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The Dopaminergic Innervation of the Supraoptic and Paraventricular Nucleus. A Light and Electron Microscopical Study

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An antiserum that has been raised against glutaraldehyde-conjugated dopamine was used to demonstrate specifically dopamine in the rat hypothalamus. This dopamine antiserum permitted an optimal fixation with glutaraldehyde and therefore enabled the simultaneous light and electron microscopic immunocytochemical localization of dopamine. It was demonstrated that the paraventricular and supraoptic nuclei of the hypothalamus were innervated by thin dopaminergic fibers, in contrast to the suprachiasmatic nucleus, which hardly received any dopaminergic input. Ultrastructural observations revealed that the dopamine fibers terminated synaptically on the magnocellular neurons and their processes. It is concluded that the present results may explain the effect of centrally injected dopamine on vasopressin and oxytocin release. In the dopamine-containing terminals the reaction product was frequently observed in 90 nm dense core vesicles and around clear vesicles.

INTRODUCTION

The release of the neurohypophyseal hormones vasopressin (VP) and oxytocin (OX) from the neural lobe is the result of information reaching the magnocellular neurons of the paraventricular (PVN) and supraoptic (SON) nuclei, and their processes. Up to now several anatomical studies have furnished evidence for numerous different afferents to the SON and even more to the PVN^{10,20,26}. It became clear that a dense catecholaminergic innervation exists on PVN and SON neurons^{2,3,19,22}. Although the noradrenergic (NA) nature of (at least part of) these fibers has been established, it remained unclear whether also a dopaminergic (DA) input existed on these hypothalamic nuclei. Since then a number of physiological studies have appeared that suggest, apart from a NA influence on VP and OX release, a role for DA in the regulation of the release of neurohypophyseal hormones. For example, intraventricular injection of DA induced or increased the release of VP and OX under various experimental conditions^{4,6,14,27}. Moreover, direct injection of DA in the

SON induced an antidiuretic response in hydrated rats²⁷.

In addition, DA was measured biochemically in the PVN and SON^{16,30} and although it could not be excluded that the amount detected was due to DA as a precursor in NA neurons, the fact that suckling induced an increase in the DA and not in the NA turnover in the hypothalamus³¹ suggested that the PVN and SON are innervated also by DA fibers. Thus far, however, only preliminary anatomical evidence for the innervation of the SON and PVN with DA fibers has been presented^{5,13}. In the present paper a recently developed specific antibody for DA⁸ was used to clarify the possible DA innervation of the PVN and SON. In addition, immunoelectron microscopy was performed to identify the DA terminals and their postsynaptic structures.

MATERIALS AND METHODS

Eight male Wistar rats (180–200 g) (CPB-TNO Zeist) were anaesthetized with Nembutal (0.1 ml/100 g b.wt. i.p.), perfused intracardially with 1%

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$\text{Na}_2\text{S}_2\text{O}_5$, followed by 5% glutaraldehyde–0.05 M cacodylate–1% $\text{Na}_2\text{S}_2\text{O}_5$ buffer, pH 7.5. A 3 mm thick transversal brain section including the hypothalamus was isolated by hand and subsequently immersed in the same fixative for 45 min at 4 °C. Fifty micrometer sections were made on a vibratome in 0.05 M Tris-HCl–1% $\text{Na}_2\text{S}_2\text{O}_5$, pH 7.2. Sections were handled

Fig. 1. Dopamine staining in transversal sections of the rat hypothalamus. A: dopaminergic innervation of the paraventricular nucleus (PVN). Note the presence of small dopaminergic cell bodies (arrows). E, ependyma of the third ventricle. B: dopaminergic innervation of the supraoptic nucleus (SON). Note the thin dopaminergic fibers (arrows). ON, optic nerve. Bar = 50 μm .

