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# Forebrain Circumventricular Organs Mediate Captopril-Enhanced Ethanol Intake in Rats

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FITTS, D. A. *Forebrain circumventricular organs mediate captopril-enhanced ethanol intake in rats.* PHARMACOL BIOCHEM BEHAV **45**(4) 811–816, 1993.—Chronic peripheral treatments with low doses of the angiotensin-converting enzyme inhibitor, captopril, enhance daily intakes of dilute ethanol solutions in rats as they do the intakes of water and saline solutions. Placing captopril into the drinking water or infusing it SC increases daily intake of 6% (v/v) ethanol from 30–100% over 4–12 days of treatment. The present study examined the effects of electrolytic lesions either of the subfornical organ (SFO) or of the organum vasculosum laminae terminalis (OVLT), on captopril-enhanced ethanol intake. Captopril was infused in minipumps at 5 mg/day for 14 days. The intake of 6% (v/v) ethanol was abolished by SFO lesions and was temporarily reduced by OVLT lesions. The SFO, in particular, is essential for the expression of enhanced ethanol intake during low-dose peripheral captopril administration. Local angiotensin II synthesis and receptor activation at the SFO appear to be the mechanism of the enhanced ethanol drinking during captopril.

Angiotensin-converting enzyme inhibitors  Subfornical organ  Organum vasculosum laminae terminalis  Thirst

DESPITE a large body of evidence showing that angiotensin II (ANG II) and angiotensin-converting enzyme (ACE) inhibitors reduce ethanol intake [reviewed in (16,17)], recent evidence from this laboratory shows that continuous, chronic treatments with low peripheral doses of the ACE inhibitor captopril consistently and reliably increase intake of ethanol solutions ranging from 4–8% (v/v) in Long-Evans rats (8,9). The difference between the findings of enhanced versus reduced ethanol intakes appears to result from differences in methodology. Continuous, chronic treatments with low doses enhance daily ethanol intake, whereas daily episodic treatments with high doses reduce ethanol intake, such as by twice daily SC injections or by single injections immediately prior to a 1-h drinking test (16).

Low-dose peripheral captopril treatments have the paradoxical effect of enhancing the intakes of water and hypertonic saline. The key to the mechanism of this enhancement is that some forebrain circumventricular organs, such as the subfornical organ (SFO), contain very high concentrations of ACE. These tiny pools of concentrated enzyme remain incompletely blocked at low doses of captopril, even though all peripheral ACE is completely blocked in major tissue sites that usually generate ANG II, such as the lungs and kidneys (3,25,26). The lack of negative feedback on renin secretion during the blockade enhances the rate of renin release, and soon the concentration of ANG I in the circulation becomes very great. This high, circulating concentration of ANG I in turn provides the substrate for a high rate of synthesis of ANG II in the SFO because of the presence of the unblocked ACE. This newly synthesized ANG II has a negligible impact upon the blood ANG II concentration, but it does lead to local dipsogenic ANG II receptor binding and thirst. This has been proven experimentally by lesions or knife cuts in the SFO, by captopril injections into the SFO to stop ACE activity locally, and by ANG II receptor blockers injected into the SFO (11,12,23,27,28). All of these abolish the thirst induced by low doses of peripheral captopril. Peripheral injections of very high doses of captopril do not arouse thirst (7), because high doses block all of the ACE in the circumventricular organs as well as in the lungs and kidneys. Thus, the dose-response curve for water intake during peripheral captopril treatments has the shape of an inverted U.

As with water intake (6,7,18,27,28) and saline intake (4,5,11,12) enhanced by peripheral captopril, excess ethanol intake during captopril treatments relies on a synthesis of ANG II in the brain. This has been demonstrated by simultaneously blocking both the periphery and the brain with captopril using combined intracerebroventricular and SC captopril infusions (8). The most logical hypothesis to explain captopril-enhanced ethanol intake is that it is controlled in much the same way as captopril-enhanced water and saline intakes; that is, by conversion of ANG I to ANG II in forebrain circumventricular organs such as the SFO and the organum vasculosum laminae terminalis (OVLT) (12,28).

