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The effects of chronic administration of captopril on the mouse median eminence

A. Castañeyra-Perdomo, E. Carmona-Calero, M. del M. Pérez-Delgado, H. Pérez-González, N. Marrero-Gordillo and R. Ferres-Torres

Department of Anatomy, School of Medicine, University of La Laguna, La Laguna, Tenerife, Spain

Summary. The effects of Captopril (an angiotensin-converting enzyme inhibitor) on the median eminence (ME) of the male albino mouse have been examined using morphometric and immunohistochemical procedures. We measured the nuclear area of the ependymocytes of the ME and of the glial cells of the reticular external zone of the ME. We also determined the cell/neuropil coefficient (CNC), which expresses the relation between cellular area and neuropil of the ME, and the global volume of the ME in each animal. For the immunohistochemical study we used rabbit anti-arginine-vasopressin, and compared the results in the different groups of mice. We detected an increase in the immunoreactive material (arginine-vasopressin, A-V) and an increase in the global volume of the organ and also an increase of the neuropil of the ME after the longest exposure to the drug. These alterations could be related to the inhibition of the brain angiotensin II by captopril and the accumulation of vasopressin in the fibrous tract that runs from the paraventricular nucleus (PVN) to the neurohypophysis.

Key words: Captopril, Vasopressin, Morphometry, Immunohistochemistry, Angiotensin-converting enzyme inhibitor, Mouse

Introduction

Axons from magnocellular neurons of the paraventricular hypothalamic nucleus (PVN) that produce vasopressin, pass through the internal zone of the median eminence (ME) and reach the neurohypophysis, where vasopressin is released (Holmes et al., 1986; Trembleau et al., 1994). The external zone of the ME receives projections from a circumscribed group of parvocellular cells of the PVN that produce corticotropin-releasing factor (CRF) and vasopressin (Antoni, 1986; Whitnall et al., 1987; De Goeij et al., 1992).

Brain angiotensin II receptors, thought to be involved in cardiovascular regulation, are localized in the ME and seem to be related here to central neural regulation of pituitary function and regulation of blood flow in the basal forebrain and pituitary gland (Palkovits, 1987; Tsutsumi and Saavedra, 1991). In fact, the release of vasopressin from the pituitary gland constitutes a major pressor mechanism of central Angiotensin-II (Severs and Daniels-Severs, 1973). Angiotensin converting enzyme inhibitors (ACEI), like captopril, are known to produce an elimination of the production of angiotensin II and, among other effects, diminish plasma levels of vasopressin whose secretion is partly dependent upon the stimulus of angiotensin II.

In a previous paper, we studied the morphometric effect of chronic captopril administration on two other circumventricular organs, the area postrema and the subfornical organ, which responded in a different and characteristic way (Castañeyra-Perdomo et al., 1993). In the present study, we analyze the variations in vasopressin immunoreactivity and in the morphometry of the ME after chronic oral administration of captopril, on the basis of the above-mentioned effects of captopril on angiotensin II production and the existence of angiotensin II receptors in the ME, to know if the decrease of the angiotensinnergic stimulus produces morphological alterations in this organ that is known to be closely related to vasopressin release.

Materials and methods

Sixty albino mice, fed with a standard diet, were sacrificed in groups of 5 at postnatal days 85, 100, 130 and 160. They were divided into 3 groups of 20 animals each, a control group and 2 experimental groups. The experimental groups received captopril in the drinking water from postnatal day 55 until sacrifice, at a dosage of 1 mg/kg bw in experimental group I (CAP-1), and 4mg/kg bw in experimental group II (CAP-4). No differences in the water intake were detected between

