



BRIEF COMMUNICATION

Intracerebroventricular Angiotensin II Increases Intraoral Intake of Water in Rats

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HOUPPT, T. A., E. S. CORP AND R. A. BERLIN. *Intracerebroventricular angiotensin II increases intraoral intake of water in rats*. PEPTIDES **19**(1) 171–173, 1998.—Ad lib and intraoral intake tests can separate the effects of drugs on the appetitive and consummatory phases of ingestive behavior. Central angiotensin II increases ad lib intake from water bottles, but its effect on intraoral intake has not been examined. Rats with both lateral intracerebroventricular (ICV) cannulas and intraoral catheters were given angiotensin II (100 ng/5 μ l ICV) followed by a 10-min intraoral infusion of water. Angiotensin II increased intraoral intake and increased ad lib water intake from bottles after the intraoral test. Thus angiotensin II increases water intake during both appetitive and consummatory phases of drinking. © 1998 Elsevier Science Inc.

Thirst Drinking Consummatory Appetitive Ingestion Motivation

MANIPULATIONS that modulate ingestion of food or drink can act during either the appetitive phase or the consummatory phase of ingestion (2). The appetitive phase includes the search for food, meal initiation, preference choices (e.g. in 2-bottle tests), and somatic behaviors that compete with ingestion and terminate meals. The consummatory phase includes the reflexive licking, chewing, and swallowing during ingestion, and reveals the animal's direct orosensory evaluation of food or drink leading to acceptance or rejection.

The two phases of ingestion can be experimentally dissociated by examining ad lib intake from a bottle, which requires both appetitive and consummatory phases, and by examining intake during infusions via an intraoral catheter that evoke the consummatory phase independent of appetitive behaviors. These two paradigms have produced evidence suggesting that the neural circuitry underlying these phases can be independently activated. For example, unlike appetitive behaviors, unconditioned consummatory behaviors can be sustained solely by the neural circuitry of the hindbrain in chronic decerebrate rats (6). Most manipulations which affect ad lib intake produce parallel effects on

intraoral intake. Intraoral intake of a sucrose solution, for example, is increased by food deprivation (8). This parallelism is not evident in all manipulations, however: neuropeptide Y (NPY), for example, does not increase intraoral intake of sucrose, although it is one of the most potent known stimulants of ad lib intake (1,13).

While most studies have examined intraoral intake of nutrients of solutes, there have been few reports of dipsogenic stimuli and intraoral intake of water. Thus, it is largely unknown whether dipsogenic stimuli increase drinking by modulating the appetitive or consummatory phases of ingestion. Eckel and Ossenkopp have reported an increase in ingestive (palatable) responses to brief intraoral infusions of water after 24-h water deprivation (3). Here we report that ICV administration of angiotensin II (Ang II), a potent dipsogenic stimulus in ad lib tests (4), increased intraoral intake of water.

METHOD

Twelve adult male rats (350–450 g) were individually housed under a 12:12 light-dark cycle at 25°C with ad lib water and rodent chow. Under chloral hydrate (153 mg/kg)-pentobarbital (35 mg/kg) anesthesia, each rat was ster-

eotaxically implanted with a 22 gauge, stainless steel, guide cannula (Plastics One, Roanoke, VA) aimed towards the lateral cerebral ventricle (1.2 mm caudal to bregma, 1.5 mm lateral to the midline, and 4 mm below the skull surface). Guide cannulas were held in place with dental acrylic bonded to stainless steel screws anchored to the skull. An obturator was inserted into each guide cannula and remained in place except during injections when it was removed and replaced with an injector that extended 1.0 mm beyond the tip of the guide cannula.

During the same surgery, rats were implanted with anterior sublingual chronic intraoral catheters as previously described (10). Briefly, PE50 tubing heat-flared at one end was drawn through the floor of the mouth midway between the root of the lower incisors and the base of the tongue. The unflared end of the catheter was externalized between the scapulas on the dorsal surface of the rat's neck, and held in place with an outer sleeve of 0.04 silastic tubing attached to a Marlex mesh disk (Bard-Parker, Billerica, MA) sutured to the dorsal neck musculature.

