Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2952–2956, April 1989
Physiological Sciences

Water intake in rats subjected to hypothalamic immunoneutralization of angiotensin II, atrial natriuretic peptide, vasopressin, or oxytocin

(angiotensin II antiserum/vasopressin antiserum/oxytocin antiserum/atrial natriuretic peptide antiserum/dehydration-induced drinking)

C. R. FRANCI*, G. P. KOZLOWSKI†, AND S. M. MCCANN†‡

*Universidade de Sao Paulo, Faculdade de Medicina, de Ribeirao Preto, 14.100 Ribeirao Preto, Brazil; and †Neuropeptide Division, Department of Physiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235–9040.

Contributed by S. M. McCann, December 23, 1988

ABSTRACT **To investigate the influence of various peptides on control of dehydration-induced drinking, water intake elicited by overnight water deprivation was analyzed in groups of male rats after intracerebroventricular (third ventricle, icv) injection of 2 μl of normal rabbit serum or an equal volume of antiserum directed against angiotensin II (Ab-AII), atrial natriuretic peptide, vasopressin, or oxytocin. There was no difference in water intake after normal rabbit serum and antiserum injections when water was offered immediately after icv injections. Water intake was greatly reduced by Ab-AII when water was offered 1 hr and 3 hr after icv injection. The other antisera were partially effective only when water was offered 3 hr after icv injection. The dipsogenic effect of icv injection of AII in normally hydrated rats was reduced only by icv injection of Ab-AII 3 hr before and not by the other antisera. Ab-AII injected icv had no effect on the drinking that occurred just before and after the onset of darkness and that was associated with eating (prandial drinking). The results indicate that AII is primarily responsible for dehydration-induced drinking, and the other peptides may play a permissive role since their antisera were partially effective, with longer latencies after antiserum injection, which is perhaps the result of gradual diffusion to effective sites within the hypothalamus. In contrast, endogenous AII appears to play little, if any, role in prandial drinking.**

Water intake is a physiological response to depletion of extracellular fluid volume, such as that which occurs in acute hemorrhage, or to cellular dehydration as occurs in prolonged water deprivation (1). Angiotensin II (AII) is a powerful dipsogen that can induce drinking after i.v., intracerebroventricular (icv), or intrahypothalamic administration (1). Central administration of the peptide is much more effective, probably because its site of action is within the central nervous system. After i.v. injection, the hormone can only reach the brain through regions that have no blood-brain barrier, such as the circumventricular organs (for example, the subfornical organ and organum vasculosum lamina terminalis) (2). Reduced extracellular fluid volume brings about release of renin from the juxtaglomerular apparatus, which leads to formation of AII by converting enzyme. Circulating AII, by means of uptake by the subfornical organ and the organum vasculosum lamina terminalis, may play a role in the drinking that follows depletion of extracellular fluid volume (1, 2). Dehydration-induced drinking has generally been thought to be mediated by hypothalamic osmoreceptors, but AII may contribute to this type of drinking as well (2). Because the existence of a brain angiotensin system has been established, it is possible that not only blood borne but also neurally derived AII could be involved in induction of drinking (2).

In contrast, atrial natriuretic peptide (ANP) has been shown to have an inhibitory effect on drinking (3). After its release from the cardiac atria, it too may act by means of uptake by the circumventricular organs to inhibit the hypothalamic areas controlling thirst. Alternatively, the peptide may be released from neurons within the hypothalamus to suppress drinking, an idea consistent with its occurrence within hypothalamic neurons (4, 5). Deficiency of vasopressin (VP) leads to the development of diabetes insipidus with a marked increase in water consumption; however, this is thought to be secondary to renal water loss rather than to a direct dipsogenic action of VP (1).

The studies were conducted to assess the physiological significance of these and other peptides in the control of water intake after dehydration by microinjecting antisera against them into the third brain ventricle (3V) to immunoneutralize the endogenous peptides.