with a paintbrush and placed in vials. The immunocytochemical procedure consisted of the following incubations: (a) dopamine antiserum diluted 1:3000 in Tris buffer containing $\text{Na}_2\text{S}_2\text{O}_5$ (and for light microscopy 0.5% Triton X-100 additionally) for 18 h at 4 °C; (b) washing and subsequent incubation in 0.9% NaCl–0.05 M Tris buffer, pH 7.6; (c) goat anti-rabbit 1:50, 60 min; (d) washing; (e) peroxidase–antiperoxidase 1:1000, 60 min; (f) washing; (g) 0.5 mg/ml 3,3-diaminobenzidine (DAB) (Sigma) and 0.01% H_2O_2 (Merck) in Tris–saline, 5–10 min, and in the case where the sections were used for electron microscopy; (h) 2% OsO_4 in 0.1 M cacodylate buffer 30 min; (i) washing; (j) dehydration in a graded ethanol series, and (k) flat embedding in Epon 812. For electron microscopy, the rostral, middle and caudal parts of the SON were studied and ultrathin sections of these regions were examined in a Philips EM400 electron microscope. Specificity controls for the anti-DA serum included, apart from the already published results with the radioimmunological assay⁸, incubations with DA- or NA-covered Sepharose beads. For this purpose lysine was coupled to CNBr-activated Sepharose beads, such that its ϵNH_2 group remained free. Subsequently, DA or NA were coupled to these lysine beads by glutaraldehyde. The cross-reaction of the DA antiserum with NA was controlled, when evaluating the staining on tissue sections, by using anti-DA serum adsorbed with DA-lysine Sepharose beads or NA-lysine Sepharose beads.

RESULTS

When applied to brain sections in the region of the hypothalamus, NA-adsorbed serum revealed no difference in staining pattern when compared with the non-adsorbed DA antiserum. No staining was observed, e.g. in the cerebellum and occipital cortex, i.e. in brain regions where only NA fibers are present. Pre-adsorption of the antiserum with DA coupled with glutaraldehyde to lysine blocked all positive staining from the brain sections.

Using non-adsorbed or NA-adsorbed antiserum, a similar dense innervation was observed on the SON and PVN. Numerous thin fibers appeared to innervate the magnocellular neurons of PVN and SON (Fig. 1A, B). In addition, these fibers innervate also parvocellular neurons in the PVN. Although staining

appeared to be more pronounced in the PVN than in the SON, the thin varicose DA fibers were found to be present throughout these nuclei. DA fibers appeared to be present in an equal density throughout the PVN and SON, from the extreme rostral to the extreme caudal part. In addition, in the region of the PVN, small DA-containing cell bodies were demonstrated. In contrast, the suprachiasmatic nucleus does not seem to receive any substantial DA input.

Electron microscopy on the SON indeed revealed that many DA-positive terminals made synaptic con-

tacts with cell bodies (Figs. 2 and 3A, B) and their processes (Figs. 3C, D and 4). Often these cell bodies appeared to contain an extensive rough endoplasmic reticulum and Golgi system and large (± 140 nm) dense core vesicles. Terminals were found to be in synaptic contact with cell bodies and their processes in all regions of the SON in the rostral, middle and caudal parts, and in the dorsal and ventral parts. In some cases it was found that one DA terminal was in synaptic contact with two cell bodies (Fig. 3B). Frequently neurons or neuronal processes on which DA

Fig. 2. Electron micrograph demonstrating dopaminergic terminals forming synapses (arrows) with a magnocellular neuron in the supraoptic nucleus. The double arrows point to large-sized dense core vesicles. N, nucleus; g, Golgi system. Bar = 500 nm.

fibers terminated synaptically were found to contain dense core vesicles of approximately 140 nm (Figs. 3 and 4). Sometimes in these processes unevenly distributed microtubules could also be demonstrated.

The DA-positive structures were small axonal processes, occasionally containing ± 90 nm dense core vesicles and many clear vesicles (Fig. 3C, D). The reaction product was present in the dense core

Fig. 3. A and B: dopaminergic terminals forming a synaptic junction with magnocellular neurons (double arrows). The single arrows point to large-sized dense core vesicles. B shows a terminal that forms simultaneously synaptic junctions with two magnocellular neurons. g, Golgi system. Bars = 500 nm. C and D: dopaminergic terminals forming axo-dendritic junctions. Note the presence of staining on small-sized dense core vesicles (arrows) and the staining around the clear vesicles and other membrane structures. Bars = 250 nm.

Fig. 4. Dopaminergic terminal forming a synaptic junction (double arrow) with a presumptive dendritic profile of a magnocellular neuron filled with large-sized dense core vesicles. Microtubules are indicated by single arrows. Bar = 250 nm.

vesicles and found scattered around membranous structures in the axonal terminal, such as clear vesicles and mitochondria.

DISCUSSION

Thus far the dense NA innervation of PVN and SON has prevented the specific localization of DA in these nuclei. The specificity of the antiserum for DA has been demonstrated previously⁸. The specificity of this antiserum in immunocytochemistry was dem-

onstrated by the preadsorption tests using lysine-Sepharose beads to which dopamine was coupled with the same fixation procedure as had been used for the tissue fixation. The solid phase absorption tests with conjugated DA and NA demonstrated that the present staining indeed elicited DA and not NA.