After surgery rats were handled daily for one week to facilitate adaptation to the central injection and oral infusion procedure. For tests of water intake, either 100 ng human Ang II (Sigma Chemical Co, St Louis, MO) dissolved in 5 μ l volume of saline (0.15 M), or saline alone, was injected ICV 2 to 5 min before oral infusion of water. The volume of all ICV injections was 5 μ l, delivered over 30 s with a handheld 50 μ l syringe (Hamilton Co, Reno, NV). After ICV injections, rats were weighed and placed in a test chamber. Tap water (11 ml) was intraorally infused over 10 min by syringe pump (Harvard Apparatus). The latency for rats to begin dripping water from their mouths during the infusion was measured; rats that did not drip were assigned a latency of 600 s. Immediately after the 10 min infusion, the rats were weighed and returned to their home cages. Intraoral water intake was determined by weight gain during the infusion. Ad lib tap water intake over 30 min from home cage water bottles was measured beginning immediately after the end of the intraoral test. All rats received both Ang II and a saline control injection, administered in a cross-over counterbalanced design on two consecutive days. Data were analyzed with paired *t*-tests.

RESULTS AND DISCUSSION

Ang II ICV increased intraoral intake of water, and increased the latency to rejection of intraoral infusions of water (see Table 1). Ang II also increased ad lib drinking immediately after intraoral infusions of water in the same test session. The increase in water intake during intraoral infusions under the control of the experimenter demonstrates that the dipsogenic effect of Ang II does not require spontaneous self-initiation of water intake (i.e. the rat seeking and contacting the spout of a water bottle). Of course,

TABLE 1
EFFECT OF ANGIOTENSIN II ON INTRAORAL AND AD LIB INTAKE OF WATER

ICV Injection	Intraoral Intake	Latency To Drip	Ad lib Intake
Vehicle	4.1 \pm 0.9 g	55 \pm 9 s	2.3 \pm 0.2 g
Angiotensin II	9.1 \pm 0.3 g	350 \pm 63 s	6.3 \pm 1.1 g
Paired <i>t</i> -test	<i>t</i> [11] = 5.32, <i>p</i> < 0.001	<i>t</i> [11] = 4.53, <i>p</i> < 0.001	<i>t</i> [11] = 3.47, <i>p</i> < 0.01

Vehicle and angiotensin II values are expressed as mean \pm SE for 12 rats.

Ang II also increases self-initiation and maintenance of ad lib water intake. Thus Ang II can increase water intake by acting both on the consummatory phase and on the appetitive phase of ingestion.

A number of manipulations that increase or decrease ad lib intake have been applied to intraoral intake tests of rats. In most cases, the effects on intraoral intake parallel the effects on ad lib intake: thus, intraoral intake of sucrose solution is increased by food deprivation (8), decreased by the satiety peptides cholecystokinin (9) or bombesin (5), and decreased after acquisition of a conditioned taste aversion (10). There are several conditions under which intraoral intake has been dissociated from ad lib intake, however. For example, amphetamine decreased ad lib intake but not intraoral intake (14).

Our results with the dipsogenic stimulant, Ang II, are in contrast to results obtained with the orexigenic stimulant, NPY. Like Ang II's effect on ad lib water intake, NPY stimulates robust ad lib intake of a sucrose solution (11). Unlike Ang II, however, NPY does not increase intraoral intake (13). Thus, of these two peptides involved in motivational systems driving ingestion, only Ang II operates on both appetitive and consummatory phases of ingestion.

Because Ang II increased the latency to drip during the intraoral infusion, Ang II appears to increase the palatability of water. Increased palatability of water is also consistent with an earlier report that Ang II increases the rate of sham-drinking in the absence of postingestive accumulation of water in the gut (12). Quantification of orofacial taste reactivity would confirm that Ang II induces a shift in water's palatability (3,7).

Finally, intraoral intake and rejection can be mediated solely by the sensory and motor circuitry of the hindbrain (6). Thus the increased intraoral intake of water by forebrain administration of Ang II is consistent with forebrain dipsogenic centers modulating hindbrain orosensory reflexes during intraoral infusions.

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