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# Drinking in the Monkey Evoked by Nicotine or Angiotensin II Microinjected in Hypothalamic and Mesencephalic Sites[1]

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MYERS, R. D., G. H. HALL AND T. A. RUDY. *Drinking in the monkey evoked by nicotine or angiotensin II microinjected in hypothalamic and mesencephalic sites.* PHARMAC. BIOCHEM. BEHAV. 1(1) 15–22, 1973.–In 7 unanesthetized rhesus monkeys (*Macaca mulatta*), spontaneous drinking was measured following a 1 μl injection of nicotine at 153 sites in the hypothalamus and mesencephalon. Nicotine in doses of 5 and 10 μg evoked the intake of water in volumes ranging from 35–365 ml within 30 min following the microinjection. A lower dose produced less drinking or had no effect. An anatomical mapping of this response revealed that nicotine exerted its dipsogenic effect at only 18 of the 153 sites at which the alkaloid was injected. These sensitive loci were clustered in and around the mammillary complex. When 1 μl of angiotensin II solution was microinjected in a dose of 1 μg at homologous sites in the hypothalamus and mesencephalon, volumes of water from 30–320 ml were consumed within 30 min after the injection. The anatomical distribution of the loci sensitive to angiotensin correspond identically to the nicotine drinking sites, i.e., the region surrounding the mammillary bodies and extending caudally into the mesencephalon. It was hypothesized that a cholinergic pathway mediated by nicotine receptors may subserve a drinking circuit in the brain stem of the primate. Further, because of the anatomical concordance of the dipsogenic substances, it was proposed that topically applied angiotensin may act to release acetylcholine within this region.

Nicotine drinking  Water intake  Hypothalamus and water balance  Angiotensin drinking
Fluid intake  Cholinergic drinking circuit  Mammillary complex and water intake

WHEN infused into the cerebral ventricles of the monkey, nicotine causes a transient and dose dependent decline in body temperature [10]. However, hyperthermia occurs if this alkaloid is microinjected within the caudal hypothalamus, but hypothermia follows its injection in rostral hypothalamic sites [11]. During the course of an investigation designed to identify anatomically these nicotine sensitive sites, we discovered that when nicotine is microinjected locally in certain regions of the brain stem, the satiated rhesus monkey will drink large volumes of water [11].

In 1962, Stein and Seifter [32] showed that crystals of muscarine applied to the hypothalamus of the rat induced a drinking response as strong as that of carbachol. On the other hand, nicotine applied similarly had only 20% potency of these two substances or about the same effect as NaCl. They concluded that muscarinic receptors mediate the polydipsic action, described by Grossman [8], of cholinergic substances on the lateral hypothalamus.

In species other than the rat it has not been possible to elicit spontaneous drinking reliably by cholinomimetic or other chemical agents injected into diencephalic or limbic structures [13, 20, 31]. In fact, cholinergic agents given intrahypothalamically may cause a blockade of the ingestive behavior of the primate, which is reversible by atropine applied at the same site [15]. In the present experiments, we have examined further the marked drinking evoked when nicotine is injected into different areas of the diencephalon and mesencephalon of the rhesus monkey. In addition, angiotensin was also microinjected to determine whether its well-known central action on drinking behavior [3, 4, 5, 27] could be localized at loci morphologically homologous to those at which nicotine exerts its dipsogenic action.

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## METHOD

Each of 7 male rhesus monkeys *(Macaca mulatta)*, weighing from 4.5–8.2 kg, was acclimated to a primate restraining chair for 3–7 days prior to surgery. They were maintained on a diet of Purina monkey biscuits, supplemented at regular intervals with oranges and bananas. In addition, 3 of the animals were trained to pull a lever to obtain 1 g banana flavored food pellets until an FR-25 schedule of reinforcement was attained. Water was always available in 1000 ml inverted cylinders fitted with a leak proof nozzle which the animal could lick. The experimental room was illuminated continuously and the ambient temperature ranged between 22–24°C.