The purpose of this study was to test the hypothesis that enhanced daily ethanol drinking during peripheral captopril

administration is also mediated by either or both of these forebrain circumventricular organs, the SFO and the OVLT. The hypothesis was tested by examining the drinking responses to SC-infused captopril in groups of rats having electrolytic lesions of either structure.

## METHOD

### *Subjects*

Male Long-Evans rats weighing 300–500 g were used. They were housed in hanging wire mesh cages with Wayne laboratory chow and tap water continuously available. The lights were on 12 h per day. Temperature in the room was constant at 23°C. The sample sizes depended on the outcome of histology and therefore are given in the Results section.

### *Fluid Intakes*

Water and 6% (v/v) ethanol intakes were measured using 100-ml graduated cylinders fitted with drinking spouts. Intakes of the fluids were recorded to the nearest milliliter. The positions of the water and ethanol tubes were alternated daily to control for position preferences.

### *Surgery*

Stereotaxic surgery was conducted under Equi-Thesin anesthesia (0.35 ml/100 g, IP), with gentamicin (0.2 ml, IM) and topically applied Betadine to control postsurgical infection. Electrolytic lesions of the SFO and the ventral median preoptic nucleus (VVMnPO) were made with a Teflon-coated tungsten wire using 1.0 mA of anodal current with the circuit completed at the rat's foot. The VVMnPO lesion includes the dorsal cap and all dorsal connectivity of the OVLT plus a small piece of the ventral MnPO (see Histology section and Fig. 1).

Coordinates for the lesions with respect to bregma and a flat skull were: SFO, three 10-s penetrations successively at AP −0.1, −0.4, and −0.7, and DV −4.5, −4.3, −4.1; VVMnPO, one 15- to 20-s penetration, AP +1.2, DV −7.4. Both lesions were made on the midline, and the dorsal-ventral readings were made from the top of the midsagittal sinus after drilling a 3.0-mm trephine hole. The sinus was not retracted during the lowering of the electrode. The VVMnPO-targeted electrode passed more than 1.5 mm rostral to the SFO and 0.2–0.4 mm rostral to anterior commissure. Sham lesions were made by puncturing the dura mater. Rats were recovered in a warm environment until they were sufficiently mobile to be returned to their cages.

Alzet 2ML2 minipumps were implanted SC under halothane anesthesia into rats having SFO, VVMnPO, or sham lesions. The pumps were loaded with 41.7 mg/ml captopril and were implanted by sterile procedure. The nominal pumping rate was 5 μl/h for 14 days, producing a total dose of 5 mg/day. The dorsal incision was closed with wound clips, Betadine was applied topically, and 0.2 ml gentamicin was given IM to control postsurgical infection. The entire procedure took 5 min per rat, and the rats were fully awake in their home cages within another 5 min.

### *Histology*

At the end of each experiment, the rats were given an overdose of pentobarbital sodium and were perfused through the heart with saline to clear blood and then with 10% formalin-saline for fixation. Brains were removed and stored in 15 volumes of formalin-saline until they were cut on a freezing microtome at 40–50 μm, mounted on subbed glass slides, and stained with thionine for subsequent single-blind analysis of the site of the lesion.

