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Somatostatin-like immunoreactivity in the amygdala of the pig

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Abstract: The distribution and morphology of neurons containing somatostatin (SOM) was investigated in the amygdala (CA) of the pig. The SOM-immunoreactive (SOM-IR) cell bodies and fibres were present in all subdivisions of the porcine CA, however, their number and density varied depending on the nucleus studied. The highest density of SOM-positive somata was observed in the layer III of the cortical nuclei, in the anterior (magnocellular) part of the basomedial nucleus and in the caudal (large-celled) part of the lateral nucleus. Moderate to high numbers of SOM-IR cells were also observed in the medial and basolateral nuclei. Many labeled neurons were also consistently observed in the lateral part of the central nucleus. In the remaining CA regions, the density of SOM-positive cell bodies varied from moderate to low. In any CA region studied SOM-IR neurons formed heterogeneous population consisting of small, rounded or slightly elongated cell bodies, with a few poorly branched smooth dendrites. In general, morphological features of these cells clearly resembled the non-pyramidal Golgi type II interneurons. The routine double-labeling studies with antisera directed against SOM and neuropeptide Y (NPY) demonstrated that a large number of SOM-IR cell bodies and fibers in all studied CA areas contained simultaneously NPY. In contrast, co-localization of SOM and cholecystokinin (CCK) or SOM and vasoactive intestinal polypeptide (VIP) was never seen in cell bodies and fibres in any of nuclei studied. In conclusion, SOM-IR neurons of the porcine amygdala form large and heterogenous subpopulation of, most probably, interneurons that often contain additionally NPY. On the other hand, CCK- and/or VIP-IR neurons belonged to another, discrete subpopulations of porcine CA neurons.

Key words: Somatostatin - Neuropeptide Y - Cholecystokinin - Vasoactive intestinal polypeptide - Amygdala - Pig

Introduction

The amygdala is formed by a set of nuclei and cortical regions in the temporal lobe and is known to be involved in several important aspects of emotion including the appreciation of affectively significant stimuli, the formation of stimulus-reward associations and emotional memories, and the generation of emotional responses [1,2]. Although the data concerning the neuronal organization [3-5] and circuitry [6,7] of the particular regions in CA are abundant, little is known about how the amygdala participates in these emotional functions at the neuronal and synaptic levels. To answer this question is to identify first the chemical coding pattern of the major cell types.

There are several lines of evidence suggesting that some of biologically active neuropeptides, including somatostatin (SOM), neuropeptide Y (NPY), cholecystokinin (CCK) and vasoactive intestinal peptide (VIP) are present in the neurons of the amygdala of animals studied so far [8-15]. For example, the levels of SOM in the amygdaloid nuclei are among the highest in the brain of monkeys and humans [16-18]. Thus, it is not surprising that this peptide is almost totally coexisted with gamma aminobutyric acid (GABA) [13,19], which is widely distributed in the amygdala of various mammals [20-23]. Until now, several studies have investigated the morphology and distribution of

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SOM-IR cells, fibers and terminals in the rat amygdala [12,13], however, data concerning the distribution of SOM-IR structures in CA of other species, are rather scarce. While there is available a detailed analysis of the distribution pattern of this substance in the monkey amygdala [8,24] and the appearance of SOM-IR fibers in the human central nucleus [25], there is no data available about the existence and distribution patter of somatostatinergic subset of neurons/nerve terminals in the porcine CA. Furthermore, data concerning the distribution (as well as colocalization with SOM) of neural elements exhibiting NPY-, CCK- or VIP-immunoreactivity, are even less numerous and were focused mostly on the rat [10-12,19,26-28]. Moreover, it should be stressed that the comparison of above mentioned data, presented by different authors and concerning different species, revealed the existence of some discrepancies or species-specific differences in the existence, distribution and co-localization pattern of these neuropeptides within different CA structures [8,12,26-28]. For example, basomedial (BM) and cortical (CO) nuclei, which seem to be very rich in SOM-IR elements in the monkey, in the rat appeared to be almost devoid of such chemically coded structures [8,12]. The existence of VIP-IR neurons in the rat central nucleus (CE) was reported by Gray [26], while Roberts [27] and Wray [28] were able to find in the same area only VIP-IR fibers.

In conclusion, there is only one report concerning the morphology and chemical coding of SOM-IR neurons in CA nuclei of human [25] and this issue has as yet been mostly studied in the rat [12,13] and monkey [8,24]. Thus, as there is a lack in data concerning both the morphology and the chemical coding of neurons in CA of the domestic pig, an animal becoming more and more important in the biomedical studies [29], we decided to provide a detailed description of the distribution and co-localization pattern of SOM and NPY, CCK or VIP in CA nuclei of this species.

Material and methods

Tissue preparation. Six sexually immature female pigs (approximately 8 weeks old, obtained from the commercial fattening farm in Niedźwiedź, Poland) were used in the present study. All experiments were carried out in accordance with the Local Ethical Committee rules. All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to produce reliable statistical data.

