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Some Biochemical Effects of Zona Incerta Lesions That Interfere With the Regulation of Water Intake[1]

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WALSH, L. L., A. E. HALARIS, L. GROSSMAN AND S. P. GROSSMAN. *Some biochemical effects of zona incerta lesions that interfere with the regulation of water intake.* PHARMAC. BIOCHEM. BEHAV. 7(4) 351–356, 1977. — The concentration of NE, DA and 5–HT in forebrain, striatum, and hypothalamus was measured after zona incerta (ZI) lesions that have been shown to result in general hypodipsia; adipsia during periods of food deprivation; impaired or abolished drinking in response to osmotic challenges (but not polyethylene glycol); impaired drinking after systemic isoproterenol or central angiotensin; and impaired or abolished feeding in response to 2-deoxy-D-glucose. The lesions produced a significant (40–50%) depletion of forebrain NE but a correlational analysis of the behavioral and biochemical effects of the lesions failed to indicate a causal relationship. The lesions did not reliably affect (a) forebrain DA or 5–HT; (b) striatal DA or 5–HT; (c) hypothalamic DA, NE or 5–HT. The results of these experiments indicate that significant impairments in ingestive behavior can be demonstrated in animals with diencephalic lesions that do not result in striatal (or forebrain) DA depletions. This confirms previous behavioral analyses showing that ZI lesions which interfere with ingestive behavior do not produce the debilitating sensory or motor dysfunctions typical of the rat with lateral hypothalamic lesions.

Zona incerta; drinking Drinking, zona incerta lesions Zona incerta; lesions Lesions, zona incerta
Zona incerta; biogenic amines Biogenic amines, zona incerta Drinking, zona incerta
Drinking biogenic amines Water intake; zona incerta Water intake, biogenic amines

BILATERAL electrolytic lesions in the zona incerta (ZI), the subthalamic region immediately dorsal to the hypothalamus, produce consistent alterations in food, water and sodium intake regulation which are different from and somewhat more circumscribed than those which follow lateral hypothalamic (LH) lesions. Damage to the ZI in rats is followed by reduction in ad lib water but not food intake [20,47], impaired response to osmotic but not hypovolemic thirst stimuli [49], adipsia or marked hypodipsia during food deprivation [47] and reduced drinking in response to systemic injections of isoproterenol or intracranial administration of angiotensin [46]. Homeostatic sodium appetite is also abolished or reduced [50]. Rats with ZI lesions fail to increase their food intake following injections of 2-deoxy-D-glucose (2DG) although they respond normally to another glucoregulatory challenge, insulin [48].

There are recent suggestions [43, 45, 51] that the effects of LH lesions on ingestive behavior may be due to the interruption of catecholamine (CA) pathways to striatum or telencephalon rather than the destruction of local nerve cell bodies. This possibility raises important questions about the nature of the fibers of passage as possibly contributing to the etiology of the regulatory disturbances observed after ZI damage.

Fluorescence histochemistry, immunofluorescence [44] and specific radioenzymatic [36] techniques have facilitated the mapping of some of the pathways to and through the ZI and the identification of their respective neurotransmitters. Numerous CA-containing fibers travel in the ZI or adjacent portions of the internal capsule, some terminate in the region, others project to hypothalamus, thalamus and other forebrain structures [12]. Most prominent are the noradrenergic (NE) projections of the locus coeruleus (the dorsal bundle) which traverse the diencephalon via the medial portions of the ZI and dorsal hypothalamus [21, 26, 44]. In addition, Maeda and Shimizu [29] have described a separate intermediate bundle which arises in the cell groups A5, A6 and A7 and travels through posterior aspects of the ZI before turning medially to enter the periventricular hypothalamus. The dorsal and ventral CA periventricular fibers, which ascend from the brain stem as part of the dorsal longitudinal fasciculus, also course through the medial ZI [26]. The lateral regions of the ZI (dorsal and dorsomedial to the internal capsule) contain portions of the ventral NE bundle and dorsal components

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of the dopaminergic nigrostriatal pathway [21, 30, 40]. Nigrostriatal fibers do not terminate in the ZI and it is unclear whether the ventral bundle contributes any of the subthalamic NE terminals. Interspersed among these CA pathways are ascending projections from the dorsal and median raphe [6,8]. The ZI contains a fairly high concentration of serotonin (5–HT) which suggests that some of the raphe fibers terminate in this region [36].