### *Surgical Procedures*

After the monkey was anesthetized with 30 mg/kg pentobarbital sodium given into a superficial branch of the saphenous vein, the head was placed in a stereotaxic instrument and the surgery was carried out following rigid aseptic precautions as described previously [14, 21]. After a midline incision was made and the aponeurosis and fascia retracted, a disc of bone 13 mm in dia. was removed by trephine. Then an array of 4–8 guide cannulae, each fitted with a corresponding indwelling stylet was lowered to a predetermined depth, so that the tips of the guide tubes rested above the intended site of injection within the diencephalon or midbrain. Each guide consisted of a 50 mm length of stainless steel Hypoflex tubing with an internal dia. sufficiently large to accept a 28 ga injector cannula. Gelfoam strips were placed in the craniotomy hole so as to cover the surface of the dura mater completely. The guide tubes were then affixed to the surface of the calvarium by cranioplast cement that was packed around them and the stainless steel anchor screws. A thermistor bead for monitoring brain temperature was placed against the falx cerebri 7–9 mm below the dura and cemented in place.

A polyethylene pedestal [16] was positioned over the entire array, screwed to the skull and filled with additional cranioplast cement. The pedestal cap was then screwed on to protect the tubes and to enable a sterile preparation to be maintained until the conclusion of the experiments.

### *Microinjection procedure*

For at least 1–4 hr before an injection was made, the intake of water as well as bar-pressing for food pellets or ingestion of freely available biscuits were recorded continuously. Body temperature was measured by the intracerebral thermistor connected to a YSI telethermometer and plotted directly on a potentiometric recorder. Simultaneous colonic temperatures were frequently obtained and peripheral vasomotor responses were estimated either by palpation of the pinna or by recording the surface temperature of the ear with a YSI disc thermistor [22]. Respiratory rate, shivering, piloerection, vocalization, the general demeanor and reactivity of the monkey were monitored closely during each experiment.

To inject nicotine, angiotensin or the control CSF solution, standard procedures were followed that have been described earlier [16]. A Hamilton microliter syringe mounted on an infusion pump was connected to a 28 ga injector cannula by way of a length of polyethylene (PE 10) tubing. After the indwelling stylet was removed, the injector cannula was lowered to one of at least three individual depths ranging from 2–7 mm below the tip of the guide tube. A droplet having a volume of 1 $\mu$l was delivered over an interval of 30 sec after which the injector cannula remained in place for another 30 sec before being replaced by the stylet.

The substances to be injected were dissolved in a pyrogen-free artificial CSF solution [18] at a pH of 7.2. Each solution was passed through a sterilized 0.22 $\mu$ Swinnex millipore filter before the injection tubing was filled. The compounds used were nicotine hydrogen tartrate (British Drug Houses) and Angiotensin II (Hypertensin CIBA); the doses were expressed in terms of micrograms of the base. The syringes and glassware were rendered pyrogen-free and the PE tubing and injector cannulae were stored in 70% ethanol. An interval of 2–4 hr elapsed between microinjections in an individual monkey and at least 48 hr between an injection at a specific site. In the nicotine series of experiments, the alkaloid was first given at a constant dose of 5.0 $\mu$g based on earlier data [11]; thereafter, doses of 2.0 or 10.0 $\mu$g were examined subsequently to ascertain a dose response relationship. In the angiotensin series, 0.5–1.0 $\mu$g doses were used since these amounts are the range that can evoke substantial spontaneous drinking in the primate [26].

### *Anatomical Analysis*

At the conclusion of a series of experiments, an overdose of pentobarbital sodium was given intraperitoneally and 1 $\mu$l of 25% solution of India ink in saline was infused at the deepest microinjection site in order to label the locus [16]. The brain of each animal was then fixed with 10% buffered formol saline which was perfused via the thoracic aorta. After the brain was blocked *in situ*, it was removed, rinsed thoroughly in deionized water and trimmed. Sections were cut at 28–30 $\mu$ on a freezing microtome and stained for cell bodies and fibers following methods described by Wolf [34].

On the basis of a detailed morphological analysis carried out under light microscopy of each histological section, the placement of each cannula was verified and the site of each microinjection was determined along the horizontal aspect ventral to the cannula tip. Anatomical maps, based on the composite analysis of all 7 monkeys were constructed in order to relate the physiological or behavioral response produced by either nicotine or angiotensin with a specific structure that had been stimulated.

## RESULTS

### *Nicotine Induced Drinking*

At 135 of 153 microinjection sites located in the hypothalamus, thalamus and mesencephalon, nicotine failed to alter water intake of the rhesus monkey. However, at the remaining loci, nicotine in doses of 5 – 10 $\mu$g evoked an intense drinking response which lasted usually from 3 – 30 min but no longer than 45 min. The total volume consumed within 30 min after the drinking began ranged from 35–365 ml.