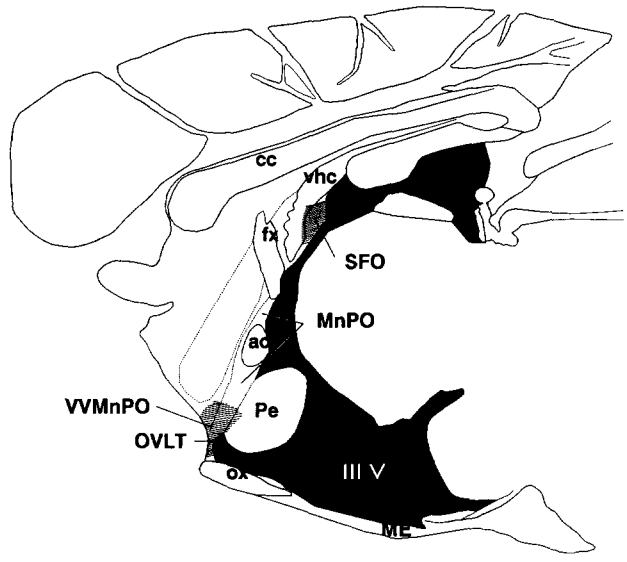


FIG. 1. Midsagittal section of a rat brain [redrawn from (24)] showing hatched areas representing minimal lesions of the subfornical organ (SFO) and the ventral ventral median preoptic nucleus (VVMnPO), including the organum vasculosum laminae terminalis (OVLT). Abbreviations: cc, corpus callosum; vhc, ventral hippocampal commissure; fx, fornix; ac, anterior commissure; ox, optic chiasm; MnPO, median preoptic nucleus; Pe, periventricular nucleus of the hypothalamus; ME, median eminence; IIIV, third ventricle.

Rats are considered to have good SFO lesions if more than 85% of the nucleus is destroyed, including all of the rostroventral stalk containing the afferent and efferent fiber systems of the SFO that relay hydromineral and cardiovascular information (19,21,23). Partial and missed lesions do less than 85% damage to the SFO and do not damage the rostroventral pole of the SFO. Lesions that do less than 85% damage to the SFO but that destroy the rostroventral pole of the SFO are categorized separately from other missed lesions because damage to the efferent and afferent fibers of the SFO produces identical effects to a lesion of the SFO itself (19,21,23). Some additional damage is often done to the overlying hippocampal commissure, the fornix, the dorsal MnPO, or the triangular septum, with less common damage to the medial septum and the paraventricular nucleus of the thalamus.

Rats are considered to have good VVMnPO lesions if the bottom third of the ventral median preoptic nucleus is completely destroyed, including especially the rostral wall of the third ventricle. The lesion must completely destroy the dorsal cap and all dorsal connectivity of the OVLT from the wall of the third ventricle to the ventral surface of the brain at the rostral edge of the OVLT. There is frequently a small amount of damage to the caudal-ventral diagonal band, but there is rarely any damage to the periventicular nucleus or especially to more caudal hypothalamic nuclei, such as the medial preoptic nuclei or suprachiasmatic nuclei. The nomenclature "VVMnPO lesion" is used instead of "OVLT lesion" to emphasize the fact that it includes a small part of the MnPO and occasionally may not completely destroy all of the most ventral

part of the OVLT where it connects with the optic chiasm. Remaining fragments of the OVLT are usually characterized by dense astroglial proliferation and chromatolysis.

*Drugs*

Captopril was a gift from Bristol-Myers Squibb Pharmaceutical Research Institute and was prepared fresh daily. It was mixed in sterile isotonic saline immediately before being injected into the reservoirs of the minipumps. Ethanol solutions were prepared daily using 95% USP ethanol in tap water.

*Statistical Analysis*

Data were analyzed using analysis of variance (ANOVA). Planned comparisons used Fisher's least significant difference test following a significant $F$ ratio. A probability of less than 0.05 was required for significance.

*Procedure*

Rats received lesions of the SFO, VVMnPO, or sham lesions 4 weeks before the minipumps infusing captopril were implanted. During this period, the rats had access to water and 6% (v/v) ethanol solution for 2 weeks immediately prior to the implantation. A similar dose of captopril (6 mg/day versus the current 5 mg/day) has been shown to greatly increase both water and ethanol intake when delivered from 2ML2 Alzet minipumps in rats (8). Water and ethanol solution intakes were measured daily for the 14 days of infusion and for 6 additional days of recovery.