Thirty minutes before the main anaesthetic, pentobarbital (Vetbutal, Biowet, Poland; 25 mg/kg b.w.) was given intravenously, the animals were pre-treated with propionylopromasine (Combelen, Bayer, Germany; 0.4 mg/kg b.w. i.m.). Then, they were transcardially perfused with 1 l of pre-perfusion solution containing 0.9% sodium chloride (Chemia, Gliwice, Poland), 2.5% polyvinylpyrrolidone (Sigma, Deisenhofen, Germany), 0.5 procaine hydrochloride (Polfa, Warsaw, Poland) and 20,000 i.U. of heparin (Heparinum; Polfa, Warsaw, Poland; added *ex tempore*), followed by 4 l of 4% ice-cold buffered paraformaldehyde (pH=7.4). It should be stressed that animals in this study were not injected with colchicine.

Following perfusion, small tissue blocks, comprised of the amygdala and adjoining structures were postfixed by immersion in the same fixative for 4 h, washed twice in 0.1 M phosphate buffer (pH=7.4) and then stored in 30% sucrose until sectioning.

Immunofluorescence experiments. Twenty-μm-thick cryostat coronal sections of the tissue samples were processed for double-labelling immunofluorescence by means of primary antisera raised in different species. Briefly, sections were air-dried for 45 min, washed 3 times in PBS prior to immunohistochemical staining and then processed for the routine double-labeling immunofluorescence using a rat polyclonal antibody against SOM (1:200; code 8330-0009, Biogenesis, UK) that was combined with rabbit polyclonal antiserum against either NPY (1:4000; code NA1233, Affinity, UK), CCK (1:4000; code H-069-04, Phoenix, UK) or VIP (1:4000; code VA 1285, Affinity, UK). Sections were incubated in these mixtures overnight in a humid chamber at room temperature. In order to visualize binding sites of antigens-antisera used, sections were then incubated (1 h, at room temperature) with a mixture of FITC-conjugated goat anti-rat IgG (1:800; code 712-095-153, Jackson ImmunoLabs, USA) and Cy3-conjugated donkey anti-rabbit IgG (1:4000; code 711-165-152, Jackson ImmunoLabs, USA). All antibodies were diluted in PBS containing Triton X-100 (0.3-0.5%) and 1% normal goat serum. After the final wash (three changes of PBS), sections were coverslipped in buffered carboxyglycerol (pH=7.8) and analysed under an Olympus BX51 microscope equipped with a CCD camera connected to a PC. Pictures were acquired with the AnalySIS software (ver. 3.02; Soft Images Systems GmbH, GER).

Standard controls, *i.e.* preabsorption for the neuropeptide antisera (1 mg of appropriate antigen per 1 ml of corresponding antibody at working dilution; all antigens purchased from Peninsula, Sigma or Dianova), and the omission and replacement of all primary antisera by non-immune sera were applied to test both antibody- and method specificity.

Cell counts and percentages. Cell counts of single-labelled and double-labelled neurons were made in 6 nuclei (i.e. the lateral, basolateral, basomedial, cortical, medial and central ones) and were pooled from 3 animals, unless stated otherwise. For each combination of antigens, sections through each subdivision were analyzed sequentially until the whole rostrocaudal extent of each subdivision was analyzed (approximately 5 sections for each subdivision in each animal). At 200× magnification, cell counts were made from the image of a 400×400 μm field displayed for merged (red/green) channels on the computer screen (double-labeled cells appear yellow). Images of the non-merged red and green channels were also displayed. Depending on the size of the nuclear subdivision at different levels of the amygdala, counts were made from either one such field positioned in the centre of the subdivision (and involving about 80-90% of its cross-sectional area), or two or more adjacent non-overlapping fields.

Results

The nuclear pattern of the pig CA

The position of the various nuclei in the pig CA is shown at the 3 different levels in the Fig. 1.

As in other mammalian species, CA of the pig is composed of a 3 subsets of nuclei. The largest and the best differentiated portion of CA is the basolateral nuclear group (BLC), consisting of the lateral (LA),