The anatomical localization of 18 sites responsive to nicotine (●) is presented in Fig. 1. Control injections of artificial CSF were without effect at these loci. From the mapping at 9 coronal planes, it is clear that the active drinking sites were widely distributed within the caudal portion of the hypothalamus and within the medial aspect of the mesencephalon. With one exeption, in the ventro-

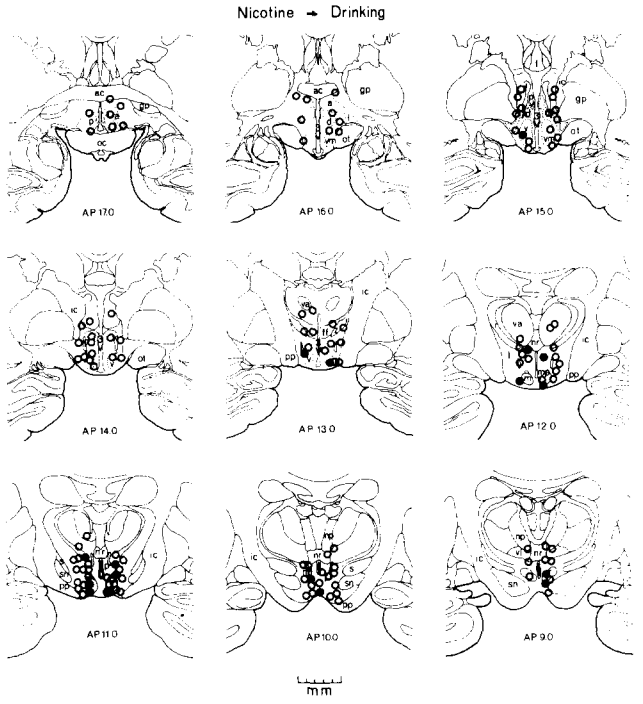


FIG. 1. Morphological mapping within 9 coronal planes of the sites in the diencephalon and mesencephalon of the rhesus monkey at which 2 to 10 μg nicotine microinjected in a 1 μl volume elicited intense drinking of water (•) or no change in water intake (○). Anatomical structures are as follows: a, anterior hypothalamus; ac, anterior commissure; d, dorsomedial nucleus; f, fornix, ff, fields of Forel; gp, globus pallidus; ic, internal capsule; l, lateral hypothalamus; m, mammillary body; mp, mammillary fasciculus princeps; mt, mammillo-thalamic tract; np, nucleus paracentralis; nr, nucleus reuniens; oc, optic chiasm; ot, optic tract; p, posterior hypothalamus; pp, cerebral peduncles; r, reticular nucleus; s, nucleus subthalamicus; sn, substantia nigra; v, ventromedial hypothalamic nucleus; vl, nucleus ventrolateralis medialis; va, nucleus anteroventralis; 3 v, third ventricle.

medial nucleus at AP 15.0, the nicotine sensitive sites were localized in the region extending from coronal plane AP 9.0–13.0. Moreover, every site was within 0.5 mm of at least one other site at which nicotine had no effect on drinking behavior (○). In this connection, some of the nicotine sensitive loci were located in close proximity to the third ventricle and the aqueduct of Sylvius, whereas other sites were much further distant from the ventricular lumen than those sites which were not responsive to nicotine. The greatest sensitivity to nicotine was found to be in the region of coronal planes AP 11.0 and AP 12.0 since at 23% and 24% of these sites respectively, nicotine elicited intense drinking. The 15 additional sites located along the midline in coronal plane AP 8.0 at which nicotine did not alter intake of water were not included on the map.

In the monkeys, in which spontaneous drinking occurred sporadically throughout the day, the amount of this intermittent water intake was averaged in ml over at least four 30 min intervals before either nicotine or angiotensin was infused. For each experiment, a drinking ratio was then calculated by dividing the ml consumed within 30 min following the microinjection by the average baseline water intake (ml). Table 1 presents those drinking responses elicited by nicotine microinjected at specific sites, which were at least twice that of the level of spontaneous drinking, i.e., a drinking ratio $\geq 2.00$. The criteria upon which this ratio value was selected were: (a) in each instance the drinking appeared to be strongly motivated since the monkey followed the drinking spout and vocalized whenever it was moved; (b) the monkey tried to grasp the nozzle when it was placed out of reach; (c) when the ratio was $\geq 2.00$ the response could be repeated; and (d) doses lower than those given in the table did not produce a reliable fluid intake which could be clearly distinguished from the baseline level of water taken.

