Immunocytochemical study of secretion by the mouse subcommissural organ after captopril treatment


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SUMMARY

We have studied the effects of chronic treatment with captopril (angiotensin-converting enzyme inhibitor, ACEI) on the secretion of the subcommissural organ (SCO) of the adult male mouse using immunohistochemistry for AFRU (antibody anti-Reissner's fiber, Rodriguez et al., 1994). Three groups of 5 animals each –a control group and 2 experimental groups– were used. The experimental groups received captopril in their drinking water from postnatal day 55 at a dose of 1 mg/kg body weight in experimental group 1 (CAP-1), and 4 mg/kg body weight in experimental group II (CAP-4). Captopril administration at a dose of 1 mg/kg did not alter the amount of AFRU immunoreactive material (AFRU-ir) in the SCO, but captopril administration at a dose of 4 mg/kg elicited a significant decrease in the SCO AFRU-ir material.

Key words: Captopril - AFRU immunohistochemistry - subcommissural organ.

INTRODUCTION

The subcommissural organ (SCO) is an ependymal gland that, in the mouse and rat, covers the entire ventral aspect of the posterior commissure. The specialized ependymal cells produce a secretory material, that is apparently a complex containing different glycoproteins (Nualart et al., 1991). The glycoproteins are mainly released into the ventricular cerebrospinal fluid, where they form Reissner's fibers (Sterba, 1967), but also gain access, via the basal processes, to the vascular network of the SCO and to the CSF-containing subarachnoid space (Rodriguez et al., 1984b). The SCO is intensely immunoreactive to the antiserum against Reissner's fibers (AFRU) (Rodríguez et al., 1984a), which allows visualization of both secretory pathways (apical and basal) of the organ.

The SCO has been implicated in several contradictory functional hypotheses, some of them relating the SCO to postnatally-induced hydrocephalus (Irigoin et al., 1990) or prenatal congenital hydrocephalus (Rodriguez et al., 1992; Castañeyra-Perdomo et al., 1994). Other important hypotheses have postulated a connection of the SCO with the mechanisms involved in blood pressure regulation (Guevas et al., 1996; Castañeyra-Perdomo et al., 1998), the salt/water balance (Foldvari and Palkovits, 1964; Rodriguez et al., 1992) and sodium homeostasis (Severs et al., 1993). The presence of angiotensin-binding sites in the SCO may be evidence of a possible role in fluid and electrolyte balance (Ghiani et al., 1988).

Captopril (an angiotensin-converting enzyme inhibitor, ACEI) produces an increase in the intake of both water and saline in rats (Thunhorst et al., 1987). In order to test the possible involvement of the SCO in the mechanisms of salt and water balance regulation, here we analyzed the effects of chronic oral administration of captopril on the secretion of the SCO by immunohistochemistry for AFRU.
MATERIAL AND METHODS

Fifteen albino mice fed with standard food and water ad libitum were divided into three groups of five animals—a control group and 2 experimental groups—and sacrificed with chloral hydrate on postnatal day 160. Water intake was measured daily with a graduated bottle from the beginning of treatment (day 55) until sacrifice, and body weight was determined weekly and before sacrifice. The experimental groups received captopril (Sigma) in the drinking water from postnatal day 55 until sacrifice, at a dose (the dose was calculated as a function of variations of water intake and body weight) of 1 mg/kg body weight in experimental group I (CAP-1), and 4 mg/kg body weight in experimental group II (CAP-4). Brains were fixed by perfusion through the mouse’s left ventricle with Bouin’s fluid, post-fixed in the same fixative and dehydrated and embedded in paraffin under standard conditions. Serial coronal sections of 10 µm were stained by the Klüver-Barrera method, while a parallel series was processed by the immunoperoxidase method of Sternberger et al. (1970). As primary antibody we used a polyclonal antibody against the glycoproteins of bovine Reissner’s fiber (AFRU, Rodríguez et al., 1984a). All sections from the control and experimental groups were incubated simultaneously in the primary antiserum over 24 hours at a dilution of 1:2500 in phosphate buffer saline (PBS) 0.01M, Triton 0.2% and sodium azide 0.1%. Anti-rabbit IgG (whole molecule) peroxidase conjugate (Sigma) at a dilution of 1:100 in the same solution as the primary antiserum was used as a second antibody over 2 hours. The peroxidase reaction product was visualized through the diaminobenzidine reaction. The intensity of the AFRU reaction was measured by optical densitometry in 6 sections of each animal corresponding to different rostrocaudal levels of the SCO, using a "Magiscan" image analysis system and the "Genias" program (Joyce Loehl, Newcastle, UK). For statistical evaluation, the control and experimental groups were compared by a 1-way ANOVA and a post hoc test (Bonferroni).