Earlier literature already described the biochemical demonstration of DA in punched-out PVN and SON^{16,31}. Up till now, however, it has remained unclear whether the measured DA was present in cell bodies (PVN) or as precursor in NA fibers. The pres-

ent study allows the conclusion that indeed the PVN and SON are innervated by DA fibers. In contrast, the vasopressin-containing suprachiasmatic nucleus appeared to receive hardly any DA innervation, which is at variance with the biochemical finding of Palkovits et al.¹⁶. Since the latter method employs punched brain regions, this discrepancy can be explained by the DA innervation of the areas close to the SCN.

The principal merit of the DA antiserum presently employed is that it allows a simple immunocytochemical localization of DA in glutaraldehyde-fixed tissue, which provides material with optimal ultrastructural features. In addition, the morphology was not much hampered by the immunocytochemical incubations, since detergents could be omitted from the incubation and washing buffers. Thus it appeared that DA-positive reaction was present in large, ca. 90 nm dense core vesicles, while the dense reaction product was also found around the smaller well-preserved clear vesicle-like structures, and not within them. Such an absence of staining suggests that DA is indeed not present in these vesicles, which raises the question whether diffusion out of the dense core vesicles might account for the reaction product around membranes. Due to artefacts that at the present time cannot be avoided during the fixation and incubation procedure, immunocytochemistry does not seem to be the adequate technique for determining whether DA is present diffusely in the cytoplasm.

Using immunoelectron microscopy without any experimental manipulation of the animal, it was found that DA-containing fibers terminated mostly on cell bodies, while synaptic contacts were also seen infrequently on dendrites or axons. The cell bodies appeared to contain large-sized dense core vesicles with a 140 nm diameter, which — certainly in the SON or PVN — is indicative of the presence of either VP or OXT^{11,29}. Vasopressin- and oxytocin-containing cell bodies are unevenly distributed over the SON and PVN. In general, the rostral part of the rat PVN and SON contains more oxytocin cell bodies, whereas the central portion of the PVN and the ventral part of the SON contain more VP cell bodies^{23,28}. It has been demonstrated that the NA fibers in the PVN and SON are preferentially located in the vasopressinergic parts of these nuclei^{19,22,24}. In contrast, the fact that DA fibers are present throughout the

PVN and SON in an equal distribution and that by immunoelectron microscopy DA terminals were found to make synaptic contacts with cell bodies and their processes throughout the SON, indicate that the DA innervation is present on both vasopressin and oxytocin cell bodies in the SON and PVN. When DA fibers established synaptic contacts with neuronal processes, these structures frequently appeared to contain also 140 nm, large-sized dense core vesicles, which is also indicative of the content of neurohypophyseal hormones. Whether these processes are axons or dendrites is extremely difficult to ascertain in the SON and PVN, since both axons and dendrites contain large-sized dense core vesicles^{1,21}. However, the presence of microtubules unevenly distributed in some of these processes indicates that at least part of these processes are dendrites and consequently that DA fibers terminate also on dendrites of magnocellular neurons. It is conceivable that by this DA innervation on VP and OXT neurons the DA system may be able to exert a direct influence on the release of VP and OXT. Since many reports indicate a stimulatory effect of centrally injected DA on VP and OXT release^{4,6,14,15}, also after injection directly into the SON²⁷, the DA input on the magnocellular PVN and SON most probably will directly facilitate the release of these neurohypophyseal hormones. It has been demonstrated that in thirsting or lactating animals, synapses terminated more frequently simultaneously on two magnocellular neurons. The fact that in the present results DA fibers were also found to terminate simultaneously on two magnocellular neurons, indeed suggests that this dopaminergic innervation of the PVN and SON provides a facilitatory input to VP and OXT release. Since the neural lobe has also been demonstrated to be innervated by DA-containing fibers^{2,3}, it can be concluded that DA fibers are able to control the release of VP and OXT, both at the level of the cell body and at the terminal. At the moment it is unclear from what cell group the DA fibers that innervate the PVN and SON are derived. It seems unlikely that the tubero-infundibular neurons that project to the neural lobe³ and probably fulfill an inhibitory role on VP and OXT release^{12,17,18,20} will project also to the SON and PVN and excite the neurons there. Retrograde transport studies demonstrated, for example, labelled cell bodies in the VTA following injection into the SON¹⁰. From these studies it

may be suggested that the VTA is the source of DA in PVN and SON. Thus, it would be more likely that at least two separate DA systems are present, which could control VP and OXT release, one at the level of the neural lobe, the tubero-infundibular system, and one at the level of PVN and SON. Further retrograde labelling or lesion studies in combination with DA localization may clarify this point.

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