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Periventricular Preoptic-Hypothalamic Lesions: Effects on Isoproterenol-Induced Thirst

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LIND, R. W. AND A. K. JOHNSON. *Periventricular preoptic-hypothalamic lesions: Effects on isoproterenol-induced thirst.* PHARMAC. BIOCHEM. BEHAV. **15**(4) 563–565, 1981.—Lesions of the periventricular tissue surrounding the anteroventral third ventricle (AV3V) have been shown to block the dipsogenic properties of many experimental manipulations, including injections of angiotensin. The present study examines the ability of rats with ablations of the AV3V to initiate drinking responses following administrations of isoproterenol, a beta-adrenergic agonist which is thought to elicit drinking in part by activating the peripheral renin-angiotensin system. It was found that rats bearing lesions of the AV3V region drank significantly less across a range of doses than animals with sham lesions. When taken together with results from other studies, the present findings suggest that destroying the AV3V region inhibits the thirst-inducing properties of endogenous, as well as exogenous angiotensin.

Thirst Drinking behavior Periventricular Angiotensin Anteroventral third ventricle (AV3V) Isoproterenol

THE periventricular tissue of the anteroventral third ventricle (AV3V) is a critical neural component in behavioral and physiological mechanisms controlling fluid balance [1]. This area of the basal forebrain was first implicated in thirst by Buggy and Fisher [2] who demonstrated that angiotensin II circulating in the cerebrospinal fluid must have access to the AV3V in order to elicit water intake. From subsequent electrolytic lesion studies a wide range of alterations in the control systems of body fluid homeostasis have been revealed.

The immediate and most profound effect of destruction of tissue surrounding the AV3V is adipsia without aphagia [7]. Impaired vasopressin release [9] accompanies the lesion-induced adipsia and results in a marked dehydration which may be fatal. Rats that survive the acute post-lesion period ultimately recover sufficient water intake under ad lib conditions to maintain themselves. However, in the chronic state animals fail to exhibit normal drinking responses to peripheral and central injections of angiotensin II and systemic dehydration induced by hypertonic saline [3].

The present work was undertaken to extend the characterization of the effects of lesions of the AV3V region on thirst mechanisms by examining the drinking response to isoproterenol treatment. We describe a dose-response analysis of drinking to isoproterenol in rats with sham lesions and AV3V lesions. The results indicate that thirst induced by this beta-adrenoceptor agonist is systematically attenuated in rats with AV3V lesions.

METHOD

Subjects

Thirty-two adult male albino rats weighing approximately 300 grams when the study began were maintained on dry chow and tap water ad lib. The animals were housed in hanging wire cages in a temperature-controlled vivarium under a 12:12 light:dark cycle (lights on at 8:00 a.m.).

Surgery

In order to produce either a sham or an AV3V lesion, the rats were anesthetized with ether and positioned in a stereotaxic apparatus. A longitudinal incision was made to expose the skull, allowing the head to be leveled between lambda and bregma. Through a midline trephination, a beldenamel-coated nichrome wire electrode was lowered 7.6 mm (from the level of the dura) into the brain at 0.3 mm caudal to bregma and zero mm lateral. Before inserting the electrode, the midsagittal sinus was retracted in order to avoid rupture. An anodal lesion was produced (rectal cathode) by passing 3 mA of current for 25 seconds with a Stoelting lesioning device. The electrode was then withdrawn and the opening closed with wound-clips. Sham lesions differed in the dorsal-ventral coordinate (5.0 mm below the dura as opposed to 7.6) and in the absence of electrolytic current.