Depletion of each of the brain monoamines (DA, NE, 5–HT) have been associated with the disruption of normal ingestive behavior. Dopamine depletion produced by hypothalamic [31], nigral [31,45], pallidal [31] or intraventricular [43] injections of 6-hydroxydopamine (6–OHDA) reproduce many aspects of the LH syndrome, including some of the behavioral changes typically seen after ZI lesions. The effects of 6–OHDA on ad lib feeding and drinking appear to be correlated with the decrease in striatal DA rather than changes in telencephalic NE [11, 31, 43]. The behavioral deficits are more severe if 6–OHDA is given in combination with uptake blockers which restrict the neurotoxic effect to dopaminergic tracts and minimize the damage to noradrenergic pathways [43]. However, some investigators [51,52] have reported that the effects of intrahypothalamic injections of 6–OHDA on water intake appeared to be related to forebrain NE depletion rather than changes in striatal amines. Anterior hypothalamic injections of 6-OHDA, which depleted to telencephalic NE but not striatal DA, produced adipsia while medial hypothalamic injections, which depleted both catecholamines, did not produce this effect in the above studies [51].

Serotonergic pathways may also contribute to the regulation of water intake. Brainstem lesions that damage the cells of origin of the raphe ascending projections produce transient hyperdipsia [9,27]. We have currently shown that knife cuts in the tegmental region increase water intake, often dramatically [18], and the magnitude of the effect correlates with the extent of forebrain 5–HT depletion [19]. Recent experiments [7,37] provide additional evidence for serotonergic involvement in the regulation of food intake. Intraventricular administration of compounds that destroy or temporarily interfere with central serotoneric transmission produce hyperphagia. Finally, the severity of the hyperphagia seen after certain tegmental knife cuts [18] correlates well with the effectiveness of the cuts in depleting forebrain 5–HT rather than any of the catecholamines [19].

The anatomical complexity of the subthalamic region indicates that numerous monoaminergic pathways (as well as cellular components of the area) may be responsible entirely, or in part, for the effects of lesions in the anterior ZI. Auer [1] has called the region a strategic bottleneck after examining the pattern of degeneration in the area following ablation of the frontal cortex. Other authors [28] have indicated that the primary linkage between brainstem and subcortical forebrain is the subthalamus rather than the medial forebrain bundle itself. It was thus important to examine the effects of our behaviorally effective ZI lesions on telencephalic, striatal, and hypothalamic amine concentration.

GENERAL METHOD

Adult male albino rats (Holtzman, Madison, WI), weighing 350–400 g at the time of surgery, were used. The animals were housed individually in an air-conditioned colony with a 12 hr light – 12 hr dark cycle. Food and water intake and body weight were monitored for one week preceding surgery.

Bilateral electrolytic lesions were produced in 15 animals under Nembutal anesthesia by passing a 1.5 mA anodal direct current for 1 sec through a No. 3 stainless steel insect pin that was insulated except for its flattened tip. The electrode was stereotaxically positioned according to the following coordinates from the de Groot atlas of the rat brain [14]: AP = 5.4; L = 1.5, H = −1.6. The control animals underwent all surgical procedures except for the lowering of the electrode and the passing of current through brain tissue.

Following surgery, food and water intake was monitored daily for 7 to 10 days at which time water intake during 24 hr of food deprivation was determined. Following recovery from this test, all animals received an intraperitoneal injection of 4 ml 1 M saline and water intake was monitored for 2 more hours. No food was present during this osmotic thirst test.