Offprint requests to: Dr. A. Castañeyra-Perdomo, Departamento de Anatomía, Facultad de Medicina, Universidad de La Laguna, 38320 Tenerife (Spain)

control and experimental animals. Animals were fixed by perfusion through the mouse's left ventricle with Bouin's fluid, postfixed in the same fixative, dehydrated and embedded in paraffin under standard conditions. Brains were cut in two alternative serial coronal sections of 10 μm. One of the serial sections was processed immunohistochemically for the A-V as follows: all sections from control and experimental groups were incubated on the same day and at the same time in the primary antisera for 24 hours (rabbit anti-arginine vasopressin; INC Immunobiologicals). We tried different dilutions until we got one in which control animals lacked vasopressin staining, while the experimental groups did stain, so that the difference in the immunohistochemical reaction could be established. So the final dilution of the primary antisera used in this study was 1: 2000, in a solution of buffer phosphate saline 0.01M, triton 0.2% and sodium acide 0.1%. Anti-rabbit IgG (Whole molecule) peroxidase conjugate (Sigma) at a dilution of 1:100 in the same solution as the primary antisera was used as a second antibody for 1 hour. The peroxidase reaction product was visualized through the diaminobenzidine reaction. The other sections were stained using the Kluver-Barrera method (Klüver-Barrera, 1953). The ependymocytes of the ME and glial cells (astrocytes and "astrocyte-like tanycytes") of the reticular-external zone of the ME [in accordance with Schiebler et al., 1978)] were measured using a "Magiscan" Image analysis system and the "Genias" program (Joyce Loebl). We determined the nuclear area (karyoplasmic area within the perimeter determined) of these elements, and the cell/neuropil coefficient (CNC) of the ME, which expresses the relation between the cellular area of the ME cells (essentially different types of glial cells) and the neuropil (including blood vessels) of the ME. We also calculated the global volume of the ME using the serial reconstruction system "SSRS" (Eutectic Electronic Inc.). For statistical evaluation we compared the control and experimental groups using a 2-way ANOVA and a post hoc test (Bonferroni) for comparison between animals of the same age.

Results

Immunohistochemistry to arginine-vasopressin

Captopril treatment produced an increase in the immunoreactive material located in the internal zone of the ME after 45 days of CAP-1 treatment (100-day-old animals). CAP-4 treatment produced at this age an increase in the immunoreactive material in the internal zone of the ME and a slighter increase in immunoreactivity in the external zone. In the animals aged 130 days, the external zone was also more immunoreactive than in the control animals in the CAP-1 group (Fig. 1a-c). Finally, with longer exposure to treatment, i.e. in animals of 160 days, the greater reaction with respect to that of the control group was limited to the internal zone of the ME in both treatment doses (Fig. 1 d-f).

Morphometry

The Nuclear area of ependymal cells (Fig. 2A) does not show significant differences between the control and experimental groups except at the last age studied, where the CAP-4 group presents higher values than the control and CAP-1 groups ($p < 0.01$).

The Nuclear area of glial cells of the ME (Fig. 2B) follows a similar evolution to that of the ependymal elements of the organ and only at 160 days we detected a significantly higher value of the area in the CAP-4 group ($p < 0.01$).

Cell/neuropil coefficient (Fig. 2C). The CNC in CAP-4 animals decreases with treatment time and reaches a significantly lower level than the control group at 160 days ($p<0.01$), suggesting an increase in neuropil. In contrast, the CNC of the CAP-1 group shows only small, non-significant variations.

Global volume of the ME (Fig. 2 D). This parameter in both experimental groups responds in a significant manner to captopril. CAP-4 shows higher values at 85, 100, 130 and 160 days ($p<0.01$). The CAP-1 group also shows higher values, although at 100 days the difference is not significant.

Discussion

Our results show that chronic administration of captopril produces an increase in immunoreactive material (arginine-vasopressin) in the ME, accompanied by a persistent and significant increase in the global volume of the organ, proportional to the dose of captopril administered. The nuclear area of the ependymocytes increases only after prolonged treatment and when the higher dose is used; the same occurs with other glial cells of the reticular-external zone of the ME. Given that the cell-neuropil coefficient (CNC) decreases in this same group of animals and at the same age (160th day) we suggest a relative increase in the neuropil at this age and in this group (CAP-4).

