation of drinking behavior [3]. The ZI carries at least 2 afferent pathways supplying the periven-

tricular areas – the noradrenergic dorsal periventricular bundle [19, 25, 36] and the dopaminergic incerto-hypothalamic system [4]. In addition the ZI receives input from the medial preoptic periventricular area [8]. Such projections not only provide the ZI with reciprocal connections with regions which may contain angiotensin receptors, but also suggest a possible involvement of the subthalamus in the control of secretion of pituitary hormones [4], particularly those related to body water balance. It is interesting to note that Wayner and his colleagues [42] have found units within the ZI itself that are particularly sensitive to electrophoretically applied angiotensin or sodium. That these neurons could function as 'receptors' which increase their firing in the presence of angiotensin or an increase in Na^+ is not impossible. Third ventricle tanycytes extending out into paraventricular hypothalamus and perhaps farther [27] could perhaps provide an access route for angiotension to affect the ZI.

The results of the water deprivation test merit further consideration. A rat with ZI lesions drinks following water deprivation, consuming more water in 1 hr than it does during 24, 48, or, in some cases, even 72 hr of food deprivation. The crucial difference between the water deprivation test and the self-imposed water deprivation when food is absent appears to be the feeding that occurs during the 24 hr of water deprivation preceding the 1 hr drinking test. Ingestion and digestion of food requires large quantities of extracellular fluid, lost isotonically as digestive fluids [24]. Intracellular fluid may decrease as well, both in response to the osmolarity of the ingested food, and in an attempt to maintain extracellular fluid volume. Since ZI animals drink in response to extracellular hypovolemia but are deficient in their response to intracellular dehydration [39], it seems likely that their thirst following water deprivation is primarily extracellular in origin. That ZI animals drink somewhat less than controls (about 26% or 4.8 ml) may reflect an impairment of the intracellular component of deprivation-induced drinking.

An alternative interpretation of the reduced water intake of the ZI animal proposes that, for the ZI rat, drinking is aversive or difficult, and thus the animal reduces water intake towards its minimal hydrational requirements [10]. Evered and Mogenson report that rats with posterior ZI lesions do not show loss of regulatory drinking but are more sensitive to quinine adulteration of water than controls and seem to suffer an impaired licking ability. Our more anterior ZI lesion, however, are followed by a different behavioral pattern. Aspects of primary as well as secondary drinking are attenuated while some drinking behaviors are left intact [38, 39, 40, 41]. Also, ad lib intake of quinine adulterated (.01%) water did not decline more in our ZI animals than in controls; in terms of absolute quantity the decrease in water intake was actually smaller among the ZI animals [37].

The hypothesis that drinking may be more aversive to these animals does not require, however, that secondary drinking be more severely reduced than primary drinking, or that all types of drinking be equally depressed, or that the rats be more sensitive to aversive fluids. Recent examination of the drinking of neurologically intact rats offered quinine adulterated water as their only fluid source [31] has revealed some striking parallels in the behaviors of ZI and quinine rats. Both are hypodipsic when food is present, nearly adipsic when food-deprived, and show impaired responses to various thirst stimuli. Most strongly affected in both groups of animals are osmotic thirst and drinking in response to isoproterenol and angiotensin; hypovolemic thirst and drinking following water deprivation are intact or only somewhat attenuated.

The zona incerta carries and receives terminal ramifications from the ascending gustatory pathways en route to the ventral thalamus [28]. It receives input from efferents of the thalamic taste nucleus as well, as they traverse the ZI to reach the cortex [29]. That this subthalamic region receives connections from several taste-related pathways suggests that it may serve as a subcortical site of taste processing. Units in the ZI-Fields of Forel show altered rates of firing in response to stimulation of the tongue with various taste stimuli [20]. In view of the intimate relationship between the ZI and taste pathways, it is conceivable that ZI lesions could, in their effect, alter taste processing in a manner similar to an external aversive stimulus like quinine. Units in the ZI are influenced by the tonic activity of the thalamic taste nucleus [9]; perhaps the ZI exerts some reciprocal influence on the ventral thalamus, an influence removed after ZI damage. Lesions located in the taste region of the ventral thalamus reproduce some of the effects of ZI lesions (hypodipsia, loss of osmotic thirst, no effect on hypovolemic thirst) [9,37].

An explanation of the effects of ZI lesions that is based on disruption of taste pathways and on the similarities between ZI and neurologically intact quinine rats would not require that ZI lesions affect neural systems specifically involved in drinking elicited by hyperosmotic stimuli or angiotensin. Whether such a hypothesis is tenable and, if so, why drinking in response to various stimuli should be differentially taste-dependent remains to be demonstrated.

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