MATERIALS AND METHODS

Animals. Adult male rats (Sprague–Dawley; Simonsen Laboratories, Gilroy, CA) weighing 250–300 g were housed under controlled conditions of light (on from 5:00 a.m. to 7:00 p.m.) and temperature (23°C–25°C) with ad libitum access to laboratory chow (Purina) and water. Stainless steel guide cannulae for microinjections were implanted into the 3V, as previously described (6), under anesthesia induced by 2.5% tribromoethanol (Aldrich; 1 ml per 100 g of body weight, i.p.). Only animals that returned to preoperative weight within 1 week were used for experiments.

In all experiments, animals were tested twice, with a 1-week interval between the tests. The animals were given an icv microinjection (2 μl) of normal rabbit serum (NRS) in the control session and antisera against AII (Ab-AII), ANP (Ab-ANP), VP (Ab-VP), or oxytocin (Ab-OT) (2 μl) in the experimental session. Injections were made during a period of 1 min. Half of the rats received NRS at the first session, and the others were injected first with antisera.

Dehydration Studies. All experiments were started between 8:30 and 9:30 a.m. Water was measured by recording from calibrated water containers attached to the cages of the individually caged rats. Drinking was measured 15, 30, 45, 60, 120, 180, and 240 min after offering water either immediately or 1 or 3 hr after icv microinjections. Thirst was stimulated by overnight water deprivation (18 hr).

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Abbreviations: AI, AII, and AIII, angiotensin I, II, and III; ANP, atrial natriuretic peptide; NRS, normal rabbit serum; VP, vasopressin; OT, oxytocin; 3V, third brain ventricle; icv, intracerebroventricular; Ab-AII, -ANP, -VP, and -OT, antiserum directed against AII, ANP, VP, and OT.

‡To whom reprint requests should be addressed.

Water Intake Elicited by AII. Normally hydrated rats were injected icv with antisera against the various peptides or NRS. Three hours later, saline (isotonic sodium chloride solution) or AII was microinjected icv, and water was offered to the animals.

Normal Water Intake. Rats with ad libitum access to lab chow and water were given icv microinjections of NRS (control session) or Ab-AII (experimental session) at 4 p.m. Lights were turned off at 7 p.m. Drinking was measured every 30 min between 4 and 10 p.m.

Microinjections. The test solutions used in these experiments were isotonic saline (0.15 M NaCl), AII (Sigma), antisera against AII and ANP (Peninsula Laboratories), and antisera against VP and OT raised in rabbits. The Ab-AII crossreacted 100% with AIII and AII, 0.5% with AI, 0.9% with renin substrate, and not at all with human ANP (hANP) and [Arg^8]vasopressin in RIA. The Ab-ANP crossreacted 100% with rat ANP ([Ile^{12}]hANP), hANP, ANP-(8–33) {[Ile^{12}]-hANP-(3–28)}, and rat atriopeptin III. It crossreacted 60% with ANP-(18–28), 5% with rat atriopeptin II, 1% with ANP-(13–28), 10% with auriculin A, and negligibly with OT and [Arg^8]vasopressin, somatostatin, and rat atriopeptin I (J.-K. Chang, Peninsula Laboratories, personal communication). The Ab-VP had 2% crossreactivity to OT and the Ab-OT had crossreactivity of <1% to VP. It failed to crossreact with AII. Chromatography of rat neural lobe extracts revealed single peaks of VP and OT crossreactivity that coeluted with the respective synthetic peptides. Both antisera appropriately stained the respective neuronal peptide systems in the hypothalamus. The microinjections in a total volume of 2 μl of antisera diluted 1 to 2 or similarly diluted NRS were carried out with a Hamilton microsyringe connected by a polyethylene 10 tube to an injecting needle filled with solution. The injections were completed during 60 sec. The brains were removed, and frozen coronal sections were examined to determine the site of microinjection.

Statistics. Significance of differences of water intake after NRS and antisera injections in the same group was calculated by the paired *t* test. The significance of differences of water intake between groups was determined by analysis of variance and Newman–Keuls test for multiple comparisons.

RESULTS

Water Intake Elicited by Overnight Water Deprivation. Fig. 1 shows cumulative intake when water was offered immediately after icv microinjection of NRS or antisera. Ab-ANP (Fig. 1*B*), Ab-VP (Fig. 1*C*), or Ab-OT (Fig. 1*D*) revealed no significant effect on fluid intake. Ab-AII caused a significant decrease in water intake only 30 min after injection (Fig. 1*A*).

