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A population of very small striatal neurons in the cat displays vasoactive intestinal polypeptide immunoreactivity

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Cells displaying vasoactive intestinal polypeptide (VIP) immunoreactivity were demonstrated in the feline striatum using a monoclonal antibody raised against natural porcine VIP. The VIP-immunoreactive neurons in the cat striatum were very small, (8 μm diameter) bipolar and multipolar cells. The VIP-positive neurons were more numerous than the cholinergic neurons but less common than the somatostatin-immunoreactive cells in the cat caudate-putamen. The VIP-immunoreactive cells were localized predominantly in the striatal matrix and tended to avoid enkephalin-immunoreactive patches. Thus VIP-immunoreactive cells comprise another neurochemically defined neuronal population which appears to observe striosomal organization.

The striatum exhibits marked heterogeneity in the organization of its neurotransmitter systems. Certain markers including opiate receptors, and enkephalin and substance P immunoreactivities are found in dense patches or striosomes, while acetylcholinesterase staining, and dense choline acetyltransferase and somatostatin immunoreactive neuropil mark the extrastriosomal matrix [6, 8, 9]. Although a few reports have mentioned the presence of striatal neurons displaying vasoactive intestinal polypeptide (VIP) immunoreactivity [12, 15, 17, 23], their relationship to striatal compartmentation has not been investigated. Indeed, VIP-immunoreactive neurons have only been well described in the rat [19]. In the present study the distribution of VIP-immunoreactive neurons in relation to striatal patch-matrix compartmentation was examined in the cat using a monoclonal antibody to VIP.

Two adult male cats were anesthetized with pentobarbital, heparinized and perfused through the ascending aorta with saline followed by fixative. The fixative for one of the cats was a buffered picric acid–paraformaldehyde mixture, while the other animal was fixed with 4% paraformaldehyde in 0.1 M sodium phosphate buffer [22].

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After perfusion, the brains were removed and post-fixed in the same fixatives for an additional 2 h, and then placed in cryoprotectant (25% sucrose, 10% glycerol in 0.05 M phosphate buffer) for 48 h at 4°C. Sections were then cut through the striatum at 30 μm thickness on a freezing microtome and serially placed into a series of nine wells containing Tris-buffered saline (TBS, 0.05 M, pH 7.4).

A previously characterized monoclonal antibody, IgG_1 (V31), raised against natural porcine VIP was used [18]. The sections were incubated free-floating with the antibody at a final concentration of 5 μg protein/ml at 4°C for 48 h. For comparison, series of sections were incubated with monoclonal antibodies against choline acetyltransferase [4] and somatostatin [21] to demonstrate the large aspiny cholinergic neurons and the medium-sized aspiny neurons containing somatostatin, neuropeptide Y and NADPH-diaphorase [20], respectively. An additional series of sections adjacent to those stained for VIP was stained using a monoclonal antibody to leu-enkephalin (Sera-Lab) [1] to differentiate the enkephalin-rich patches from the striatal matrix [9].

All the sections were processed for immunohistochemistry using the avidin–biotin complex method with the appropriate ABC Kits (Vector Labs) as previously described [21, 22]. Immunoperoxidase activity was demonstrated using either the standard 3,3′-diaminobenzidine procedure, or the nickel ammonium sulfate intensification protocol [21, 22]. Control sections in which the diluted peptide antisera were preabsorbed with 1 nmol/ml of the respective peptide showed no specific staining.

Many VIP-immunoreactive neurons were detected in the cat brain with this monoclonal antibody. In the forebrain a major population of VIP-positive neurons was present in the cortex. In addition, the feline striatum contained many VIP-immunoreactive neurons (Fig. 1A,B). These were small, their major diameter averaging about 8 μm, and they often appeared to have a bipolar or multipolar shape. These neurons frequently gave rise to varicose processes that extended up to 100 μm in a single section. These small VIP-positive neurons were scattered throughout the caudate nucleus, putamen and nucleus accumbens. Similar cells were also present in the claustrum.