At the fifth postoperative week both the operated controls and the experimental animals were sacrificed by decapitation. The brain was quickly removed from the cranium and dissected on ice. Striatal and telencephalic tissue samples were weighed, wrapped in foil and stored in liquid nitrogen; the remainder of the brain was discarded. The samples were assayed for 5–HT, DA and NE according to the procedure outlined by Barchas *et al.* [2] with minor modifications [19].

The present data reflect the results of several experiments. A pilot study, conducted in the Spring of 1975, included 5 rats with subthalamic lesions that displayed the complete pattern of water-intake disturbances which we have described earlier [47], 5 rats with small and inconsistent behavioral effects on the three screening tests used and 10 operated controls. A replication of the pilot study included 17 additional animals of which 12 had lesions in the subthalamic region. The results of these two pilot studies were very similar in all respects and therefore the data were combined and are presented under Results: Experiment 1 (vide infra). A second experiment, using identical procedures was conducted in the Winter of 1977 in order to verify the pattern of results observed in the first series. The second experiment consisted of 21 operated controls and 21 rats with ZI lesions and the data are presented under Results: Experiment 2 below.

Results: Experiment 1

(a) Behavioral. All animals with lesions in the subthalamic region displayed normal food intake, accompanied by some disturbances in water intake regulation. Twenty-three of the 27 rats with subthalamic lesions were hypodipsic during food deprivation; 17 displayed reduced responsiveness to the osmotic challenge. Data from 10 animals that showed the most consistent drinking effects typical of rats with ZI lesions (reduced ad lib water intake without hypophagia, little or no water intake during food deprivation, and sever reduction or absence of drinking responses to an osmotic challenge) were selected to form the ZI lesion (ZI) group. Data from 10 experimental animals that displayed the smallest and least consistent effects on these measures were grouped together to form the lesion control (LC) group. The food and water intake of the 2 groups, expressed as a percentage of control intake, is shown in

Fig. 1. Neither of the groups with subthalamic lesions showed any effect on food intake, but both displayed some disruption of drinking behavior. The ZI lesion group averaged a 33% reduction in daily water intake (as compared to a 19% reduction in the LC group), a 78% decrease in drinking during food deprivation (LC rats had a 60% decrease), and a 62% reduction in the drinking response to injections of hypertonic saline (on the average the LC group actually drank slightly more than the control animals on this test).

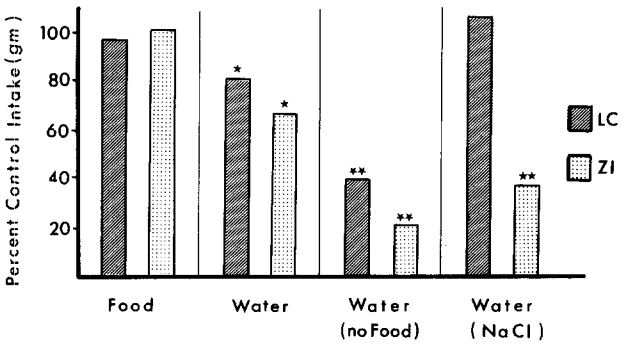

FIG. 1. Mean ad lib food and water intake during food deprivation or following injection of 4 ml of 1 M NaCl, of the ZI lesion group (N = 10) and lesion control (LC) group (N = 10), expressed as a percentage of control (N = 10) intake. * = $p<0.05$; ** = $p<0.01$.

(b) Biochemical. The only systematic neurochemical effect observed after subthalamic lesions was a consistent reduction in telencephalic NE in the ZI group (see Table 1). ZI lesions which reliably impaired both water intake in food-deprived rats and the response to an osmotic challenge in our drinking tests also produced a significant reduction in telencephalic NE ($\bar{X}$ ZI group = 231 ng/g, $\bar{X}$ lesion control group = 388 ng/g, $p<0.05$).