Intake of water decreased significantly at all times from 15 to 240 min when it was offered 1 hr after Ab-AII (Fig. 2*A*), but there was no significant effect of ANP or OT antisera (Fig. 2 *B* and *D*). Water intake decreased at 180 and 240 min after offering water when the animals were given Ab-VP (Fig. 2*C*).

Drinking was reduced significantly in all groups when water was offered 3 hr after icv injection of antisera against AII, ANP, VP, or OT (Fig. 3).

When the difference in cumulative water intake from that of the NRS-injected rats was calculated, there was no significant effect of any of the antisera if water was offered immediately after their injection; Ab-AII but not the other antisera was highly effective in suppressing drinking if water was offered 1 hr after injection. Although all antisera had a suppressive effect if water was offered 3 hr after injection of antisera, Ab-AII was significantly more effective between 2 and 4 hr after availability of water than the other three antisera.

Water Intake Induced by AII. The dipsogenic effect of AII was blocked only by antiserum against AII injected 3 hr prior to icv injection of AII (Fig. 4). The other antisera had no effect. None of the rats drank in the control session when

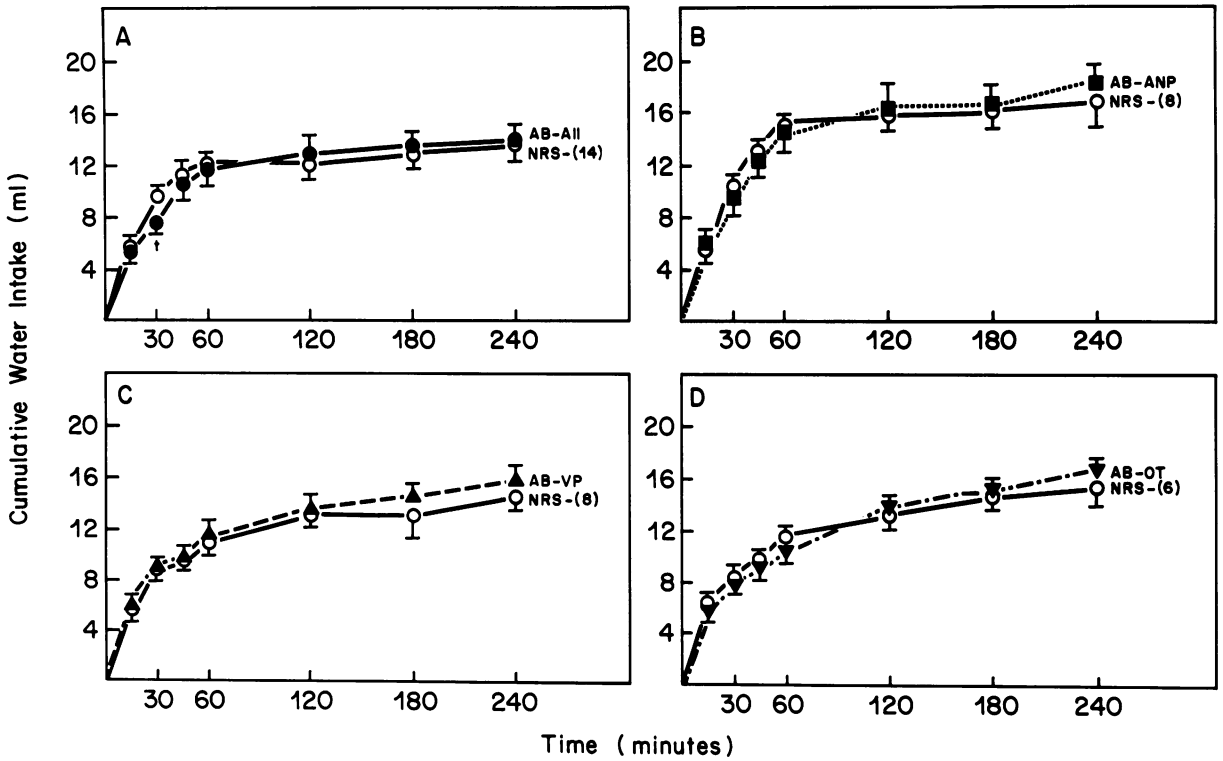

FIG. 1. Cumulative water intake in water-deprived rats when water was offered immediately after icv injection of NRS (control) and Ab-AII (*A*), Ab-ANP (*B*), Ab-VP (*C*), or Ab-OT (*D*). Values are the means ± SEM. Numbers of animals are in parentheses. †, $P < 0.05$ vs. control value.

