THE SPECIFIC LOCALISATION OF VASOPRESSIN AND OXYTOCIN PATHWAYS IN THE RAT CENTRAL NERVOUS SYSTEM.

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SUMMARY

By means of the unlabeled antibody enzyme method and purification of the first antiserum, vasopressin and oxytocin pathways were specifically demonstrated in the rat brain. From the suprachiasmatic nucleus only vasopressin containing fibres run towards the limbic system where they terminate with punctate pericellular structures. From the paraventricular nucleus and probably also from the supraoptic nucleus vasopressin and oxytocin fibres reach several brain regions ranging from the septum to the substantia gelatinosa of the spinal cord. These findings are discussed in relation with brain development, monoamine metabolism, avoidance behavior and water balance.

INTRODUCTION

In the last decades data that hypophysal hormones effect brain function are accumulating. The neurohypophysal hormones vasopressin and oxytocin were shown to affect avoidance behavior in rats. When applied centrally, these hormones affect monoamine metabolism in certain brain regions. In addition there are data suggesting that vasopressin is involved in brain development.

In view of these results the specific localization of vasopressin and oxytocin in the brain is of particular importance. Since E. Scharrer demonstrated by means of the Gomori-staining the existence of an exohypothalamic tract from the paraventricular nucleus (PVN) in the garter snake, evidence has been gathered on the existence of such exohypothalamic tracts in a variety of species. In the present study the specific localization of vasopressin and oxytocin pathways is described from the magnocellular nuclei to the limbic system and spinal cord. Moreover it is demonstrated that the vasopressin producing suprachiasmatic nucleus SCN has extensive projections towards the lateral septum and lateral habenular nucleus.

MATERIALS AND METHODS

Six male Wistar rats and three male rats of the Brattleboro strain homo-
zygous for diabetus insipidus were perfused and the brains were fixed in buffered 2.5% glutaraldehyde-1% paraformaldehyde 24 h at 4°C and subsequently embedded in paraffin, 6 µm sections were made in the sagittal or transversal plane. Staining of the sections was accomplished after incubation for 60 minutes with the following antisera: (1) purified antivasopressin or antioxytocin plasma, (2) swine anti-rabbit serum, (3) peroxidase-antiperoxidase complex. Prior to each incubation the sections were incubated for 10 minutes with a 10% solution of swine serum, and after each incubation with antiserum the sections were washed in buffer. Thereupon the sections were stained for 25 minutes with diaminobenzidine according the procedure of Graham and Karnovsky. See for detailed information on the used controls, the staining procedure and purification of the first antibodies.

Fig. 1. Diagram illustrating fibre pathways of the SCN and possible sites of termination in sagittal plane, reproduced from the stereotaxic atlas of König and Klippel. CA anterior commissure; CFV vertral commissure of fornix; CO optic chiasm; F fornix; Hi hippocampus; PVS paraventricular nucleus; S subiculum; SCN suprachiasmatic nucleus; TCC truncus corpus callosi; TD tractus diagonalis; lh lateral habenular nucleus; PVS periventricular nucleus; sl lateral septum; tam anterior thalamic nucleus.
RESULTS

From the SCN thin vasopressin containing fibres fan out in several directions (fig.1), rostrally to the organum vasculosum laminae terminalis (fig.2), rostro dorsally the lateral septum and dorsocaudally via the nucleus periventricularis hypothalami to the lateral habenular nucleus. The latter fibre pathway is partly crossing the PVN. Whether fibre terminations exist in this nucleus could not be established. In the lateral septum and the lateral habenular nucleus these SCN fibres appear to end with punctate pericellular structures. The largest part of fibres in the lateral septum is derived from the SCN and is thus vasopressinergic. In addition all fibres in the lateral habenular nucleus seem to be derived from the SCN as appeared e.g. from the finding that in the Brattleboro rat no oxytocin fibres were found in this nucleus.