We have found an increase in the immunoreactive material to vasopressin after 45 days of captopril treatment in the internal zone, i.e., the zone where we localized the axons that from the PVN magnocellular neurons direct to the neurohypophysis to release vasopressin. In some cases, with the higher dose of captopril or a more prolonged time of treatment, we also detected an increase of immunoreactivity in the external zone of the ME, which is known to receive projections from a circumscribed group of parvocellular neurons of the PVN that produce corticotropin-releasing factor (CRF) and vasopressin.

Brain ANGII may be involved in the control of vasopressin and other endocrine hormones (Schölkens et al., 1980). The increase that we detected in the vasopressin content in the fibres of the internal zone of the ME after prolongued treatment with captopril, could

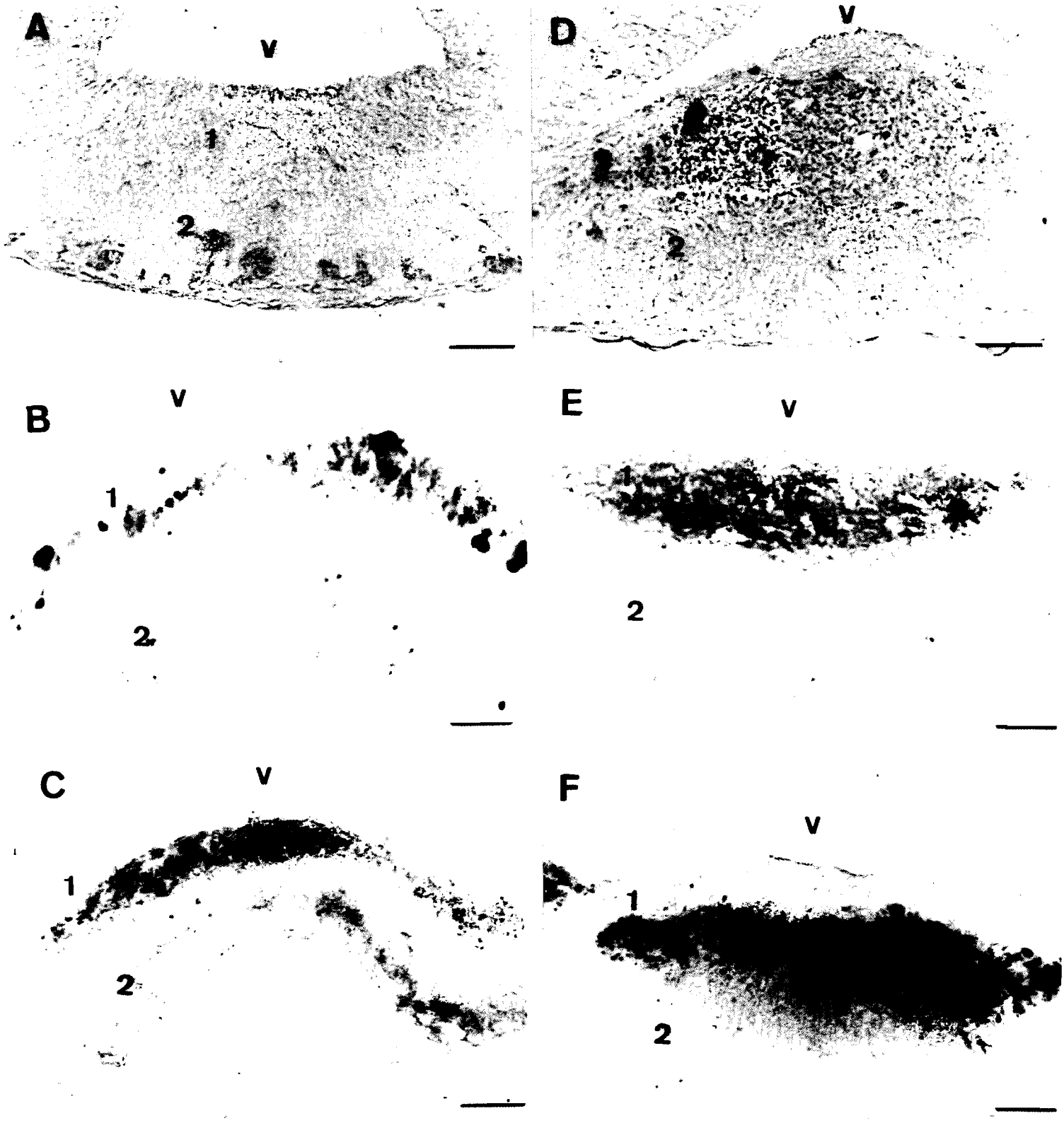

Fig. 1. Immunohistochemical reaction to arginine vasopressin in the median eminence of the male mouse at the 130th day (A, B, C) and 160th day (D, E, F). **A.** Control animal, 130 days. **B.** Animal of 130 days, treated with captopril (1 mg/kg bw), from postnatal day 55 (CAP-1). **C.** Animal of 130 days, treated with captopril (4mg/kg bw) from postnatal day 55 (CAP-4). **D.** Control animal, 160 days. **E.** Animal of 160 days, CAP-1. **F.** Animal of 160 days, CAP-4. 1: internal zone of the median eminence; 2: external zone of the median eminence. V: third ventricle. Bar= 30 μm.

be related to the inhibition of vasopressin release in the neurohypophysis, as plasma levels of vasopressin diminish after administration of angiotensin- converting enzyme inhibitors. The hormone is not released and then accumulates along the fibrous tract.

Vasopressin nerve terminals in the zona externa of the ME have been reported to increase after chronic psychosocial stress, in association with an increase in vasopressin secretory activity of vasopressin-containing CRF neurons of the PVN (De Goeij et al., 1992). We have detected a transient increase in the vasopressin content of the external zone of the ME at the 130th day, that disappears at the 160th day. The possible meaning of this transient increase requires further investigation. New groups of animals with longer exposure to the treatment are being prepared in order to test if the increase at the 130th day was only casual or if it appears in other ages, and then may be related to some kind of regulation of AngII (and then to an effect of the inhibition produced by captopril on the production of this peptide) on vasopressin release from the CRF terminals.