Neither the behaviorally effective ZI lesions nor the control lesions that failed to produce consistent impairments in food-deprivation drinking and responding to osmotic challenges affected telencephalic or striatal DA significantly (see Table 1). The small and statistically unreliable difference in the group means reflects the influence of data from a few animals (n = 4 both in the ZI and the control lesion group) that had striatal DA depletions of 27–61% in the ZI group and 14–41% in the lesion control group. The remaining 6 animals with ZI lesions, as well as the remaining 6 rats with control lesions, had striatal DA levels well within the range of normal values. There was no indication of a relationship between striatal DA and the magnitude of the behavioral deficits. Telencephalic DA was within normal limits in all animals with lesions in the subthalamic region.

There was no significant effect of the lesions on either striatal or telencephalic 5–HT. The small 5–HT decreases seen in the ZI lesion group are the result of larger (about 50%) depletions in 2 of the 10 rats whereas values for the rest of the group were at control level. These 2 animals were not behaviorally distinct from the 8 which had normal levels of 5–HT. Similarly, there was 1 animal in the lesion control group with a 44% depletion of telencephalic 5–HT which showed no greater impairment of ingestive behavior than the other members of that group that showed little or no loss of 5–HT.

TABLE 1

MEAN REGIONAL CONCENTRATIONS OF MONOAMINES (NG/G ± SE)

	Striatum		Telencephalon		
	DA	5-HT	NE	DA	5-HT
Control	8730 ±451	460 ±31	408 ±35	1054 ±38	491 ±39
Lesion Control	7668 ±475	479 ±31	388 ±67	1010 ±128	463 ±39
Zona Incerta Lesion	7593 ±728	445 ±33	231* ±54§	1056 ±96	431 ±48

N = 10 in all groups except for telencephalic DA, where N = 5.
*$p<0.02$ compared to control.
§$p<0.05$ compared to lesion control.

Results: Experiment 2

(a) Behavioral. The results of the second series of experiments were quite uniform. None of the 21 animals with subthalamic lesions were significantly hypophagic after the first 24–48 hr postoperatively; 18 rats drank less than the control mean (23.4–29.8 ml as compared to a control mean of 31.2) under ad lib conditions; all 21 animals drank less than the control mean (0.0–12.00 ml compared to a control mean of 16.8 ml) when food was not available; and all but one of the 10 experimental animals tested with hypertonic NaCl drank less than the controls in the 2 hr after the osmotic challenge. The overall results are summarized in Fig. 2 which presents a stricking similarity to the pattern of results obtained in the first experiment (Fig. 1). Because there was relatively little variability in the behavioral data we did not distinguish between effective and ineffective lesions as had been done in Experiment 1.

(b) Biochemical. The results of the biochemical assays are summarized in Table 2. As in Experiment 1, the only significant biochemical effect of the lesions was a drop in telencephalic NE. Telencephalic DA and 5–HT were within control range. The measure of principal interest, striatal DA, was not significantly reduced by the lesion (although a few animals showed small depletions), and striatal 5–HT was at control level. This pattern of results is an exact replication of the data from the first experiment (see Table 1).

In view of a recent report of an incerto-hypothalamic DA projection in the rat [4] and of a dorsomedial trajectory of NE projections through the subthalamus to the paraventricular hypothalamus [21, 26, 44], we included assays of hypothalamic amines in the second experiment. The data summarized in Table 2 indicate that the ZI lesions did not interfere with these projections, possibly because the lesions were localized dorsal and rostral to the DA and NE projections.

DISCUSSION

The results of the present experiments demonstrate that neither striatal nor telencephalic DA depletions are neces-

TABLE 2

MEAN REGIONAL CONCENTRATIONS OF MONOAMINES (NG/G ± SE)

	Striatum		Hypothalamus		Telencephalon		
	DA	5-HT	NE	5-HT	NE	DA	5-HT
Control	8886	371	1476	622	433	900	569
	±229	±34	±63	±79	±10	±21	±14
	(17)	(13)	(21)	(20)	(20)	(21)	(20)
Zona Incerta	8791	438	1438	588	270*	915	584
Lesion	±453	±109	±96	±17	±17	±41	±16
	(17)	(8)	(21)	(20)	(20)	(20)	(22)

The number of samples in each group is given in parentheses.
*$p<0.001$.

