Drinking to Intracerebral Angiotensin II and Carbachol: Dose-Response Relationships and Ionic Involvement

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SWANSON, L. W., L. G. SHARPE AND D. GRIFFIN. Drinking to intracerebral angiotensin II and carbachol: dose-response relationships and ionic involvement. PHYSIOL. BEHAV. 10(3) 595–600, 1973.—Dose dependent drinking responses were elicited by microinjections (in 0.1 μl volumes) of carbachol and angiotensin II directly into a midline region of the rat brain including the medial septal and medial preoptic areas. It was found that angiotensin II had a lower threshold dose, a lower maximally effective dose, and a shorter response latency than carbachol in eliciting drinking. No drinking was produced by hypertonic solutions of 300 or 800 mM NaCl when injected alone into this area. However, hypertonic NaCl solutions mixed with threshold doses of angiotensin II (9.71 pmol) potentiated the drinking response but had no effect when mixed with low doses of carbachol (13.7 pmol). Water intake following higher doses of angiotensin II and carbachol was not altered by hypertonic vehicles of NaCl or LiCl, whereas hypertonic KCl inhibited angiotensin induced drinking. Responses to angiotensin II (48.6 pmol) and carbachol (548 pmol) were unaffected when the injection vehicle was distilled water or 154 mM sucrose.

DRINKING in the rat may be elicited by hypertonic NaCl solutions [8], certain cholinomimetics [19,32], or angiotensin II (AII) and many of its analogs [14, 17, 33] when applied directly to tissue in the basal forebrain including parts of the hypothalamus, preoptic area, and septum. The neural receptor mechanisms by which these compounds induce polydipsia are not well understood, although a synaptic action for cholinergic drugs has been postulated [21], and activation of osmoreceptors by hypertonic solutions of NaCl has been hypothesized [8].

Anatomical localization and pharmacological characterization of receptors for each group of dipsogens is essential for an understanding of central neural mechanisms involved in the drinking response. It has been shown, for example, that while both carbachol and AII elicit drinking in the preoptic area [14], separate receptors for the drugs appear to exist since atropine sulfate blocks drinking to carbachol but not AII [18, 33, 36]. Evidence that osmoreceptors localized in the lateral preoptic area (LPO) are anatomically separate from receptors involved in thirst arising from extracellular hypovolemia has also been presented [8]. In the goat, the magnitude of drinking evoked by AII appears to be directly related to the concentration of Na⁺ ions in the infusion vehicle [2,3].

The present work investigated the effect of various ions on the drinking response to carbachol or AII microinjected into a restricted region of the brain which included the medial septum and medial preoptic area (MS–MPO), using an injection volume of 0.1 μl. This was done in order to further characterize AII and carbachol receptors in this brain region, which is quite sensitive to both drugs, and to look for evidence of the involvement of osmoreceptors in the response. No data indicating the presence of osmoreceptors in this region was obtained. However, increased Na⁺ and K⁺ levels were found to differentially affect the drinking response to carbachol and AII.

METHOD

Animals

Adult male albino rats (Holtzman; N = 130) were used for this study. Animals were housed in individual chambers 19 cm wide by 30 cm long by 58 cm high, equipped with a single 100 ml Richter tube and a food cup containing ground laboratory chow. Food and water were available at all times. The animals were kept on a 12-hr light: 12-hr dark schedule throughout. Before surgical implantation of a cannula system, 24-hr water intakes were determined for

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comparison with postoperative levels. No experiments were performed before postoperative intakes returned to normal (about one week).

Canula Implantation

Animals were anesthetized with an intraperitoneal (IP) injection of 40 mg/kg sodium pentobarbital (Diabutal) and placed in a stereotaxic apparatus. Following exposure of the calvaria, a single 17.0 mm guide cannula constructed of 23 ga stainless steel tubing was lowered, through a hole drilled in the sagittal suture, to a predetermined depth aimed at the MS--MPO. The cannula was fixed to the skull with 4 anchor screws and cranioplastic cement. Ether and atropine sulfate were administered as required to maintain proper anesthesia and to control the secretion of fluids into the respiratory tract, respectively. Stereotaxic coordinates (A2.8 to A1.4) were predetermined using the atlas of Pellegrino and Cushman [30].

Microinjection Procedure

Microinjections were made in a volume of 0.1 µl as previously described [34]. In brief, an injection was made by advancing the plunger of a 10 µl microsyringe with the aid of a micrometer, which forced drug containing solution from the tip of an injection cannula attached to the microsyringe by a 50 cm length of PE-10 tubing. The volume delivered was determined by the movement of a short air space in a calibrated segment of the PE-10 tubing.