RESULTS

General observations

The total amount of fluid intake in the CAP4 group was significantly higher than in the control and CAP1 animals (p<0.05) (Fig. 1A). However, no significant differences in body weight were found between the control and captopril-treated groups, and hence the data are not given here.

Fig. 1.—A: Average daily water intake in ml from the 55th to 160th day.
B: Densitometry of immunoreactive material of antiserum against Reissner’s fiber (AFRU).
control = control rats
CAP1 = captopril-treated group at 1 mg/kg body weight
CAP4 = captopril-treated group at 4 mg/kg body weight
IRM = Immunoreactive material
% of IRM = densitometric percentage of immunoreactive material (AFRU)
+ = Standard error
**Immunobistochemistry**

In the untreated mouse SCO, most AFRU-ir material is located in the perinuclear and supranuclear cytoplasmic regions of the specialized ependymal cells (Fig. 2A,B). The CAP1 group only showed a slight and statistically non-significant increase in AFRU-ir material (Fig. 1B) and a redistribution of this material, which now filled the supranuclear region or the basal pole of the SCO cells in the form of a coarse granular secretion product slightly more than in the control group (Fig. 2C,D). Only with the highest dose, in the CAP4 group, did we notice a considerable and generalized decrease (p<0.01) in the amount of AFRU-ir material (Fig. 1B, Fig. 2E,F).

**DISCUSSION**

Captopril (angiotensin-converting enzyme inhibitor) treatment blocks the conversion of angiotensin I (AGI) to angiotensin II (AGII) by decreasing the activity of the renin-angiotensin system and has a hypotensive effect on hypertensive mice but not on control mice (Webb et al., 1986). In normotensive animals, captopril produces an increase in water intake and a loss of potassium through the kidney (Thunhorst et al., 1987). The SCO has been related to the renin-angiotensin system, since specific receptors for angiotensin II are located in the subnuclear region of the ependymal secretory cells of the subcommissural organ (Ghiani et al., 1988). Our results suggest that a

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**Fig. 2.** Coronal view microphotographs of the SCO in the control group (A,B), CAP1 group (C,D) and CAP4 group (E,F). AFRU-immunostained sections. A, C and E: x 200. B, D and F: x 400.

PC = Posterior Commissure
V = Third ventricle
SCO = subcommissural organ
redistribution and a non-significant increase in AFRU-ir take place in the SCO after low-dose captopril treatment; only the high dose of captopril produced a consistent decrease in the secretory product. The role of the SCO in salt/water balance has been repeatedly suggested, e.g., by Foldvari and Palkovits (1964), who observed variations in the nuclear volume in the SCO after a sodium-and potassium-deficient diet. Rodriguez et al. (1992) have implicated the SCO in the metabolism of water and electrolytes, while Severs et al. (1993) observed that aldosterone affects the SCO as result of sodium loss. In agreement with Thurhorst et al. (1987), our results in the CAP4 group show that high-dose captopril treatment produces an increase in water intake.

Despite this suggested involvement of the SCO in electrolyte homeostasis, no changes in the AFRU-ir material were found in the rat SCO organ after salt and water loading and after water deprivation (Rodriguez et al., 1992). However, we wish to emphasize that most authors who have studied the SCO under different experimental conditions modified sodium and water intake, without changing potassium intake, and therefore the original observation by Foldvari and Palkovits (1964) has never been confirmed. These results were only recently confirmed by Carmona-Calero et al. (1996), who found an initial but transient increase in the overall volume of the SCO after captopril treatment and the consequent loss of potassium. In keeping with the results of Foldvari and Palkovits, our present observations suggest that the secretory activity of the SCO is linked to salt-water balance, as a consequence of changes in potassium levels.

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REFERENCES