From the drinking ratios in Table 1, it can be seen that nicotine evokes a response four times the baseline level, or more, when the alkaloid was microinjected within the mammillary region at a locus adjacent to the fornix. In the four experiments in which over 200 ml were ingested within 30 min after the microinjection of 5.0–10.0 μg of nicotine, the sites were located either in an area adjacent to the mammillary body or its contiguous fiber tract, the mammillary fasciculus princeps.

A marked drinking response following the local application of nicotine is exemplified in Fig. 2. When 5.0 μg of the alkaloid were microinjected at the site shown in the inset, 200 ml were consumed within 15 min and another 165 ml during the next 15 min interval. Thereafter, the water

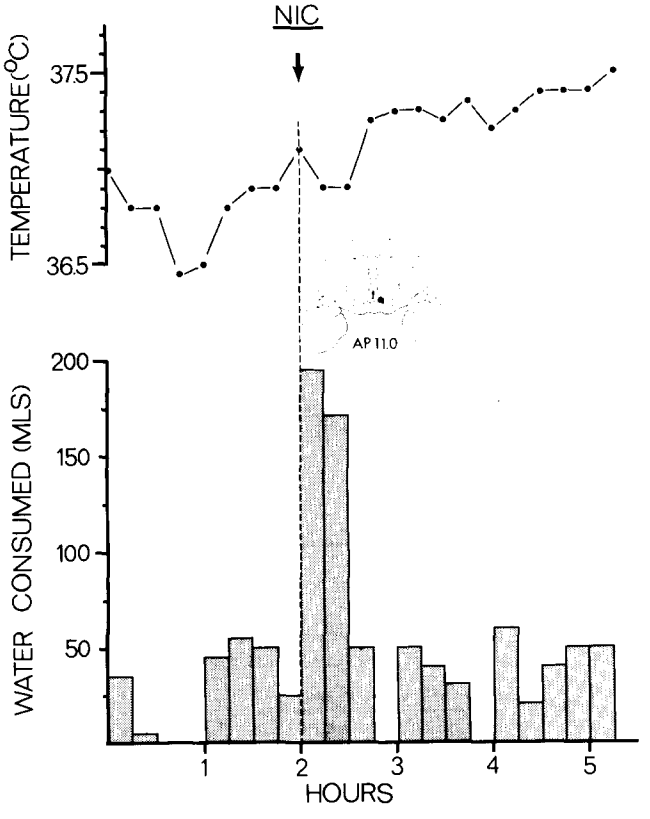


FIG. 2. Drinking responses and temperature record of a rhesus monkey after 5 μg nicotine was injected at coronal plane AP 11.0 in a volume of 1 μl at a site within the fasciculus princeps dorsal to the mammillary body (inset).

TABLE 1

DRINKING IN THE RHESUS MONKEY INDUCED BY INTRACEREBRAL MICROINJECTIONS OF NICOTINE

| Monkey | Microinjection Sites* | Dose (μg) | Water Intake (ml)† | Spontaneous Drinking (ml)‡ | Drinking Ratio§ |
|---|---|---|---|---|---|
| A | Dorsomedial hypothalamus | 10.0 | 180 | 47.3 | 3.80 |
| | Posterior hypothalamus | 5.0 | 90 | 42.2 | 2.13 |
| | Fornix | 5.0 | 145 | 34.5 | 4.20 |
| | Lateral hypothalamus | 5.0 | 130 | 42.2 | 3.08 |
| | Mammillary fasciculus princeps | 5.0 | 365 | 82.3 | 4.43 |
| " | " | 5.0 | 250 | 81.3 | 3.08 |
| | Mammillary Body | 5.0 | 150 | 61.3 | 2.45 |
| | Ventral interpeduncular space | 5.0 | 170 | 80.0 | 2.13 |
| B | Mammillary body | 10.0 | 100 | 29.2 | 3.42 |
| " | " | 10.0 | 80 | 18.3 | 4.37 |
| " | " | 10.0 | 50 | 16.5 | 3.03 |
| | Mammillo-thalamic tract | 10.0 | 50 | 21.3 | 2.34 |
| C | Mammillary body | 5.0 | 90 | 39.6 | 2.27 |
| | Nucleus subthalamicus | 5.0 | 55 | 17.7 | 3.10 |
| " | " | 5.0 | 35 | 11.2 | 3.13 |
| D | Ventromedial hypothalamus | 2.0 | 90 | 0 | — |
| | Mammillary fasciculus princeps | 5.0 | 220 | 0 | — |
| E | Mammillary body | 5.0 | 80 | 0 | — |
| " | " | 10.0 | 250 | 0 | — |
| F | Lateral hypothalamus (posterior) | 2.0 | 20 | 0 | — |
| | | 5.0 | 90 | 0 | — |
| | | 10.0 | 120 | 0 | — |