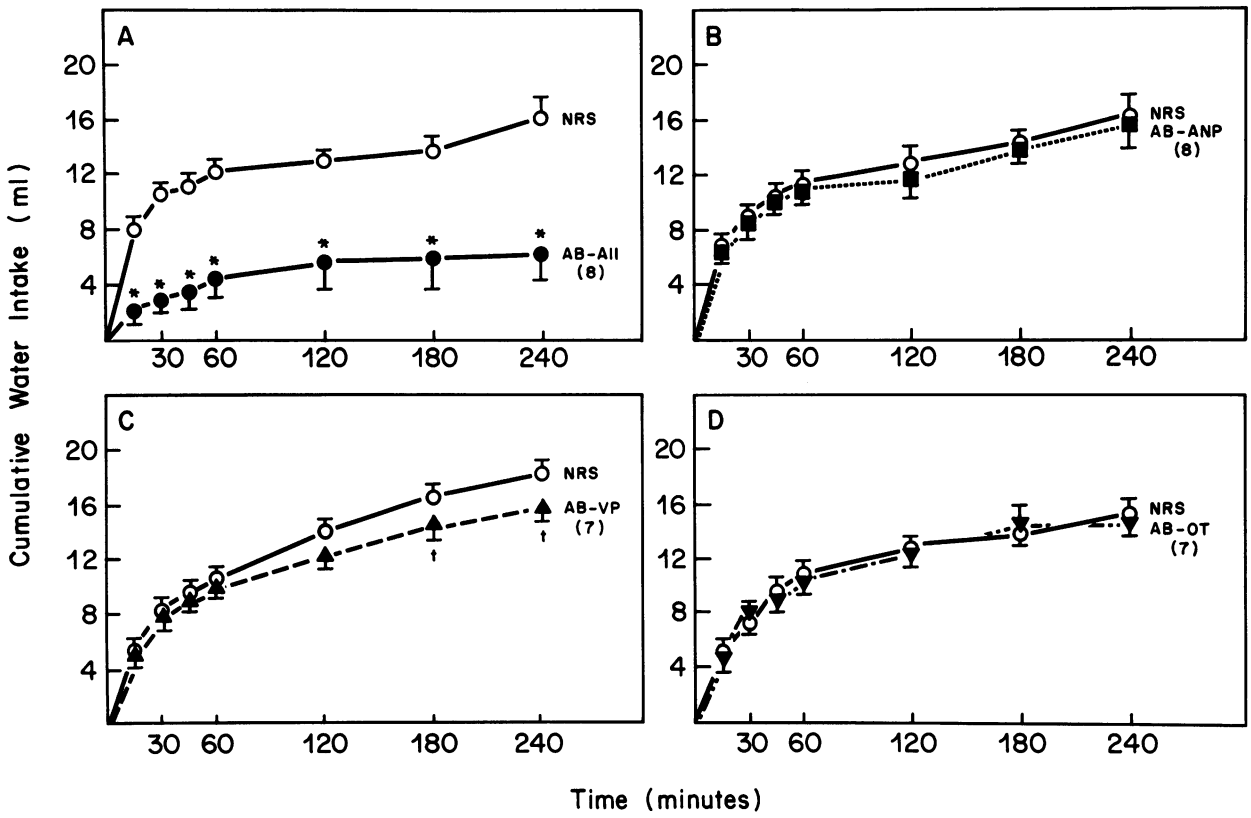

FIG. 2. Cumulative water intake in water-deprived rats when water was offered 1 hr after icv injection of NRS (control) and Ab-AII (*A*), Ab-ANP (*B*), Ab-VP (*C*), or Ab-OT (*D*). Values are the means ± SEM. Numbers of animals are in parentheses. *, $P < 0.005$ vs. control; †, $P < 0.05$ vs. control values.

isotonic saline was injected 3 hr after NRS, Ab-AII, Ab-ANP, Ab-VP, or Ab-OT.