## RESULTS

*Histology and Analysis*

Histological analysis of the brains of the rats showed that seven rats had sham lesions, four rats had complete SFO lesions, five rats had rostroventral SFO lesions that destroyed all rostral connectivity of the SFO, five rats had VVMnPO lesions, and six rats had missed VVMnPO lesions that did only partial or no damage to the OVLT and failed to disconnect the OVLT from the overlying MnPO along the rostral wall of the third ventricle. One other rat that had a missed SFO lesion that did not damage the rostroventral pole was excluded from further analysis.

Thus, 27 rats were included in the statistical analysis. The data for the 6 days prior to the implantation of the minipumps were averaged to provide a stable baseline point, and the rest of the 14 infusion and 6 recovery days were grouped into 2-day blocks as a within-subjects variable in the ANOVA. Type of fluid (water or saline) was included as another within-subject variable, and the different lesion groups were analyzed as a between-subject variable. The SFO-lesioned rats and the rostroventral SFO-lesioned rats were combined into a single group of nine rats for the analysis because of the expectation, confirmed by preliminary analysis, that the two lesions were functionally identical and therefore would not differ (10,22). This group is hereafter referred to as the SFO-lesioned group.

The three-way interaction of lesion groups by fluid type by days was significant, $F(30, 230) = 2.20$, $p < 0.001$, indicating that the captopril infusions caused different changes across the days of infusion according to the type of fluid ingested and the location of the lesion. All further comparisons thus reflect planned comparisons among the different lesion groups and the days of captopril treatments. Intakes of water and ethanol did not differ among the different lesioned groups during the baseline period except for the VVMnPO-lesioned group, which showed a significant water polydipsia typical of this lesion relative to the sham-lesioned group (13). Basal blood chemistries in VVMnPO-lesioned rats exhibiting water polydipsia (13) indicated that the rats were not out of balance in either the intracellular (osmolality, sodium concentration) or extracellular (hematocrit, plasma protein) compartments.

*Water Intake*

The results for water intake are shown in Fig. 2. After the implantation of the minipumps, all groups except the SFO-lesioned group increased water intake during the first two or three 2-day blocks. The VVMnPO-lesioned group remained significantly greater than the sham group during the first 2-day block. Thereafter, however, the sham-lesioned group continued increasing water intake and exceeded the other three groups until the last 2-day block of the recovery period. At this time, it was not significantly greater than the SFO-lesioned rats but did remain greater than both of the ventrally lesioned groups.

The important feature of the water intake data is that the SFO-lesioned group never changed its intake during the captopril infusion, whereas both the complete and missed VVMnPO-lesioned groups at first increased and then decreased water intake during the course of the infusion.

*Ethanol Intake*

The results for ethanol intake are shown in Fig. 3. As with the water intake, the sham-lesioned rats increased ethanol intake progressively over the first 6 days of infusion, but the SFO-lesioned rats never altered their intake of ethanol. The SFO-lesioned group drank significantly less ethanol than the sham-lesioned rats during the entire captopril infusion and during the first 2-day block of the recovery period, after which the two groups did not differ.

Contrary to the water intake data, the VVMnPO-lesioned group failed to increase ethanol intake early in the infusion period and drank significantly less than the sham-lesioned group during the first three 2-day blocks. By the end of the infusion, however, the VVMnPO-lesioned group increased ethanol intake to an absolute level greater than all other groups, and the enhanced intake persisted throughout the recovery period.

The missed VVMnPO-lesioned group never differed significantly from the sham-lesioned group, and drank significantly more ethanol than the SFO-lesioned group during the fourth 2-day block of the infusion and during the first and third blocks of the recovery period.

## DISCUSSION

Hypertonic saline intake during captopril is unaffected by the SFO lesion or by ventral SFO knife cuts (23,27). Indeed, infusions of ANG II directly into the SFO generated water intake but no saline intake. By contrast, identical infusions into the tissue near the OVLT elicited both water intake and dose-related saline intake (12). The sensitive tissue was localized within 0.25 mm of the dorsal cap of the OVLT, including a small piece of the most ventral part of the MnPO, and this area of sensitivity is the functional definition of the VVMnPO. Lesions of this VVMnPO tissue reduced salt appetite aroused by captopril or by sodium depletion, but produced very few other drinking or regulatory disturbances (13).