*Sites were included only when drinking ratio ≥ 2.00. Microinjections of CSF or lower doses of nicotine yielded drinking ratios < 2.00.

†ml of water consumed within 30 min after a microinjection.

‡Mean ml of water consumed spontaneously per 30 min interval prior to microinjection.

§ $\frac{\text{drug-induced drinking}}{\text{mean spontaneous drinking}}$

intake returned to the baseline level which averaged approximately 40 ml every 15 min. Although there was a slight fall in the monkey's body temperature of about 0.2°C during the drinking episode, which was due probably to the water load, the temperature remained relatively unchanged following the microinjection.

*Angiotensin II - Induced Drinking*

In 3 of the monkeys, angiotensin II was microinjected at 20 sites homologous to many of those at which nicotine elicited drinking. As shown in Fig. 3, an intense drinking response occurred when 1 μg of angiotensin was injected at

sites distributed from coronal planes AP 8.0 – AP 12.0. Although microinjections were not given at loci more rostral than AP 15.0, an increase in water intake did not occur when the polypeptide was applied at 4 sites rostral to AP 12.0 in coronal planes AP 14.0 and AP 15.0. Figure 3 shows that the greatest sensitivity to angiotensin, however, was within the caudal hypothalamus and mesencephalon of the monkey, since at all 5 sites in coronal planes AP 8.0 and AP 10.0, drinking followed the injection of 1 μg of angiotensin. In 6 out of 8 loci in coronal plane AP 11.0, a polydipsic response was likewise elicited.

Two anatomical observations are particularly noteworthy: (1) at sites located within 1.0 mm of the monkey's third ventricle, i.e., in coronal plane AP 12.0 (Fig. 3), the injection of angiotensin did not evoke drinking; and (2) at other loci also illustrated in Fig. 3, which were over 4.0 mm distant from the ventricle, the peptide did stimulate the water intake of the primate.

The range of individual volumes of water consumed by the monkey corresponds to the magnitude of drinking elicited by nicotine. Table 2 presents the drinking responses following the administration of angiotensin within specific anatomical structures, which were at least twice that of the spontaneous drinking level, i.e., a drinking ratio $\geq 2.00$. The criteria used to establish this ratio were identical to those for nicotine drinking. Table 2 shows that in the three experiments in which the drinking of water exceeded 200 ml within 30 min after angiotensin was microinjected, the sites of stimulation were associated with the mammillary bodies or contiguous structures. It is interesting that the drinking ratios of 5.88 and 4.25 following the injection of 1.0 μg of angiotensin in the third ventricle were less than those observed when the same dose was microinjected into the region between the mammillary bodies and cerebral peduncle, i.e., interpeduncular mammillary region, or directly into the mammillary body (Monkeys B and C).

A representative example of the pattern of angiotensin evoked drinking is presented in Fig. 4. At a point when the monkey's baseline water intake averaged 38.5 ml per 15 min interval, 1 μg angiotensin injected at a site impinging on the mammillary body (inset) caused sustained drinking over the next 30 min. Two and one-quarter hr later a subthreshold dose of 2 μg nicotine microinjected within the same region but contralaterally did not alter the water intake of the monkey. Again, the temperature of the monkey declined slightly during the period of drinking but increased during the course of the experiment in correspondence with the diurnal fluctuation of temperature in this species [17].

In another experiment, a subthreshold dose of nicotine again failed to alter the monkey's low baseline intake of water. However, as shown in Fig. 5, 1 μl angiotensin microinjected in the mammillary body at AP 11.0 and, 3 hr later, within the medial mesencephalon at AP 10.0, elicited two patterns of drinking response. In the first case, the latency was 15 min and the duration of the drinking was 15 min. The second injection of angiotensin at the more posterior locus caused an intake of 70 ml but the latency of the response was only 2 min.