The morphology, distribution and density of the VIP-positive striatal neurons were compared with those of the striatal cholinergic- and somatostatin-immunoreactive neurons on series of adjacent sections through the cat caudate nucleus. In representative 30-μm-thick sections through the head of the caudate, there were about twice as many neurons per section immunoreactive for somatostatin (409 cells) as there were positive for VIP (200 cells), however, there were somewhat more VIP-immunoreactive neurons than cholinergic neurons (144 cells). The VIP-positive neurons were dramatically smaller than the cholinergic and somatostatin striatal neurons and in fact resembled miniature versions of these striatal cell types (Fig. 1).

The distribution of VIP-immunoreactive neurons in the cat caudate nucleus was compared to that of the strongly enkephalin-positive patches [9] detected on an adjacent series of sections. The VIP-positive neurons were almost always found within the striatal matrix outside of these patches (Fig. 2).

The present report describes the presence of a large population of VIP-immunoreactive neurons in the cat striatum. Obata-Tsuto et al. [15] have previously men-

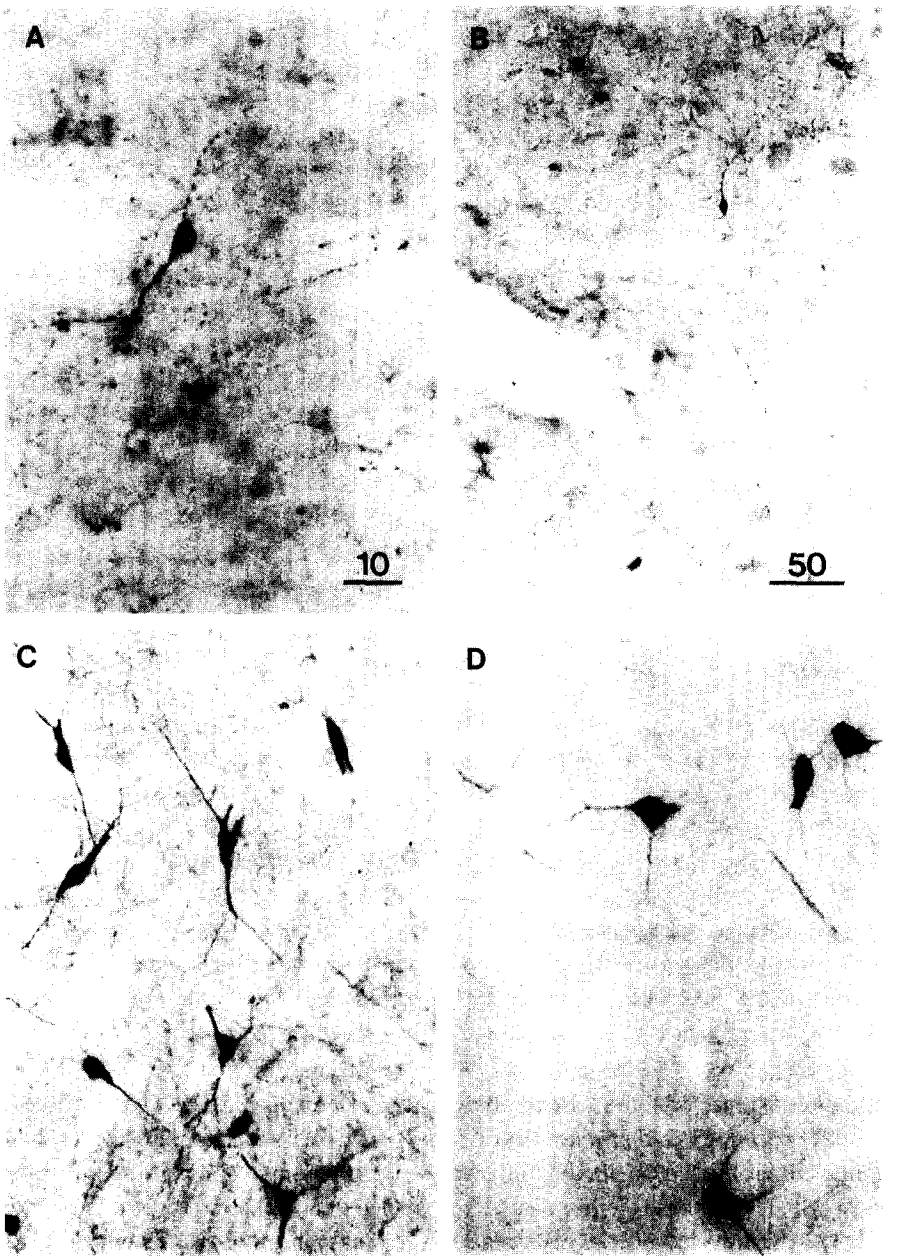

Fig. 1. The size and morphology of the vasoactive intestinal polypeptide (VIP)-immunoreactive neurons (A, B) can be compared with those of the somatostatin- (C) and choline acetyltransferase-immunoreactive (D) neurons in the cat caudate nucleus. Note that micrographs B–D were all photographed and printed at the same magnification illustrating the extremely small size of the striatal VIP-immunoreactive neurons. Bar = 10 μm for (A) and 50 μm for (B–D)

Fig. 2. Camera lucida drawing illustrating the relationship of the VIP-positive neurons (asterisks) to the intensely enkephalin-immunoreactive patches (shaded areas) present in an adjoining serial section of the cat caudate. Note that almost all of the VIP-immunoreactive neurons are found in the extrastriosomal matrix.

tioned the existence of some VIP-immunoreactive neurons in the cat caudate nucleus, but the morphology and distribution of these neurons was not described. The caudate nucleus of the cat has been analyzed in detail using Golgi staining and electron microscopy. Using these techniques, Kemp and Powell defined 6 types of striatal neurons [11]. Attempts have since been made to correlate immunohistochemically identified neurons of the striatum with the cell types identified