Because of the intermingling of the fibres from the supraoptic nucleus (SON) with those from the PVN, it could not be established to what extend or even if the SON contributes to the exohypothalamic fibres. From the magnocellular bipolar and multipolar neurosecretory cells of the PVN, vasopressin and oxytocin fibres could be traced towards the following brain regions: the septum, stria medullaris, via the stria terminalis towards the amygdala, along the fornix and via the subiculum into the ventral hippocampus and entorhinal cortex. Caudally fibres project via the substantia nigra and substantia grisea towards the medulla oblongata and spinal cord. In the spinal cord, where the fibres run into the substantia gelatinosa and around the central canal more oxytocin than vasopressin fibres are visualized. In the amygdala these fibres appear to terminate with punctate pericellular structures while in the ventral hippocampus these fibres end with fibre branching on the apical dendritic tree of the pyramidal layer.

DISCUSSION

The wide spread distribution of vasopressin and oxytocin fibres in the rat brain suggests that these pepitderic fibres might be involved in a variety of processes. The observed bi- and multipolarity of the cells of the PVN suggest that the same cell projects to the neurohypophysis as well as to extrahypothalamic brain regions. An important question in this respect is whether these fibre projections are axon-like or dendritic, or whether such a distinction cannot be made by these neurosecretory cells. Another point of discussion is the question whether such cells would release the neurohypophysial hormones simultaneously in all directions. An argument for this is the simultaneous
elevation of vasopressin and oxytocin levels in the CSF and blood following vagal stimulation \textsuperscript{13} or hemorrhage \textsuperscript{14}. On the other hand the occurrence of the high density of oxytocin fibres in the substantia gelatinosa, which serves as a gate for peripheral information to central brain regions \textsuperscript{15} might indicate that these neurosecretory fibres are not primarily secretory but receptive for the signal for the milk ejection that would then be a monosynaptic reflex towards the magnocellular nuclei.

Up till now three different kinds of nerve endings could be demonstrated, 1. punctate pericellular endings in the lateral septum, lateral habenular nucleus and amygdala, 2. fibre branching in the ventral hippocampus and 3. termination of fibres in the neurohaemal organs, e.g. the neurohypophysis, eminencia

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*Fig.2. Sagittal section of Wistar rat brain, vasopressin fibre termination in organum vasculosum laminae terminalis after incubation with purified vasopressin antiserum 1:200. 3V third ventricle. 375 x*
mediana and organum vasculosum laminae terminalis. (Fig. 2) The various forms of fibre termination is another argument for the involvement of vasopressin/oxytocin fibres in several different processes in the central nervous system. One of such processes might be the regulation of water balance. Lesions in the anterior wall of the third ventricle including the lamina terminalis induce a disturbed water balance and inappropriate vasopressin release in the goat \(^{16}\) and the rat \(^{17, 18}\). In these very regions vasopressin fibres that are mainly derived from the SCN either terminate (lamina terminalis) or pass through towards the lateral habenular nucleus and the lateral septum suggesting that the SCN might be involved in the regulation of water balance. This is the more plausible since the septum, which is rich in vasopressin containing SCN fibres, is also involved in the regulation of water balance \(^{19}\), while latero-caudal and dorsal isolation of the SCN abolishes drinking rhythms \(^{20}\).

The suprachiasmatic nucleus is one of the most important contributors of vasopressin fibres in the limbic system, which seems to be of considerable importance in avoidance behavior that can be influenced by vasopressin \(^{21}\). The punctate pericellular structures in these brain regions suggest neurohypophysial hormone release. These neuropeptides could function here as neurotransmitters or modulators influencing membrane properties \(^{22}\) possibly via monoamine metabolism \(^{23}\).

An indication for such an effect on behavior is that in the lateral septum where mostly vasopressin fibre terminations occur injection of vasopressin does and oxytocin does not affect avoidance behavior. While in the hippocampus where fibre terminations of both peptides occur, oxytocin influences avoidance behavior opposite to vasopressin, suggesting the presence of receptors for both peptides.

REFERENCES


24. Kovács, G.L., pers. comm.