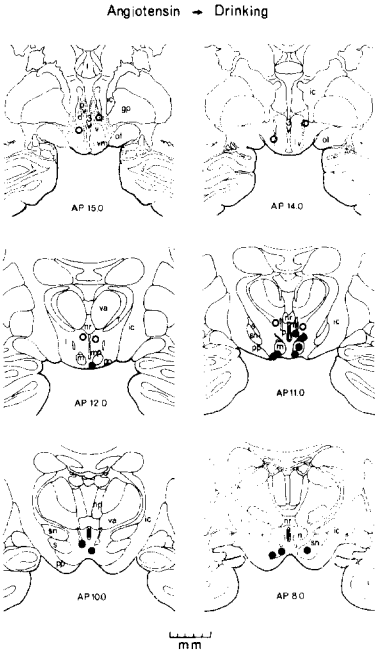


FIG. 3. Morphological mapping within 6 coronal planes of the sites in the diencephalon and mesencephalon of the rhesus monkey at which 1 μg angiotensin in a 1 μl volume elicited intense drinking of water (•) or no change in water intake (○). Anatomical structures are abbreviated as in Fig. 1.

## DISCUSSION

On the basis of pharmacological evidence, Stein and Seifter [32] proposed that the central cholinergic drinking system in the rat [8] was mediated by muscarinic receptors in the hypothalamus. However, in the rhesus monkey, intrahypothalamic carbamyl choline causes a blockade of drinking as well as feeding behavior, the pharmacological specificity of which was demonstrated by its reversal with atropine, a muscarinic antagonist, applied at the same diencephalic site [15, 30]. From the present experiments, in which nicotine induced a marked consumption of water without any bar-pressing for food or other responses, one could envision that cholinergic synapses could subserve the anatomical pathways underlying the intake of water. Taken together with the previous findings from our Laboratory, it would appear that nicotinic receptors may mediate the efferent pathway for drinking behavior, which originates in the diencephalon, whereas muscarinic receptors could underlie an inhibitory component of fluid intake. In preliminary experiments in which mecamylamine was microinjected at nicotine- and/or angiotensin - sensitive sites, water intake was attenuated or absent in several cases following a subsequent injection of the alkaloid or peptide; however, additional experiments are required before a clear delineation of receptor specificity can be obtained.

The differences between the primate and rat in terms of the anatomical localization of the nicotine induced ingestion of water seem to parallel the pharmacological discrepancy as well. In the monkey, the strongest drinking response was elicited when nicotine was given at sites encompassing the mammillary complex, whereas in the rat the local muscarinic effects are apparently more rostral [32]. Surprisingly, the morphological distribution of angiotensin sensitive loci in the monkey again centered within the caudal hypothalamic and mesencephalic planes. In his classical study, Booth reported that the injection sites at

TABLE 2

DRINKING IN THE RHESUS MONKEY INDUCED BY INTRACEREBRAL MICROINJECTIONS OF ANGIOTENSIN-II

| Monkey | Microinjection Sites* | Dose (μg) | Water Intake (ml)† | Spontaneous Drinking (ml)‡ | Drinking Ratio§ |
|---|---|---|---|---|---|
| A | Mammillo-thalamic tract | 1.0 | 220 | 93.6 | 2.35 |
| | Interpeduncular mammillary region | 1.0 | 170 | 77.0 | 2.21 |
| | Mammillary body | 1.0 | 200 | 77.0 | 2.60 |
| B | Interpeduncular mammillary region | 1.0 | 140 | 18.6 | 7.50 |
| " | " | 1.0 | 320 | 39.6 | 8.10 |
| " | " | 1.0 | 155 | 22.5 | 6.89 |
| | Substantia nigra | 1.0 | 95 | 27.3 | 3.48 |
| | Mammillary body | 1.0 | 90 | 15.0 | 6.00 |
| " | " | 1.0 | 70 | 15.0 | 4.67 |
| | Mammillo-thalamic tract | 1.0 | 30 | 15.0 | 2.00 |
| | Substantia nigra | 1.0 | 50 | 24.1 | 2.07 |
| " | " | 1.0 | 100 | 17.9 | 5.59 |
| C | Third ventricle | 1.0 | 120 | 20.4 | 5.88 |
| " | " | 1.0 | 85 | 20.0 | 4.25 |
| | Mammillary body | 1.0 | 120 | 15.0 | 8.00 |
| | Mesencephalon (ventromedial) | 1.0 | 70 | 15.0 | 4.67 |

*Sites were included only when drinking ratio ≥ 2.00. Microinjections of CSF or a lower dose of angiotensin yielded drinking ratios < 2.00.