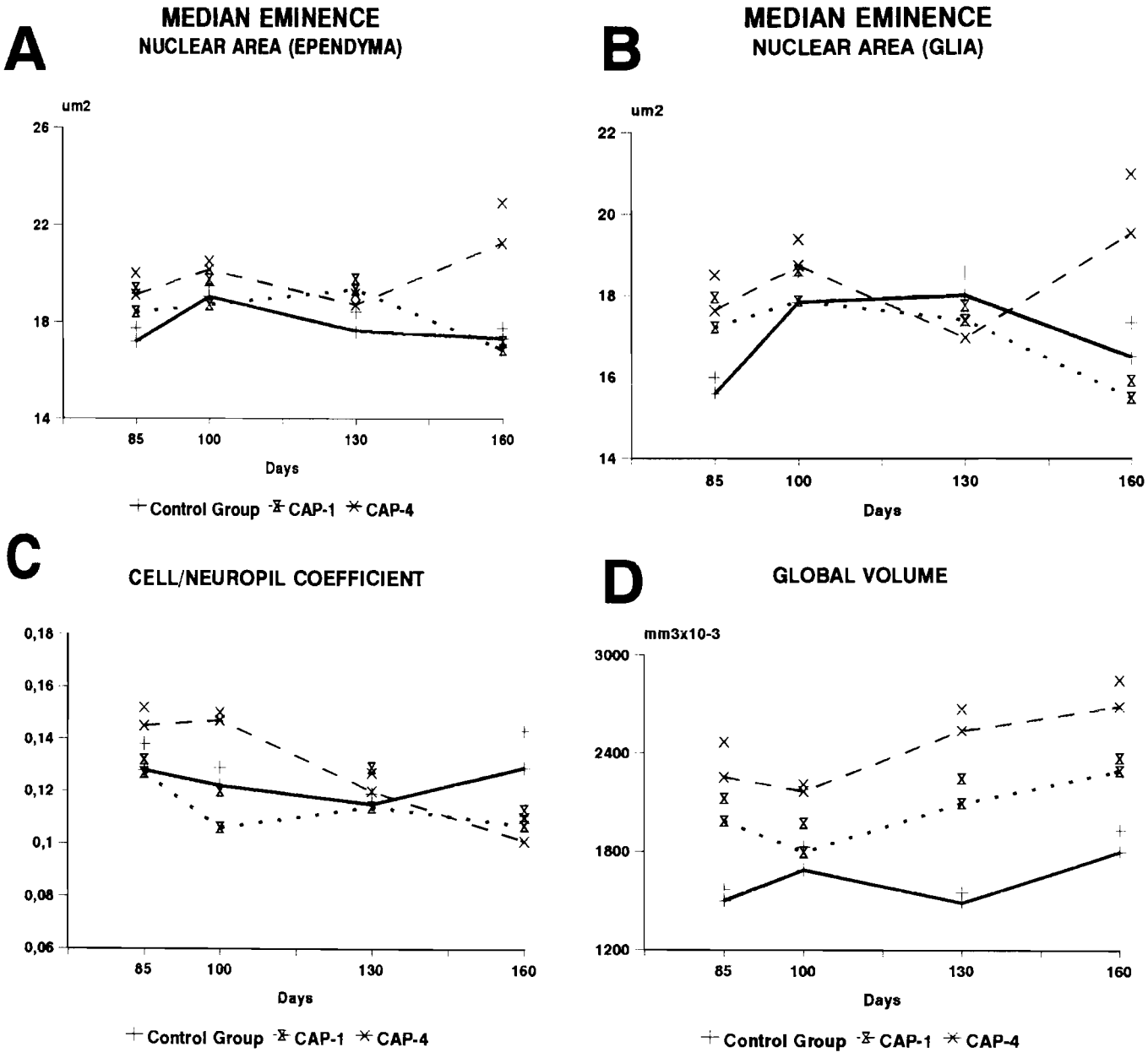

Fig. 2. Values from the 85th to the 160th postnatal days of different morphometric parameters of the median eminence (ME) of the male mouse in control and experimental animals treated with oral captopril at two different doses: 1 mg/kg bw (CAP-1); and 4mg/kg bw (CAP-4). **A.** Nuclear area of the ME ependymocytes. **B.** Nuclear area of the ME glial cells. **C.** Coefficient cell/neuropil in the ME. **D.** Global volume of the ME.

The present study shows that the ME responds to chronic captopril administration, with an increase in its global volume and a late alteration of the nuclear area of its cells and the ME-CNC. The increase in global volume could be due in part to an enlargement of the neuropil compartment, which in the ME includes blood vessels, axonal fibers and tanycytic processes, that could be explained partly by the increase in the vasopressin content in the axonal fibers (detected in the immunohistochemical study) and also by an increase in the volume of the tanycytic processes from the ependymal cells (as the area of these cells also increases in the last group studied of the CAP-4 animals, indicating a higher functional activity of the cell (Edstrom and Eichner, 1960)). This increased activity of the ME ependymocytes could be related to an increment of their trophic and transport functions into the ME (Fuxe et al., 1986). In a previous work we also described an increase of the global volume of the subfornical organ (SFO) and a late decrease of the CNC in this organ, that is known to send angiotensinergic projections to magnocellular neurons of the PVN that produce vasopressin. The similar response to captopril treatment of the ME and the SFO (Castañeyra-Perdomo et al., 1993) could be related in some way to the axonal connections that exist between both through the PVN (Ferguson, 1988).