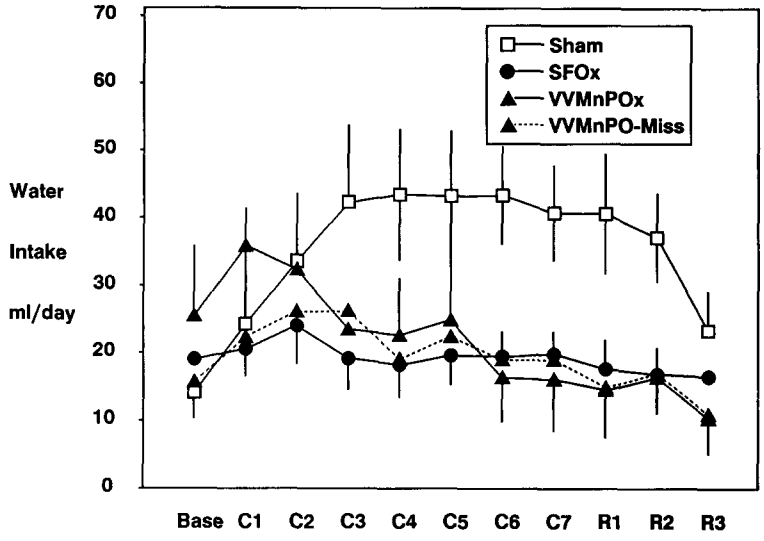


FIG. 2. Mean daily water intakes in water/ethanol choice by lesioned groups during minipump infusions of 5 mg/day SC captopril. Base: mean of 6 days of baseline. C1 to C7: 2-day block means during 14 days of infusion. R1 to R3: 2-day block means during 6 days of recovery. SFOx: Subfornical organ lesion; did not increase during captopril, lower than sham group. VVMnPOx: ventral ventral median preoptic nucleus lesion including organum vasulosum laminae terminalis. VVMnPO-Miss: missed lesions. The ventral lesion groups temporarily increased intake. Bars are SE.

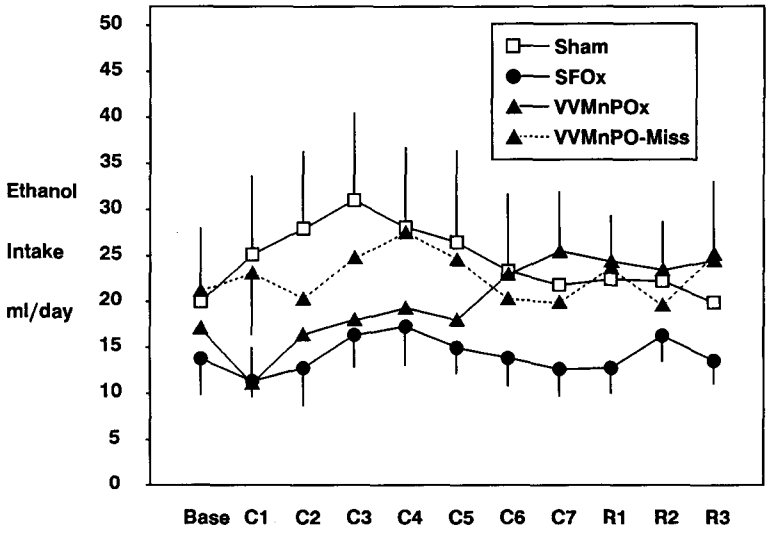


FIG. 3. Mean daily ethanol intakes in water/ethanol choice by lesioned groups during minipump infusions of 5 mg/day SC captopril. Base: mean of 6 days of baseline. C1 to C7: 2-day block means during 14 days of infusion. R1 to R3: 2-day block means during 6 days of recovery. SFOx: Subfornical organ lesion; did not increase intake during captopril, lower than sham. VVMnPOx: ventral ventral median preoptic nucleus lesion including organum vasulosum laminae terminalis; lower than sham early, increased late in infusion. VVMnPO-Miss: missed lesions; did not differ from sham. Bars are SE.