†ml of water consumed within 30 min after a microinjection.

‡Mean ml of water consumed spontaneously per 30 min interval prior to microinjection.

§ $\frac{\text{drug-induced drinking}}{\text{mean spontaneous drinking}}$

which angiotensin produced an average water intake of 9.75 ml in the rat were located rostral to the medial forebrain bundle just posterior to the preoptic area [3]. Similarly, Epstein *et al.* [4] have described the regions most sensitive to the local application of the polypeptide as being within the rostral hypothalamus and preoptic area. It should be recognized, however, that with a carrier volume as large as 1.0 or 2.0 μl, the question of localizing the direct neuronal action of an injected chemical in the brain stem of a rat may be extremely difficult in view of the fact that pharmacologically active substances injected in a 1 μl volume in the lateral hypothalamus of the rat can diffuse readily, with substantial concentrations found even in the mesencephalon [23]. In any event, the present data correspond with the brief report of Setler [26], who found that intracranial angiotensin elicited an excessive intake of water in 1 of 3 monkeys (based on our drinking ratio criterion of ≥ 2.00), although anatomical verification of the injection site was not given.

The latency of the nicotine or angiotensin induced drinking was often difficult to quantitate on a consistent basis, because of the sporadic and intermittent drinking which occurs frequently in many restrained monkeys. When a sustained and unequivocal drinking response did occur, the latency was usually 1.5 – 5.0 min which is much shorter than that reported by Sharpe and Myers [30] for the water consumption elicited by serotonin injected at several diencephalic points as well as in the fields of Forel. In response to nicotine or angiotensin there was no overt display of emotional behavior unless the drinking spout was moved or taken beyond the monkey's reach. Further, the intake of water could not be viewed in any way as prandial, since food was not consumed during the 30 min interval immediately following the microinjection. Moreover, angiotensin is known to possess an anorexic effect even in a fasted animal [12]. There were also no consistent changes

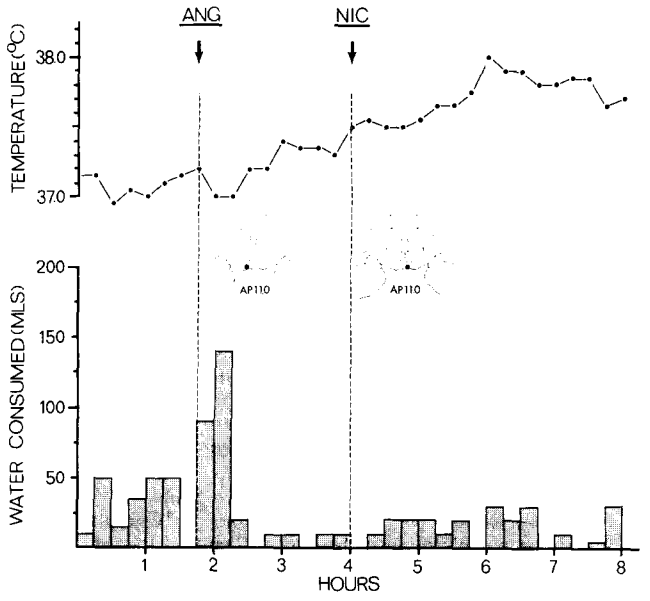


FIG. 4. Drinking responses and temperature record of a rhesus monkey after 1 μg angiotensin was injected (first arrow) at coronal plane AP 11.0 in a volume of 1 μl at a site adjacent to the mammillary body (inset). At second arrow, 2 μg nicotine was similarly injected at a contralateral site (inset) within the same coronal plane.

