Distribution of Somatostatin Immunoreactivity in Sheep Hypothalamus: A Comparison with That of the Rat*

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Summary. The distribution of somatostatin immunoreactivity was determined throughout the hypothalamus of the sheep and comparisons were made with the known distribution of somatostatin immunoreactivity in the rat. Immunopositive perikarya were present in the sheep periventricular region from as far rostral as the supraoptic recess of the third ventricle to the posterior optic chiasm. In the basal hypothalamus, a thick shell of immunopositive neurons surrounded the ventromedial nucleus (VMH), and there were also neurons in the caudal arcuate nucleus. Somatostatin immunoreactive fibres were concentrated in the dorsal VMH and arcuate nucleus as well as in the median eminence. The distribution in sheep was similar to that in rats, but immunoreactive neurons around sheep VMH were distinctive, a characteristic that might relate to differences in growth hormone physiology in this species.

The central nervous system distribution of somatostatin-containing neurons has been described in several mammalian species, including the sheep (PAPADOPOULOS et al., 1986; POLKOWSKA et al., 1987) and has been described in detail in the rat (KRISCH, 1978; BENNETT-CLARKE et al., 1980; KAWANO et al., 1982; JOHANSSON et al., 1984). Comparison of findings in the rat, sheep, hedgehog (PAPADOPOULOS et al., 1986), dog (HOFFMAN and HAYES, 1979) and garden doormouse (RICHOUX and DUBOIS, 1980) reveal a common pattern of organization, with numerous somatostatin immunopositive perikarya in the periventricular preoptic and anterior hypothalamic areas and a few perikarya in the arcuate nucleus and around the ventromedial nucleus (VMH). In these species, there is also an easily recognizable somatostatin-immunopositive fibre innervation of the arcuate nucleus and VMH. Periventricular somatostatin neurons have been demonstrated to be the source of the median eminence innervation by somatostatin fibres (KAWANO et al., 1982; WILLOUGHBY et al., 1989) while it is likely that the innervation of the arcuate nucleus and VMH arises from local neurons (KAWANO et al., 1982).

The use of sheep for neuroendocrine studies has increased as it permits collection of both pituitary portal and venous blood for hypothalamic regulatory factor and pituitary hormonal level determination in unanaesthetised animals (FROHMAN et al., 1990). In advance of the use of the sheep for intra-hypothalamic injection studies, we herein aim to clarify more precisely the distribution of somatostatin neurons in this animal, with an emphasis on the impressive shell of immunopositive neurons around the VMH, consistent with previous findings (POLKOWSKA et al., 1987).

MATERIALS AND METHODS

Sheep somatostatin immunohistochemistry

Sheep were injected intravenously with 25,000 IU heparin (Heparin sodium, CSL, Parkville, Australia) and then killed by intravenous injection of pentobarbitrate sodium (1 ml/2 kg, Lethabar, Virbac, Parkhurst, Australia). Their heads were removed and the brains were perfused via the carotid arteries with normal saline until the blood was washed out, and then with approximately 2 l of a mixture of 0.2% picric acid and 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4) solution. The brain was removed for post-fixation for 2-4 days in the same fixative. The fixed brains were then placed base down on a flat surface and, based on preliminary findings, coronal slices were made perpendicular to the brain-base at a level through...
the mid-optic chiasm and through the mammillary region, thereby producing a coronal slice approximately 1 cm thick that contained all hypothalamic regions in which somatostatin immunoreactive perikarya were located. A hypothalamic block was then cut from the slice and placed in 30% sucrose in water for 2-9 days in preparation for sectioning on a freezing microtome. Sections of 50 μm were cut and washed successively in 50% alcohol three times and, in some animals, preincubated in 0.5% H2O2 to minimize the activity of endogenous peroxidases. Sections were next incubated for 1 h in 20% normal horse serum in Trisma buffer and then overnight in rabbit anti-somatostatin antibody (1: 5,000), previously used for immunohistochemistry (WILLOUGHBY et al. (1989) and prepared from thyroglobulin-conjugated-somatostatin 14. After washing, sections were incubated with biotinylated goat anti-rabbit secondary antibody (Vector, Burlingham, USA) which was then revealed using a streptavidin-horseradish peroxidase, glucose oxidase, nickel enhanced, diaminobenzidine reaction. Pre-incubation of the somatostatin antibody with synthetic somatostatin (Peninsula, California, USA), completely abolished neuronal labelling in sheep and rat.

Rat somatostatin immunohistochemistry
Rats were anaesthetised with intraperitoneal pentobarbitrate 60 mg/kg and perfused through the heart with 1 l of the same fixative as used for the sheep, and thereafter tissue was processed in the same way as for the above sheep.

Sections were mounted and viewed at 20-200× magnification on an Olympus BH-2 microscope, and hypothalamic regions were mapped using the Magellan morphometry programme (P. Halasz, University of New South Wales, Sydney, Australia) on a computer interfaced to the microscope via a drawing tube. The positions of immunopositive perikarya were plotted on representative sections taken at different levels through the hypothalamus in both sheep and rats. Fibre rich areas were photographed for presentation as photomicrographs.

RESULTS

In the sheep, dark immunoreactivity for somatostatin was clearly identifiable in fibres in the median eminence, and there were immunopositive perikarya in the periventricular region. The rostral most extent of the immunoreactive cells in the periventricular medial preoptic area was basally just caudal to the vascular organ of the lamina terminalis. The caudal most extent of the immunoreactive cells in the periventricular region was anterior to the retrochiasmatic area, a similar but more anteriorly located distribution than is observed in the rat (KRISCH, 1978; BENNETT-CLARKE et al., 1980; KAWANO et al., 1982; JOHANSSON et al., 1984).