For a microinjection, animals were lightly restrained by hand while the fluid filled injection cannula was inserted to its proper depth in the guide cannula. Following the microinjection, the rat was returned to its home chamber where water intake was measured for a 45-min period. Response latency (time between placement of animal in its chamber and the first sign of drinking) was measured in appropriate experiments. An average of 21 ± 2 sec (± 1SE) elapsed between the end of a microinjection and the placement of an animal in its chamber. Microinjection experiments for a given animal were spaced a minimum of 24-hr apart, and were always made between 1:00 and 3:00 p.m.

Drugs used included carbamylcholine chloride (carbachol), All (Hypertensin CIBA), glutamic acid, and 1-arterenol hydrochloride (1-norepinephrine). All drug solutions were prepared using sterile isotonic NaCl as the vehicle except in the case of hypertonic salt solutions (i.e., when quantities of the appropriate salt were added to sterile distilled water).

The drinking response to various microinjected dipoles is subject to considerable variability, especially when different animals are compared. In order to minimize this, data concerning direct comparisons of the effects of a particular solute on the response to either carbachol or AII has only been included when each treatment was given to each animal in a group. Whenever possible, a t-test for related samples was used to test for significant differences between means [15]. The results of all microinjections into the MS-MPO, regardless of magnitude, were included in the analysis of dose response relationships. However, only animals displaying a drinking response to a given dose of carbachol or AII were included in the analysis of ionic effects on response magnitude.

Histology

Canula tip placements were carefully determined in most animals and only data from those animals injected in the MS--MPO were included. Animals were anesthetized with 50 mg/kg sodium pentobarbital (IP) and administered 0.5 ml of a 0.9% solution of sodium nitrite intracardially for its vasodilatory effect. A transcardial perfusion with 100--150 ml of 1.5% glutaraldehyde--1.0% paraformaldehyde fixative was performed. Fixed brains were sectioned at 24 µ in a cryostat and stained following a method developed by Klüver and Barrera [22]. Cannula tip placements were verified with the aid of a microscope.

![Graph](image-url)

**FIG. 1.** Dose-response relationships for All and carbachol microinjected into the MS--MPO region of the rat brain in 0.1 µl volumes. Each point represents the mean water intake for all animals injected at that dose (vertical lines = ± 1 SE; numbers near circles = number of microinjections).

**RESULTS**

**Dose-response Relationships**

Figure 1 shows that the dose-response curves for All and carbachol were quite similar in shape and magnitude, although the latter was shifted to the right, i.e., had a higher threshold and a higher maximally effective dose. All microinjected into the midline, involving the MS--MPO (see below), in a volume of 0.1 µl elicited a significant drinking response at a threshold dose of 1.21 pmol (1.25 ng) while that for carbachol in the same volume was 13.7 pmol (2.5 ng). Peak response magnitudes for the two drugs were not statistically different (t-test), although that for carbachol
required approximately 10 times as much drug on an equal molar basis. The highest dose of each drug tested elicited a less than maximal response. The reason for this was not apparent in the gross behavior of animals following All microinjections. However, 3300 pmol of carbachol caused subconvulsive behavior in 53% of the animals, indicating toxicity to the high dose.

Microinjections of glutamic acid, a general neural excitant [9], in doses from 3.55 to 29.6 nmol did not elicit drinking; neither did 1-norepinephrine (4.86 and 9.72 nmol).

Response Latencies

Mean response latencies did not differ significantly among effective doses of either All or carbachol. However, when the two drugs were compared, mean drinking latencies in the water replete animals differed significantly (p<0.01) between the two drugs. All, in a dose range of 1.21 pmol to 971 pmol, elicited drinking with a mean latency of 2.1 ± 0.3 min (± 1 SE; N = 126). The latencies for carbachol and All are quite long when compared with that measured when 24-hr water deprived rats were either microinjected with saline or taken through a sham microinjection procedure (0.3 ± 0.02 min, N = 5).

No potentiating effect of hypertonic NaCl was observed, however, at doses equal to those required to elicit an approximately 50% maximal response, or ED50 (Fig. 2C, E). This was tested with concentrations of 300 and 800 mM NaCl for All (Fig. 2C). Thus, hypertonic NaCl potentiated drinking only when given with a threshold dose of All.

The responses to All and carbachol were further differentiated on the basis of the effects of hypertonic KCl and CaCl2, both of which inhibited drinking following the former but not the latter (Fig. 3). Equimolar LiCl had no effect on All or carbachol elicited drinking.