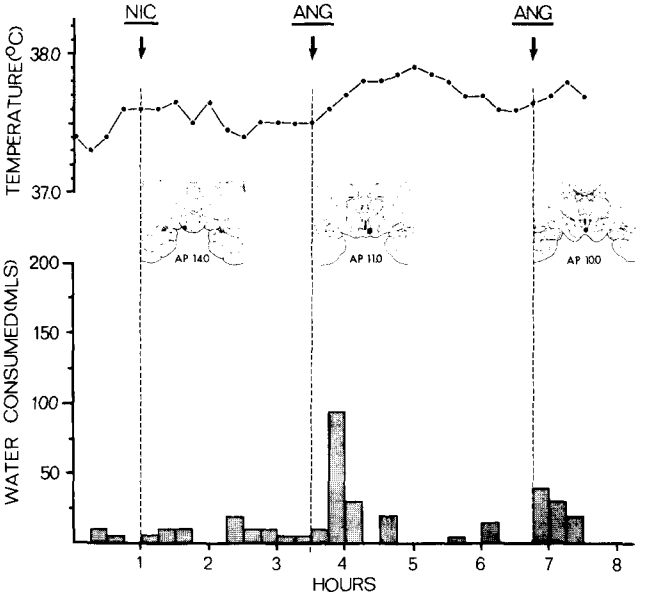


FIG. 5. Drinking responses and temperature record of a rhesus monkey following the successive microinjections in a 1 μl volume of: 2 μg nicotine (first arrow) at a site lateral to the mammillary body (inset); 1 μg angiotensin (second arrow) at a site within the contralateral mammillary body (inset); and 1 μg angiotensin (Third arrow) at a midline locus just caudal to the mammillary complex.

in the monkeys' temperature produced by either substance. Nevertheless, within the posterior hypothalamus nicotine can cause a sharp rise in temperature, the physiological characteristics of which are similar to the hyperthermic response produced by acetylcholine applied at the same site [11].

Although the cellular mechanisms within the hypothalamic mesencephalic axis underlying angiotensin induced drinking are of course not understood, there are several explanations for this phenomenon. Angiotensin is known to act at the cellular level to affect vascular transport and cellular metabolism [25]. In this connection, Andersson and Westbye have proposed that the polypeptide acting via the intracerebral route causes drinking by means of facilitating the transport of sodium into cells involved in thirst or ADH release [1]. This concept was further substantiated by the finding that angiotensin infused intraventricularly in isotonic glucose or urea solutions has a much weaker dipsogenic effect than when given in NaCl solution [2]. In accord with this view is one put forth by Severs *et al.* that angiotensin is intimately related to salt balance and may cause the cells in the brain to perceive a state of hyperosmolality [29].

Along with their findings that angiotensin-renin drinking may result from an aggravated hypovolemia rather than from a signal to hypothalamic receptors, Haefeli and Peters [9] have suggested that topically applied angiotensin simply causes a local vasoconstriction with a concommitant loss of fluid from hypothalamic vessels. A subsequent decline in hypothalamic blood volume could then trigger a drinking response due to a hypovolemic stimulus [33].

An alternative view is based on the fact that angiotensin can act to release acetylcholine from cholinergic synapses in the periphery [24]. Recently, Severs *et al.* [27] demonstrated that a high dose of atropine administered in the rat by the intraventricular route abolished central angiotensin drinking. On the other hand, Giardina and Fisher [7], and Fitzsimons and Setler [6] have found that muscarinic blockade with centrally injected atropine in lower doses fails to reduce angiotensin induced drinking. These results suggest that angiotensin exerts its dipsogenic action at nonmuscarinic sites. Of course, it is possible that angiotensin may act at local adrenergic sites [6, 28] which atropine would not affect, but may require more distant cholinergic circuits which are blocked by intraventricular atropine. Although our data do not resolve the inconsistency in these results, they do favor the view that in the primate, a cholinergic link may be involved in angiotensin-drinking. This interpretation is based on three findings: (1) the identical characteristics of nicotine and angiotensin-induced drinking in terms of the latency, magnitude, duration and motivated nature; (2) the striking anatomical concordance between nicotine and angiotensin-sensitive sites since the strongest drinking was elicited by either substance when injected at homologous loci associated with the mammillary complex; and (3) the similar percentage of active loci within the caudal coronal planes.

Taking these results together, one could envision that in the primate angiotensin is acting to release acetylcholine within a posterior efferent circuit involved in the volitional intake of fluid. Presumably, the choline ester is liberated presynaptically onto nicotinic receptor sites, and the transport of sodium ions [1,29] may well be involved in this process. Further research will be required, however, to: (a) elucidate the receptor properties of the nicotine- and angiotensin-sensitive regions; and (b) show that the resting release of acetylcholine within the diencephalon and mesencephalon, which is now possible to demonstrate [19], does in fact change in the water-deprived primate as well as in response to elevated angiotensin levels in the serum.

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