The lesion is otherwise characterized by an immediate postsurgical adipsia lasting 1 to 3 days in most animals and lasting long enough to necessitate euthanasia in about 15% of lesioned rats without artificial hydration or provision of palatable solutions. After the period of adipsia, the rats rebound to a permanent pattern of polydipsia 25–100% greater than their presurgical baseline water intakes. Another characteristic is a greatly enhanced salt appetite during daily treatments with DOCA (10,13).

The VVMnPO lesion is much smaller than two other preoptic region lesions that are commonly used to study forebrain brain effects on body hydration, the anteroventral third ventricle (AV3V) lesion and the complete ventral median preoptic nucleus lesion from the anterior commissure to the dorsal cap of the OVLT (1,2,14,15,20). These two lesions produce hypernatremia, hyperosmolality, profound adipsia in all animals, and insensitivity to stimuli for thirst, including hypertonic saline, water deprivation, and isoproterenol. The VVMnPO lesion has none of these effects (10,13). Thus, the VVMnPO lesion is ideal for studying the specific functions of the OVLT without the confounding effects of the much larger lesions.

Once it was determined that ethanol intake increased during treatments with low doses of peripheral captopril (8), it was of interest to determine whether either or both of these forebrain circumventricular organs accounted for the enhancement. The findings show that SFO lesions completely abolish captopril-enhanced water and ethanol intakes. This result is supported by an unpublished experiment in which SFO lesions abolished the enhanced ethanol intakes resulting from an adulteration of both the drinking water and the ethanol solution with 0.1 mg/ml of captopril (own unpublished data). A preliminary report of this experiment with a much smaller sample size reported negative results because of insufficient power (9).

The results from the VVMnPO lesions showed interesting effects that are not easily interpretable. As usual, the VVMnPO group was slightly but significantly polydipsic during the baseline condition. The reason for this polydipsia is currently not known. The initial captopril-induced rise in water intake in the good-lesion and missed-lesion groups is consistent with the fact that the SFOs of these rats were intact. However, water intake declined again before the end of the infusion and never matched the high level of intake of the sham-lesioned group. In fact, the decline in water intake in the VVMnPO-lesioned group persisted even during the posttreatment recovery period, despite the expectation that it would regain a high level because of the chronic polydipsia of VVMnPO-lesioned rats. What appeared to happen instead was a gradual increase in ethanol intake from a significantly depressed level early in the infusions to a significantly enhanced level relative to the SFO-lesioned group during the postinfusion recovery period. During the last 2-day block of the recovery, the VVMnPO-lesioned group had both the highest absolute ethanol intake and the lowest absolute water intake.

The missed-lesion VVMnPO group showed similar changes to the complete-lesion group, although the deviations in intakes were not as extreme, and the group never differed from the sham-lesioned group in ethanol intake.

In the absence of systematic data from rats with VVMnPO lesions and no captopril treatment, it is difficult to determine if the dramatic change in ethanol preference in that group resulted from the captopril treatment or simply from a passage of time. In the unpublished experiment cited above, three rats with VVMnPO lesions had 9 days of access to 6% (v/v) ethanol before the oral captopril phase began. On the last day of baseline, these three rats drank 2, 1, and 6 ml of water and 52, 64, and 23 ml of ethanol, respectively. This suggests that the exaggerated ethanol preference in VVMnPO-lesioned rats may develop independently of the captopril stimulation, although the evidence is weak because of the small sample size. However, the impressive and persistent voluntary ethanol intakes of 3–8 g/kg/day in VVMnPO-lesioned rats, whether stimulated by captopril or not, warrants further investigation.

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