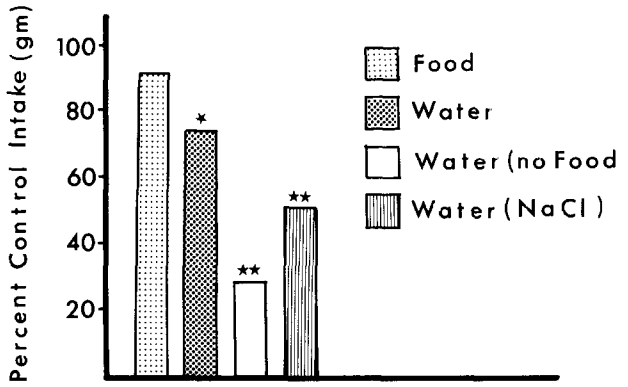

FIG. 2. Mean ad lib food and water intake, and water intake during food deprivation of 21 rats with ZI lesions. Ten randomly selected animals were given 4 ml of 1M NaCl. Their water intake in the two waters subsequent to the injection is also shown. All data are expressed as a percentage of the intake of 21 (or 10) operated control animals of comparable age, weight, and test experience. * = $p<0.05$; ** = $p<0.001$.

sary for the appearance of severe impairments in regulatory functions that control ingestive behavior. Many studies have suggested that a major portion of what is known as the LH syndrome may be the result of damage to the nigrostriatal system [11, 31, 34, 43, 45]. It is apparent from our data that ad lib hypodipsia, food deprivation adipsia, and abolished or reduced response to osmotic thirst stimuli can be produced by lesions which spare the dopaminergic projections. Although the present experimental animals were exposed only to these drinking tests before the biochemical assay, their response to these tests was all-but-identical to those of rats with ZI lesions that received more extensive behavioral scrutiny in previous experiments [46, 48, 50]. It therefore seems likely that glucoprivic feeding, homeostatic sodium appetite, and drinking in response to systemic isoproterenol or intracranial angiotensin may also be lost or significantly impaired in animals that have normal striatal (and telencephalic) dopamine concentrations.

These results indicate that the zona incerta contains neural elements that are specifically concerned with the organism's response to particular hydrational and gluco-regulatory signals. The findings are not incompatible with the hypothesis of Stricker and Zigmond [43] that disruption of the dopaminergic nigrostriatal pathway – which may be essential for general arousal – interferes nonspecifically with ingestive behavior in such a way that many similar impairments in the response to regulatory challenges result. Lateral hypothalamic lesions may well interrupt arousal-related pathways that are critical for all complex behaviors as well as pathways (or local cellular components) that contribute more specifically to the regulation of ingestive behavior.

The behaviorally effective ZI lesions similarly failed to produce any significant striatal, telencephalic, or hypothalamic 5–HT depletions. This indicates that the deficits in ingestive behavior we observed after ZI lesions are probably not related to a gross depletion of serotonin from any major brain region (a large loss in a small compartment would not be detected by our assays). We cannot rule out the possibility that serotonergic pathways that do not course through the subthalamic region may contribute to regulatory processes related to feeding and drinking as a number of recent experiments [7, 18, 19, 37] as well as earlier lesion studies [9,27] have suggested.

Our behaviorally effective ZI lesions did produce significant NE depletions from telencephalon. In view of the anatomical complexity of the area, this finding may not necessarily reflect a causal relationship but it may merely indicate that successful lesions must involve the medial aspects of the subthalamic region that is traversed by the dorsal noradrenergic bundle [22, 26, 44]. A more specific relationship is indicated by the results of the first experiment which showed significant telencephalic NE depletions only in animals with consistent and large behavioral deficits. However, a formal correlational analysis of the data obtained in the second experiment, using the magnitude of the behavioral impairment (water drunk during periods of food deprivation) and the extent of telencephalic NE depletion, failed to yield a significant correlation coefficient ($r = -.24$). Telencephalic NE has been implicated in the regulation of water intake by Osumi *et al.* [35] as well as the results of some of our own studies [18,19] since electrolytic lesions as well as surgical knife cuts in portions of the tegmentum which are traversed by NE projections to the telencephalon resulted in hyperdipsia of varying duration. However, we [19] have been unable to demonstrate significant correlations between the behavioral and biochemical effects of these lesions. We wish to emphasize, in this context, that such correlational analyses