TABLE 1

AD LIB WATER INTAKE (TOP) AND BODY WEIGHTS (BOTTOM) OF EXPERIMENTAL (E) AND CONTROL (C) RATS ON THE DAY BEFORE AND AFTER SURGERY (MEANS + SEM)

	E	C
	Water Intake	
Prelesion	34.9 (+1.72) ml	34.2 (±2.6) ml
Postlesion	11.6 (+4.80) ml	26.2 (±2.1) ml
	Body Weight	
Prelesion	393.1 (+11.1) g	392.2 (±12.3) g
Postlesion	363.6 (+13.4) g	387.6 (±13.5) g

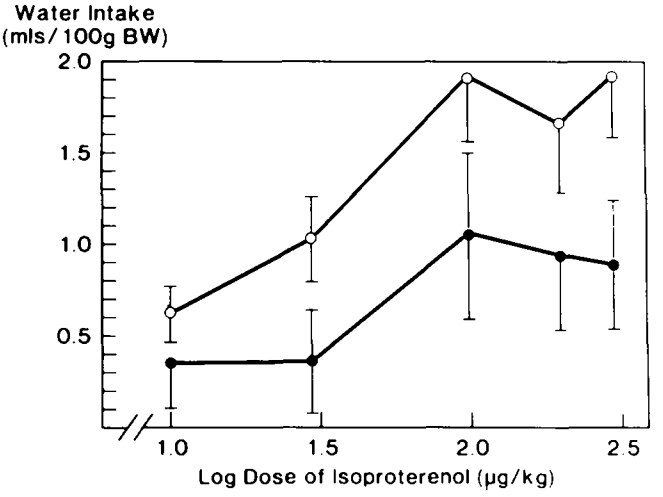

FIG. 1. Dose-response curves of drinking to subcutaneous isoproterenol by rats with sham lesions (open circles, n=9) and AV3V lesions (closed circles, n=11). Intakes are converted to mls drunk per 100 g body weight (mean±S.E.M.) and doses are expressed in log units (μg/kg).

Drinking Tests

All drinking tests were conducted in the home cage during the light period. Fluid consumption during three hours after injections was measured to the nearest 0.1 ml by using a chemical burette fitted with a metal drinking spout. All doses of isoproterenol and the vehicle control were delivered subcutaneously in the back in volumes of 1 ml/kg.

Protocol

Two pre-lesion screening tests with isoproterenol (100 μg/kg) demonstrated that all animals drank reliably when treated with an adequate dose of this drug. Three days after the completion of these pre-lesion drinking tests, the rats received either an AV3V (n=14) or a sham (n=9) lesion.

Animals were weighed to the nearest gram at the time of surgery and on the next day. Twenty-four hour water intake in the home-cage was measured to the nearest ml.

Over a two-week recovery period following surgery, tap water intake resumed and body weights stabilized in the surviving experimental rats (n=11). A dose-response analysis for drinking to isoproterenol was then conducted in the following order: 10, 30, 100, 300, and 200 μg/kg. An isotonic saline control injection was given after the last isoproterenol test. At least 2 days separated consecutive testing periods. After the completion of testing, the animals were perfused with Formalin and brains were prepared for histological analysis by sectioning with a freezing microtome and staining with cresyl violet. Lesions were characterized by examination with a light microscope and by reconstructions in the midsagittal plane. This latter procedure involved the use of a projecting scope to plot the lesioned area and prominent midsagittal structures from successive sections such that a midline "profile" view of the lesion could be produced.

RESULTS

The mean body weight and water intake for the two groups of rats on the day before and after surgery are presented in Table 1. Analyses of variance indicate that the AV3V lesion group manifested a body weight loss and a hypodipsia that are typical of rats with lesions of the AV3V region (trials by groups interaction for drinking, $F(1,22)=5.3$, $p<0.05$; for body weight, $F(1,22)=13.1$, $p<0.005$). Three experimental animals did not survive the post-lesion adipsia.

Because rats with destruction of the AV3V region remain chronically underweight (in this study, the experimental subjects were still significantly lighter than control animals at the time of perfusion), drinking responses have been converted to mls drunk per 100 g body weight. The average intakes of both groups to the saline control injection were less than 0.1 ml per 100 g. The mean responses over the three-hour observation period that followed isoproterenol injections are graphed in Fig. 1. An analysis of variance indicates a significant difference between the groups, $F(1,18)=5.07$, $p<0.05$, with the experimental rats drinking less than the control animals at all doses. The absence of a significant trials by groups interaction points out that the dose-response curves of the experimental and control animals are approximately parallel (i.e., the groups differed consistently across the dose-range that was employed). A significant effect of dose, $F(4,72)=5.07$, $p<0.005$, indicates that drinking responses to the drug injections were dose-dependent.