using the Golgi method. In this scheme, the cholinergic neurons appear to correspond to the large aspiny neurons [22] and the somatostatin-immunoreactive neurons to a medium-sized aspiny cell type [9, 20, 21]. GABA, substance P, dynorphin, and Leu- and Met-enkephalin are predominantly found in medium spiny neurons, by far the most common of the striatal cell types. The VIP-immunoreactive cells identified in the present study may correspond to the small cells identified by Kemp and Powell [11] which were 5–9 μm in diameter and made up less than 1% of the neurons in the caudate nucleus.

A recent report has described the presence and ultrastructure of VIP-positive neurons in the rat striatum [19]. In this study, only one or two VIP-positive striatal neurons were detected per section. In sections of rat striatum processed together with our cat sections we also could detect only very few VIP-immunoreactive neurons. In contrast, a typical 30-μm-thick section through the head of the cat caudate nucleus contained about 200 VIP-immunoreactive neurons. The ultrastructural observations of Theriault and Landis indicate that the rare VIP-positive neurons in the rat striatum are a type of medium-sized aspiny cells of 12–17 μm diameter [19]. In contrast, our results indicate that VIP is contained in a substantial population of much smaller neurons in the cat.

VIP has previously been found to coexist with acetylcholine in peripheral secretomotor neurons in the cat [13] and in small bipolar choline acetyltransferase-immunoreactive neurons in the rat cortex [3]. However, from the present observations, it is clear that the striatal neurons containing VIP are distinct from the large aspiny cholinergic neurons. Within the cortex, there is good evidence for a role for VIP in regulating local glucose metabolism, and a similar role for this peptide in the striatum has been suggested [14]. In addition, a neurotransmitter role for VIP is likely, since VIP has been detected in striatal synaptosomal preparations [7] and VIP binding sites [2] and a VIP-stimulated adenylate cyclase [16] are also found in the caudate-putamen.

It may be of importance to note that no changes in striatal VIP levels have been found in Huntington's disease [5]. Thus it will be important to determine whether VIP-immunoreactive neurons are present in the human caudate-putamen, and whether these neurons are spared in Huntington's disease.

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