are only as good as the data that are used for them. Since we have analysed only major subdivisions of the brain, it is quite possible that the behavioral effects of our lesions could, in fact, be due to NE depletions at the level of individual nuclei or terminal projection areas, even though the correlation coefficients were extremely low.

Lastly, the fact that the behaviorally effective ZI lesions did not produce significant effects on hypothalamic amine concentrations is worth further discussion. The dorsal and ventral periventricular CA pathways project to and through the ZI [26] and a dopaminergic incertohypothalamic projection to the dorsal and anterior hypothalamus originates in the medial ZI [4]. In addition, there are noradrenergic as well as dopaminergic cell bodies in the caudal part of the ZI (usually spared by our lesion) [3, 4, 26] some of which project to or through the hypothalamic region. The present results allow at least two interpretations. The rather dorsal and rostral subthalamic lesions may miss most of the CA pathways to the hypothalamus; or, these projections constitute such a small percentage of the total catecholaminergic innervation of the hypothalamus that their destruction fails to result in a significant overall depletion. Considerable experimental evidence suggests that catecholaminergic innervation of the hypothalamus contributes significantly to the regulation of water (and food) intake. However, there is little information, as yet, about the precise pathways involved. Intraventricular or intrahypothalamic injections of NE inhibit drinking in water deprived rats (as well as animals given hypertonic saline systemically) but have no apparent effect on water intake in response to extracellular stimuli [39]. This pattern of effects is, in many respects, similar to that seen after our ZI lesions. Intrahypothalamic injections of the beta-adrenergic agonist isoproterenol [25] or NE itself [10,39] have also been reported to elicit drinking in sated animals but the problem of leakage into the systemic circulation has not been ruled out in these experiments [15]. Finally, intracranial administration of adenosine 3',5'-monophosphate (cAMP) which interacts with postsynaptic noradrenergic mechanisms, produces an increase in water intake, particularly when cAMP is injected directly into the zona incerta [38].

At this point, it is worth mentioning that the ZI contains numerous cell bodies and fibers of passage that have not been associated with any of the biogenic amines. The area is rich in L-glutamic acid decarboxylase (used to estimate gamma-amino-butyric acid) [22]. Cholinergic cell bodies [21] as well as cholinergic fibers of passage [21,41] have also been described. The latter is of particular interest in view of the internally consistent, if as yet, poorly understood literature which indicates that central cholinergic mechanisms play an important role in the regulation of water intake (see [15] for review). Intrahypothalamic [16] as well as intracertal [23] injections of cholinergic agonists elicit drinking in sated animals and administration of anticholinergic compounds into these areas inhibit drinking in response to deprivation [17] or experimental treatments that result in cellular dehydration or extracellular hypovolemia [5,17].

Our behaviorally effective lesions almost certainly interrupt major components of the ascending projections from the pontine taste-area [33] which appear to convey high-order visceral afferents to the thalamus and amygdaloid region [32]. The projections originate just lateral and slightly posterior to the locus coeruleus where lesions have been reported to affect ad lib water intake [35], they follow the course of the dorsal noradrenergic bundle (which has been related to both food and water intake by several recent studies [18], they project to and through the ventral thalamus (where we have consistently found lesion effects similar to those seen after ZI damage) and they finally end in the region of the central nucleus of the amygdala which has, over the years, been a center of curiosity for investigators of ingestive behavior [13]. The close association of the higher-order visceral afferents with the dorsal noradrenergic bundle could explain the apparent association between telencephalic NE depletions and impaired responding to cellular thirst stimuli observed in the present series of experiments.

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