Figure 2 presents a midsagittal reconstruction of a representative lesion. Included in the damaged area are the organum vasculosum of the lamina terminalis, the median preoptic nucleus, the periventricular stratum of the preoptic area, and the most medial part of the medial preoptic area.

DISCUSSION

Electrolytic destruction of the AV3V attenuated thirst induced by subcutaneous administration of isoproterenol. This reduction of beta-adrenoceptor agonist-induced drinking occurred in a group of experimental animals that manifested post-lesion adipsia and weight loss, signs that are typical of rats with AV3V lesions. A histological analysis showed the cerebral ablations to be similar to AV3V lesions described previously [4].

Current evidence indicates that drinking to isoproterenol may be mediated by both renal and extrarenal mechanisms.

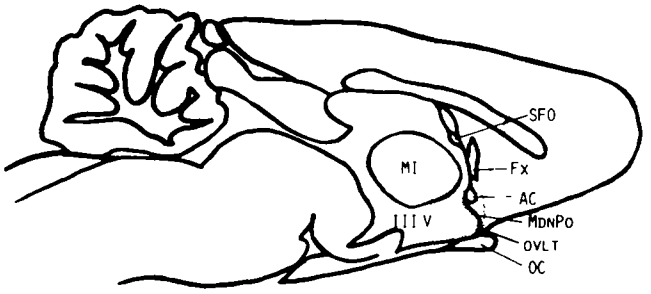

FIG. 2. Midsagittal view of a representative lesion of the AV3V region. The stippling depicts the area of electrolytic damage and the electrode track (SFO, subfornical organ; Fx, columns of the fornix; AC, anterior commissure; MdnPo, median preoptic nucleus; OVLT, organum vasculosum of the lamina terminalis; OC, optic chiasm; IIIV, third ventricle; MI, massa intermedia of the thalamus).

Isoproterenol stimulates renin release by lowering arterial pressure [10] and by directly stimulating beta-adrenoceptors in the kidney [12]. The isoproterenol-induced renin release and the demonstration that nephrectomy abolished isoproterenol associated drinking lead Houpt and Epstein [6] to conclude that the renin-angiotensin system was the sole mediator of the dipsogenic response. However other investigators [5] have provided evidence that extrarenal mechanisms, perhaps associated with arterial baroreceptors or low pressure volume receptors, may also subserve isoproterenol-induced thrist. This redundancy of controls requires that at least two interpretations of the present data be entertained.

The first possibility to consider is that AV3V ablations inhibit isoproterenol-induced water intake by rendering the animal insensitive to the dipsogenic properties of angiotensin. Consistent with this hypothesis are reports that angiotensin II delivered either systemically [3] or centrally [4] loses its dipsogenic potency after destruction of the AV3V region. A recent study [8] has demonstrated that the plasma levels of angiotensin II rise well above the dipsogenic threshold [11] when rats are injected with isoproterenol (30–300 mg/kg). Thus, it would appear that rats with AV3V lesions are insensitive to angiotensin regardless of whether the source of this effector peptide is *exogenous* or *endogenous*.

A second interpretation of the current findings proposes that destruction of the AV3V region inhibits isoproterenol-induced drinking by interrupting mechanisms of extracellular thirst (e.g., high-pressure baroreceptors or low pressure volume receptors) other than the renin-angiotensin system. Although these postulated mechanisms have yet to be specifically identified, they have been implicated to a reasonable degree of certainty [5,13]. The present data may constitute evidence that these proposed extra-renal mediators of hypovolemic thirst have neural representations in the AV3V region.

It is possible that both of the preceeding interpretations are correct. Given the redundancy of fluid regulatory systems and the generalized effects of AV3V ablation on body fluid controls, it might be expected that deficits in both postulated mechanisms contribute to produce the present observation that AV3V lesions disrupt thirst induced by a beta-adrenoceptor agonist. Further work is required to elucidate the mechanisms of isoproterenol-induced thirst as well as the finer details of the effects of AV3V lesions on water intake.

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