Computer assisted mapping of immunoreactive perikarya in sheep conveniently displayed the distribution of perikarya throughout the hypothalamus as shown in Figure 1. Intensely immunopositive perikarya were found in the periventricular regions. Of special interest was a distinct shell of lightly labelled perikarya around the VMH that extended from its rostral to caudal pole, while the VMH centrally contained no immunoreactive neurons (Fig. 1). Cells were most numerous laterally. Lightly labelled perikarya were also present in the posterior arcuate nucleus as far caudally as the mammillary recess of the third ventricle (Fig. 1).

Distinct concentrations of somatostatin-immunoreactive fibres were also evident in the dorso-lateral part of the VMH and in the arcuate nucleus (Fig. 2), all of which bear close resemblance to the fibre distribution in the rat (KRISCH, 1978; BENNETT-CLARKE et al., 1980; KAWANO et al., 1982; JOHANSSON et al., 1984). In addition, less dense concentrations of immunoreactive fibres were distributed through all the hypothalamic regions examined. Fibres were also observed in the sheep in the vascular organ of the lamina terminalis where, as in the rat, no immunoreactive neurons were present (Fig. 2). Unlike in the rat, an increased density of immunoreactive fibres was not observed in the area of the suprachiasmatic nucleus.

Because somatostatin immunoreactive neurons were observed in the shell region around the VMH of the sheep, careful attention was directed to the corresponding area in the rat. Lightly immunoreactive neurons could be identified in the rat, although they were rather more diffusely distributed (Fig. 3). Overall, the ventromedial hypothalamic shell region provides the most distinctive difference between the distributions of somatostatin perikarya in the rat and sheep. Other, though less impressive differences, are the disappearance of the periventricular somatostatin neuronal population caudal to the retrochiasmatic region—whereas periventricular neurons in the rat can be identified as far caudally as the VMH (Figs. 1, 3), and the close approach of basal hypothalamic immunoreactive perikarya to the third ventricular wall.

DISCUSSION

In agreement with observations in other species and earlier observations in the sheep (PAPADOPOULOS et
Fig. 1. Low power maps of the distribution of somatostatin perikarya in the sheep and rat, plotted using the Magellan morphometry program. Numerous somatostatin immunoreactive cells are present in the periventricular preoptic region (level A and B) in the sheep. A distinctive shell of immunoreactive perikarya is present around the ventromedial nucleus (D), its rostral and caudal limits being seen in the adjacent sections (C, E). A less robust, but similar appearance is evident in the rat. Relative to the retrochiasmatic region, somatostatin immunoreactive perikarya are present more posteriorly in the rat (C). Bar = 1 mm. OC optic chiasm, OT optic tract, ME median eminence, VMH ventromedial hypothalamic nucleus.
al., 1986), we have demonstrated that there is somatostatin immunoreactivity in hypothalamic perikarya in the anterior periventricular region as well as in the arcuate/VMH regions in the sheep. Although we did not recognize somatostatin-immunoreactive perikarya in the suprachiasmatic nucleus as previously described (PAPADOPOULOS et al., 1986; TILLET et al., 1989), we did not use colchicine in our study. Rather noticeable in the sheep is a shell-like distribution of immunoreactive perikarya around the VMH, although the cells are lightly labelled. This distribution is consistent with the findings of PAPADOPOULOS and associates (1986), and is demonstrated clearly in our computer-mapped data. A few neurons can be seen around the VMH in the rat, and some neurons in this area usually appear in anatomical studies of other species, viz the garden doormouse (RICHOUX and DUBOIS, 1980) and dog (HOFFMAN and HAYES, 1979). Given our use of the same methods in the sheep and rat and the complete immunoneutralisation of immunoreactivity in the VMH shell area, it seems likely in the sheep that the shell of somatostatin perikarya around the VMH is a significant species characteristic.

While there are species differences in metabolism and growth hormone regulation between sheep and other mammals that might suggest hypotheses about function of the VMH shell neurons, the role of this region in growth hormone regulation is undefined. The distribution of somatostatin immunoreactive neurons around the VMH may be independent of any growth hormone function; however, the VMH itself has been implicated in body weight homeostasis (PLUM and VAN UITERT, 1978) and is likely to be indirectly involved in somatotrophe regulation. The clear correspondence in the distribution of somatostatin neurons in the different species suggests similar connectivity. Thus, it would be expected that sheep periventricular somatostatin neurons innervate the median eminence, periventricular neurons innervate the vascular organ of the lamina terminalis (CAMACHO and PHILLIPS, 1981)

Fig. 2. Fibres immunoreactive for somatostatin in the median eminence (A), vascular organ of the lamina terminalis (B), ventromedial hypothalamic nucleus/arcuate nucleus (C) and posterior arcuate nucleus (D) regions in the sheep. Sections are 50 μm thick, so that not all structures are on the same focal plane. v Ventromedial hypothalamic nucleus, a arcuate nucleus. A, C, D: ×15, B: ×150
Fig. 3. Lightly immunoreactive perikarya around the ventromedial nucleus (v) in the sheep (A) and rat (B). Somatostatin immunoreactive fibres can be seen within the ventromedial nucleus. Sections are 50 μm thick, so that not all structures are at the same focal plane. Larger neurons are evident in the sheep. A: ×145, B: ×290
and the arcuate and VMH shell neurons innervate local nuclei, proposals that are suitable to testing. Furthermore, the clear anatomical separation of periventricular somatostatin neurons from the basal hypothalamus and median eminence makes this population of neurons easily manipulated by intrahypothalamic microinjections.

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REFERENCES