Although some of the effects of chronic captopril treatment may be rather unspecific, we think that the significant effects on the ME are specifically directed on a circumventricular organ closely linked to vasopressin and angiotensin II.

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References

Antoni F. (1986). Hypothalamic control of adrenocorticotropin secretion: advances since the discovery of 41-residue corticotropin-releasing factor. Endocr. Rev. 7, 351-378.

Castañeyra-Perdomo A., Meyer G., Carmona-Calero E., Pérez-González H., Pérez-Delgado M.M., Bañuelos-Pineda J. and Ferres-Torres R. (1993). The effects of chronic administration of captopril on the mouse subfornical organ and area postrema. Exp. Neurol. 120, 145-148.

De Goeij D.C.E., Dijkstra H. and Tilders F.J.H. (1992). Chronic psychosocial stress enhances vasopressin but no corticotropin releasing factor in the external zone of the median eminence of male rats: relationship to subordinate status. Endocrinology 131, 247-253.

Edstrom J.E. and Eichner D. (1960). Qualitative und quantitative Ribonukleinäureuntersuchungen an den Ganglienzellen der NN supraopticus und paraventricularis der Ratte unter normalen und experimentellen Bedingungen (Kochsalzbelastung). Anat. Anz. 108, 312-319.

Ferguson A.V. (1988). Systemic angiotensin acts at the subfornical organ to control the activity of paraventricular nucleus neurons with identified projections to the median eminence. Neuroendocrinology 47, 489-497.

Fuxe K., Andersson K., Härfstrand A., Agnati L.F., Eneroth P., Janson A.M., Vale W., Thorne M. and Godstein M. (1986). Medianosomes as integrative units in the external layer of the median eminence. Studies on GRF/catecholamine and somatostatine/catecholamine interactions in the hypothalamus of the male rat. Neurochem. Int. 9, 155-170.

Holmes M.C., Antoni F.A., Aguilera G. and Catt K.J. (1986). Magnocellular axons in passage through the median eminence release vasopressin. Nature 319, 326-329.

Klüver H. and Barrera E. (1953). A method for the combined staining of cells and fibres in the nervous system. J. Neuropathol. Neurol. 12, 400-403.

Palkovits M. (1987). Summary of structural and functional aspects of the circumventricular organs. In: Circumventricular organs and body fluids. Gross P.M. (ed). Vol. II. CRC Press. Boca Raton, Florida. pp 209-218.

Schiebler T.H., Leranth C., Zaborszky L., Bitsch P. and Rützel H. (1978). On the glia of the median eminence. In: Brain-endocrine interaction III. Neural hormones and reproduction. Scott D.E., Kozlowski G.P. and Weindl A. (eds). Karger. Basel. pp 46-56.

Schölkens B.A., Jung W., Rascher W., Schömig W. and Ganten D. (1980). Brain angiotensin II stimulates release of pituitary hormones, plasma catecholamines and increases blood pressure in dogs. Clin. Sci. 59, 53s-56s.

Severs W.B. and Daniels-Severs A.E. (1973). Effects of angiotensin on the central nervous system. Pharmacol. Res. 25, 415-449.

Trembleau A., Morales M. and Bloom F.E. (1994). Aggregation of vasopressin mRNA in a subset of axonal swellings of the median eminence and posterior pituitary: light and electron microscopic evidence. J. Neurosci. 14, 39-53.

Tsutsumi K. and Saavedra J.M. (1991). Angiotensin-II receptor subtypes in median eminence and basal forebrain areas involved in regulation of pituitary function. Endocrinology 129, 3001-3008.

Whitnall M.H., Smyth D. and Gainer H. (1987). Vasopressin coexists in half of the corticotropin-releasing factor axons present in the external zone of the median eminence in normal rats. Neuroendocrinology 45, 420-424.

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