Effect of Ions

Microinjections of 300 and 800 mM NaCl directly into the MS--MPO elicited no more drinking than control injections of 154 mM NaCl or sterile distilled water (Fig. 2A). Thus, while both All and carbachol induced dose-dependent drinking in this region, hypertonic NaCl solutions microinjected alone showed no dipsogenic action.

Drinking due to a threshold dose of All (9.71 pmol), but not carbachol (13.7 pmol), was significantly (p<0.02) increased by the addition of hypertonic NaCl to the injection vehicle (Fig. 2B, D; t-test for related samples). A group of 4 animals was used. Each rat received the threshold dose of both drugs in a vehicle of isotonic (154 mM) or hypertonic (800 mM) NaCl, for a total of 12 separate microinjections, made in a counterbalanced sequence.

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Additional experiments (Fig. 4) showed that drinking due to All and carbachol microinjected into MS--MPO was unaffected when: (a) the isotonic NaCl injection vehicle was replaced by 154 mM sucrose, and (b) the vehicle was sterile distilled water. This evidence suggests that the drinking response to All (and carbachol) in the water replete rat does not require the presence of elevated levels of NaCl.

Location of Sensitive Tissue

All microinjection sites were in the midline and confined to tissue in the medial septum dorsally and the medial preoptic area ventrally. Intermediate loci in the bed nuclei of the stria terminalis and anterior commissure were also stimulated. Microinjections into antero--ventral sites usually involved the introduction of drug containing solution directly into the third ventricle and immediately adjacent periventricular and medial preoptic tissue. This entire midline region was sensitive to both All and carbachol, with no nuclear area displaying greater sensitivity to one
drug or the other (a quantitative analysis of positive and negative sites for both drugs throughout the diencephalon is in preparation). An example of a microinjection site in the medial preoptic area is shown in Fig. 5.

**FIG. 4.** The magnitude of drinking responses to AII and carbachol was unaffected when the injection vehicle contained 154 mM sucrose or distilled water rather than isotonic (154 mM) NaCl (vertical lines = 1 SE; numbers in parentheses = number of different animals microinjected).

**FIG. 5.** Frontal section of brain with cannula tip placed in medial septum immediately dorsal to the anterior commissure. All sites tested were in the medial septum or ventral thalamus, i.e., the bed nuclei of the anterior commissure and stria terminalis, and the periventricular and medial preoptic areas. The arrow in this Figure indicates the center of the microinjection site.
drinking response.

It has been reported that AII and Na⁺ may act on the same cell type in tissue immediately surrounding the third ventricle in the goat, to elicit drinking [2]. However, since these experiments involved intraventricularly applied drugs, it is possible that the AII and increased levels of Na⁺ were acting at different anatomical loci. It is also possible that the mechanism of AII-induced drinking in the goat differs from that in the rat. For the goat, Andersson et al. [3] reported that solutions containing AII and isotonic glucose or urea were less effective (less than 20%) than solutions of AII in isotonic NaCl. In the MS–MPO of the rat, however, AII in solutions of sucrose or even distilled water (Fig. 4) were as potent as an equal amount of AII in isotonic NaCl.

Evidence that elevated Na⁺ levels may increase the response to threshold doses of AII (Fig. 2B) is not surprising since Na⁺ has been shown to influence the response to AII in a number of peripheral systems [7,24]. Some of these effects may result from an alteration of the steric conformation of the AII molecule by Na⁺ [6].

While carbachol has been assumed to act upon postsynaptic cholinergic receptors to activate the drinking response [21], the mechanism by which AII elicits drinking remains more obscure. Three possibilities have been examined. (1) AII is known to release acetylcholine (ACh) in certain peripheral systems (e.g., [28]). The most straightforward explanation of its central dipsogenic action, therefore, would be that AII acts presynaptically to release ACh, or postsynaptically on ACh receptors. This is unlikely, however, since atropine pretreatment blocks carbachol induced drinking but has no effect on that due to AII [18, 33, 36]. (2) AII is known to release catecholamines in some instances (e.g., [13]). However, norepinephrine was not found to induce drinking consistently in this study or others [19,20]; nor was dopamine [20]. (3) It has been suggested [2] that AII may directly sensitize or otherwise modify osmoreceptors or Na⁺ receptors which are responsible for thirst. In the present work no such receptors were found in the tissue responsive to AII. The cellular mechanism of AII action in the MS–MPO is therefore problematic and requires further study. It is possible that the hormone may have a direct depolarizing action on cells surrounding the third ventricle [35], since it is known to do so to other central neurons [23,27], and to cells in the periphery [12,13].

REFERENCES