Normal Water Intake. Animals with ad libitum food and water that were given NRS or Ab-AII at 4 p.m. began to drink

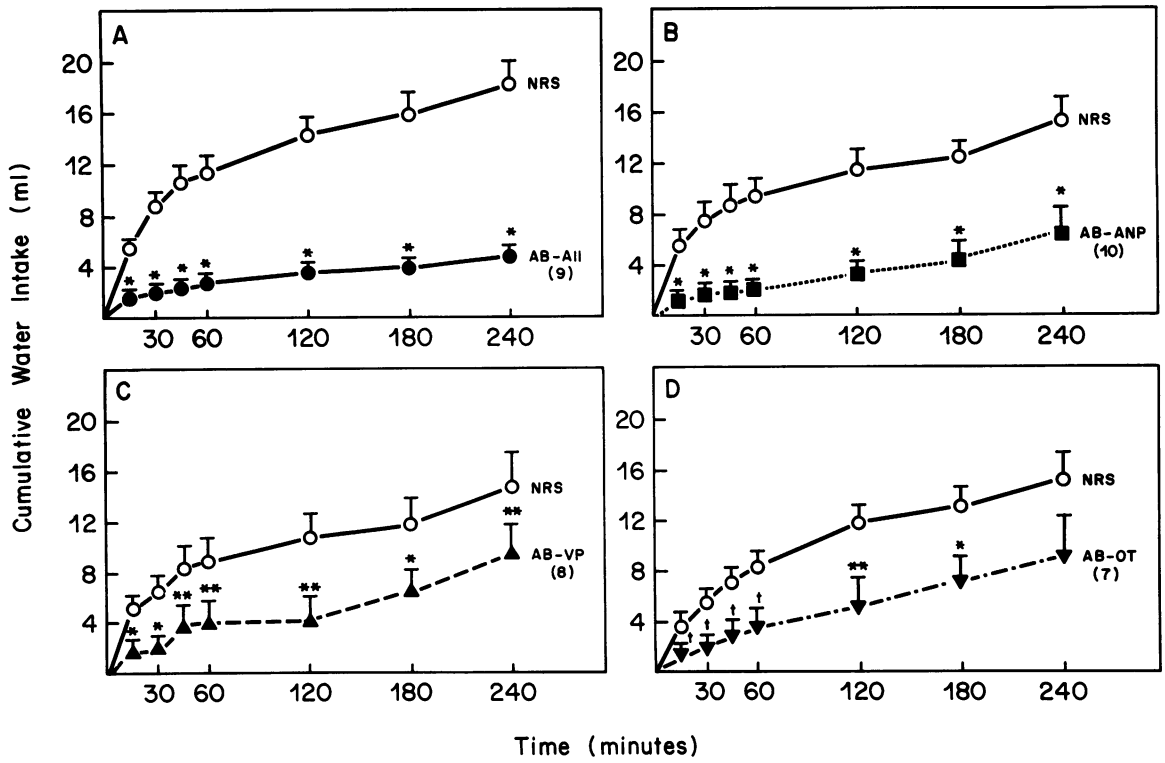

FIG. 3. Cumulative water intake in water-deprived rats when water was offered 3 hr after icv injection of NRS (control) and Ab-AII (*A*), Ab-ANP (*B*), Ab-VP (*C*), or Ab-OT (*D*). Values are means ± SEM. Numbers of animals are in parentheses. *, $P < 0.005$; **, $P < 0.025$; and †, $P < 0.05$ vs. control values.

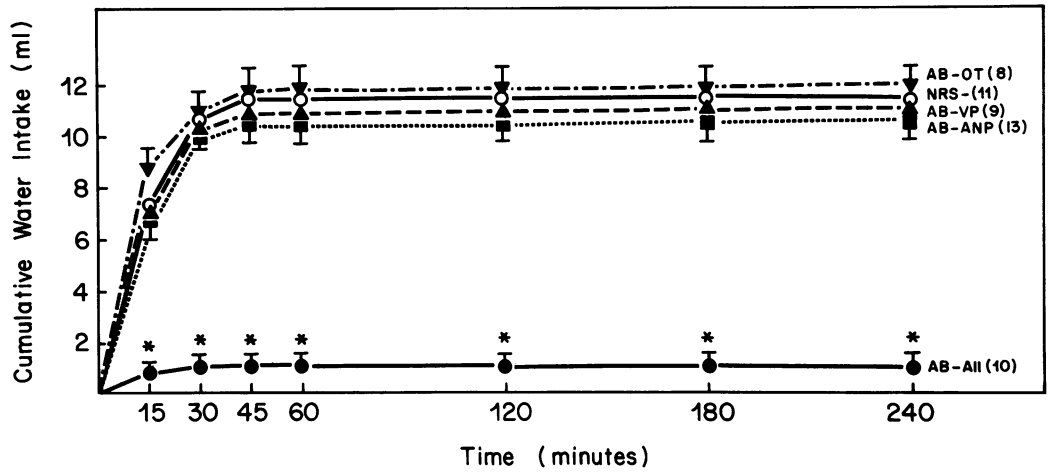

FIG. 4. Cumulative water intake in normally hydrated rats when drinking was elicited by AII 3 hr after icv injection of NRS, Ab-AII, Ab-ANP, Ab-VP, and Ab-OT. Numbers of animals are in parentheses. Values are means ± SEM. *, $P < 0.005$ vs. other four groups.

at 6 p.m. (Fig. 5). There was no difference in the water intake between the control session (NRS) and experimental session (Ab-AII). As expected, there was considerable water intake during the period from 6 to 10 p.m., associated particularly with feeding, which occurred when the lights were turned off at 7 p.m.

DISCUSSION

The earlier onset of the blockade of dehydration-induced drinking following icv injection of Ab-AII than the suppression from the other antisera indicates that the sites of action of the latter may be more distant from the 3V or that their mechanisms of action may be different (perhaps background activity of these peptidergic neurons must be present for drinking to take place); the more effective inhibition by Ab-AII indicates a primary action of AII on drinking behavior.

The normal prandial drinking of rats that occurs with the onset of darkness and accompanies food intake was not changed by microinjection of Ab-AII. This indicates that AII may not be essential to normal water intake. Rather the action of AII to induce drinking behavior may be vital only for the drinking that occurs following dehydration.

In previous research, antisera against a variety of brain peptides have been found to have effects opposite to those of the peptide itself following their injection into the 3V, which suggests that gamma globulins may penetrate into adjacent central nervous system tissue after injection and immunoneutralize endogenous peptides. The time required for antisera to act varies markedly depending on the antiserum (7, 8). The time required for antisera to act is presumably related to the period required for uptake from the ventricle and diffusion to the site of neutralization of endogenous peptide. This time was <1 hr in the case of AII antiserum, which suggests that the site of action was near the ventricle. Our results do not disclose whether dehydration-induced drinking requires the presence of AII in the brain, which has reached that site by way of the circumventricular organs or release of neuronally derived AII.

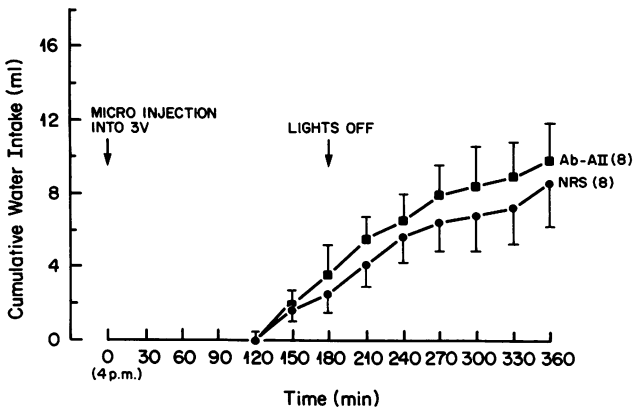

FIG. 5. Cumulative water intake in animals with ad libitum food and water that were submitted to the 3V microinjections of NRS or Ab-AII at 4 p.m.

There have been several attempts to block dehydration-induced drinking with the AII antagonist, saralasin (9–12). These attempts have been ineffective except for one instance in which infusions of saralasin into the lateral ventricle of rats prior to and after the presentation of water caused approximately a 50% decrease in drinking during the ensuing 30 min after presentation of water (13). The relative failure of saralasin to block dehydration-induced drinking in the prior studies was probably related to incomplete saturation of the AII receptors by the infusions or injections of saralasin into the lateral ventricle. In contrast, using antisera injected into the 3V, we were able to obtain a complete blockade of dehydration-induced drinking, which was presumably due to complete immunoneutralization of hypothalamic AII.

There are interactions between osmotic and hypovolemic stimuli in the drinking and VP secretion caused by water deprivation. In this situation, drinking is stimulated not only by hyperosmolality of body fluids but also by decreased blood volume. The osmotic threshold for drinking is lower in water deprivation than after hypertonic saline infusion when the volume component is absent (14). Hypovolemia and low doses of VP decreased the osmotic threshold for drinking, whereas high doses of VP, which produce a pressor effect, were not as effective (14). If it is true that VP release occurs in response to stimuli of small magnitude and precedes the triggering of thirst mechanisms, the facilitatory interaction between VP-releasing and drinking-controlling mechanisms would have great meaning (15, 16). Our results show that Ab-VP decreased drinking in water-deprived rats, probably by inhibition of this facilitatory interaction between VP release and other mechanisms that control drinking.

However, there are situations in which VP release and drinking occur independently. For example, hypervolemia can be sufficient to inhibit VP secretion and to yield a marked diuresis without altering drinking (17). Thus, there are situations in which VP release may facilitate drinking behavior, whereas in others this does not occur. Icv injection of AII in

normally hydrated rats could be an example of this last situation since VP release would be low because of plasma osmolality below the threshold for its release (14) and the permissive action of VP on drinking would not occur. Consequently, Ab-VP had no effect. There are no studies of the possible participation of OT in drinking behavior. Our results indicate a possible permissive action.

It was observed in rats that water deprivation increased the number and decreased the affinity of ANP binding sites in the subfornical organ, indicative of a central role for ANP in the regulation of water balance (18). Intraventricular injection of ANP decreased water intake in water-deprived rats, whereas higher doses were effective by means of the i.v. route (3, 19). The peptide also decreased drinking induced in normally hydrated rats by AII (20). Although Ab-ANP had no effect upon water intake in normally hydrated rats and failed to alter drinking induced by i.v. AII, it had a delayed suppressive effect on dehydration-induced drinking. This result is puzzling since the peptide itself had a similar effect. One explanation could be that background activity in the ANP neuronal system is necessary for induction of dehydration-induced drinking. Thus, ANP seems to have no action on normal drinking behavior; it acts only when drinking mechanisms are stimulated.

The fact that Ab-AII decreased drinking elicited by water deprivation or AII, whereas other antisera (Ab-ANP, Ab-VP, or Ab-OT) decreased drinking caused only by water deprivation, may indicate that the actions of endogenous ANP, VP, and OT on drinking are effective when there is an interaction of osmotic and volemic stimuli but are not effective in a situation in which brain AII is artificially increased through icv injection. Perhaps in this last situation the dipsogenic effect of AII is present, but permissive actions of other peptides (ANP, VP, and OT) are not displayed because other conditions determined by water deprivation are absent. The failure of antisera directed against VP, OT, and ANP to alter AII-induced drinking argues for the specificity of the effects of these antisera.

The results reported in this work suggest that endogenous AII is primarily responsible for dehydration-induced drinking behavior whereas ANP, VP, and OT may have a permissive action on mechanisms controlling drinking when osmotic and volemic stimuli are both present, as in water deprivation. By contrast endogenous AII appears not to be involved in prandial drinking that occurs when rats become active and are eating at the onset of the dark period.

We thank Judy Scott for typing the manuscript. This work was supported by National Institutes of Health Grants AM10076 and HD09988.

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