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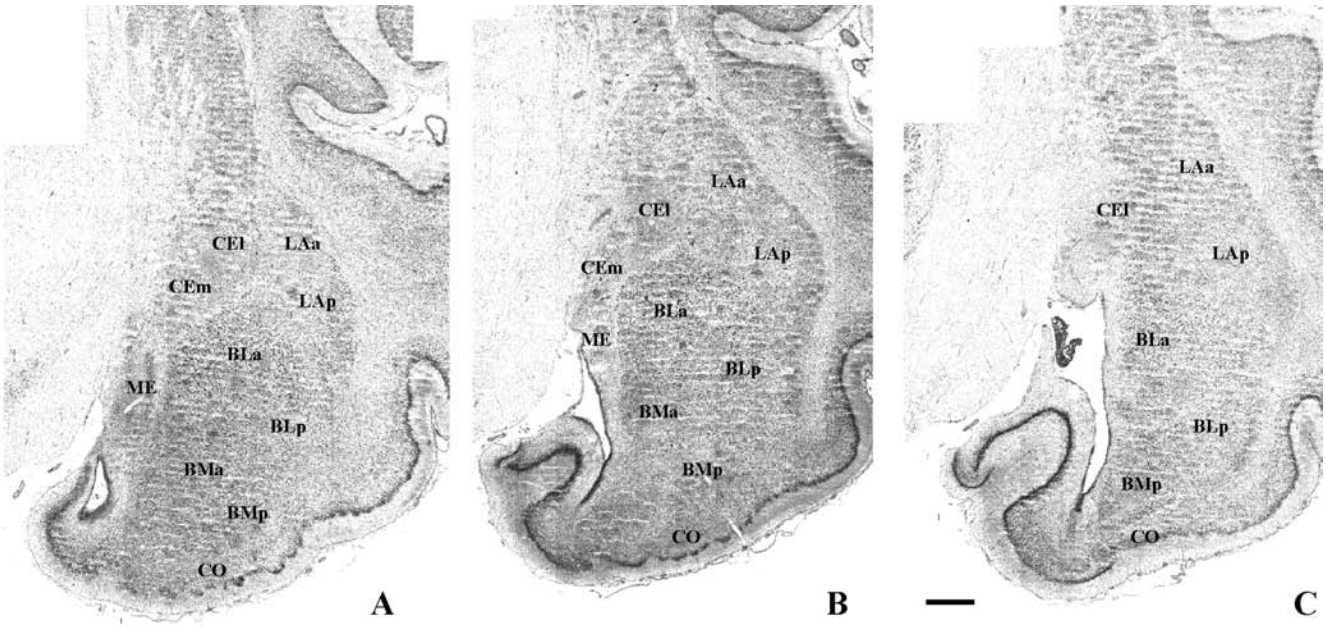

Fig. 1. Microphotographs of Nissl-stained sections through the anterior (**A**), middle (**B**) and caudal (**C**) portions of the amygdala in the pig. Scale bar=1 mm.

basolateral (BL) and basomedial (BM) nuclei. The second is the central group (CC), composed of the anterior amygdaloid area (AAA) as well as the central (CE) and intercalated nuclei (I). The third portion of CA is the corticomedial group (CMC), formed by the cortical (CO) and medial nuclei (ME) as well as the nucleus of the olfactory tract (NLOT) and the amygdalohippocampal area (AHA).

The general morphology and cellular structure of these different amygdaloid regions in the pig CA and their parcellation into the finer parts are very similar to these described by Krettek and Price in the rat and cat [30]. Briefly, on the basis of more subtle differences in the density and cell size within LA, BL and BM, they can be further subdivided into the anterior (LAa, Bla and BMa) and posterior (LAp, BLp and BMp) parts. LAa, BLp and BMp are formed by medium-size and small neurons where as LAp, BLa and BMa are large-celled. Two subdivisions are also present within CE, although the boundaries between them are not as clear-cut as in the basolateral nuclei. The medial part (CEm) is located more rostrally and is composed of the larger, darker and more tightly-packed cells. The lateral part (CEl) contains the smaller, lighter and more loosely arranged neurons. In ME it is also possible to distinguish 2 regions, according to differences in the density and size of the cells. The anterior part (MEa) forms the rostral and main body of the nucleus, whereas the posterior part (MEp) constitutes the small thin caudal portion. Finally, CO consists of 3 distinct regions: the anterior cortical nucleus (COa), the periamygdaloid cortex (COc) and the posterior cortical nucleus (COp).

In the present study, six of the above mentioned amygdaloid nuclei (LA, BL, BM, CE, CO and ME) and their subdivisions were taken into consideration as far as the distribution and morphology of SOM-IR somata and fibres are concerned. In all these regions colocalization of SOM with NPY, CCK or VIP was also evaluated.

The distribution pattern and morphology of SOM-, NPY-, VIP- or CCK-IR structures in the porcine CA

The distribution pattern of SOM-, NPY-, CCK- or VIP-IR structures within the pig CA is demonstrated in details in Fig. 2. The morphology and characteristic features of all studied structures were visualized in Figs. 3-9.

All antibodies used produced robust immunohistochemical staining in the pig amygdala and several other forebrain areas. However, SOM-IR structures always outnumbered the neurons and terminals immunoreactive for remaining peptides in any section studied. Moreover, the pattern of immunohistochemical staining for each of these peptides was slightly different in details:

i. SOM-IR cell bodies and fibers were present in all subdivisions of the pig CA, however, their number and density were different depending on the nucleus studied, and even within the different subdivisions of the single nucleus (Fig. 2). The highest density of SOM-IR somata was observed in the layer III of the CO, in the anterior (magnocellular) part of BM and in the caudal (large-celled) part of

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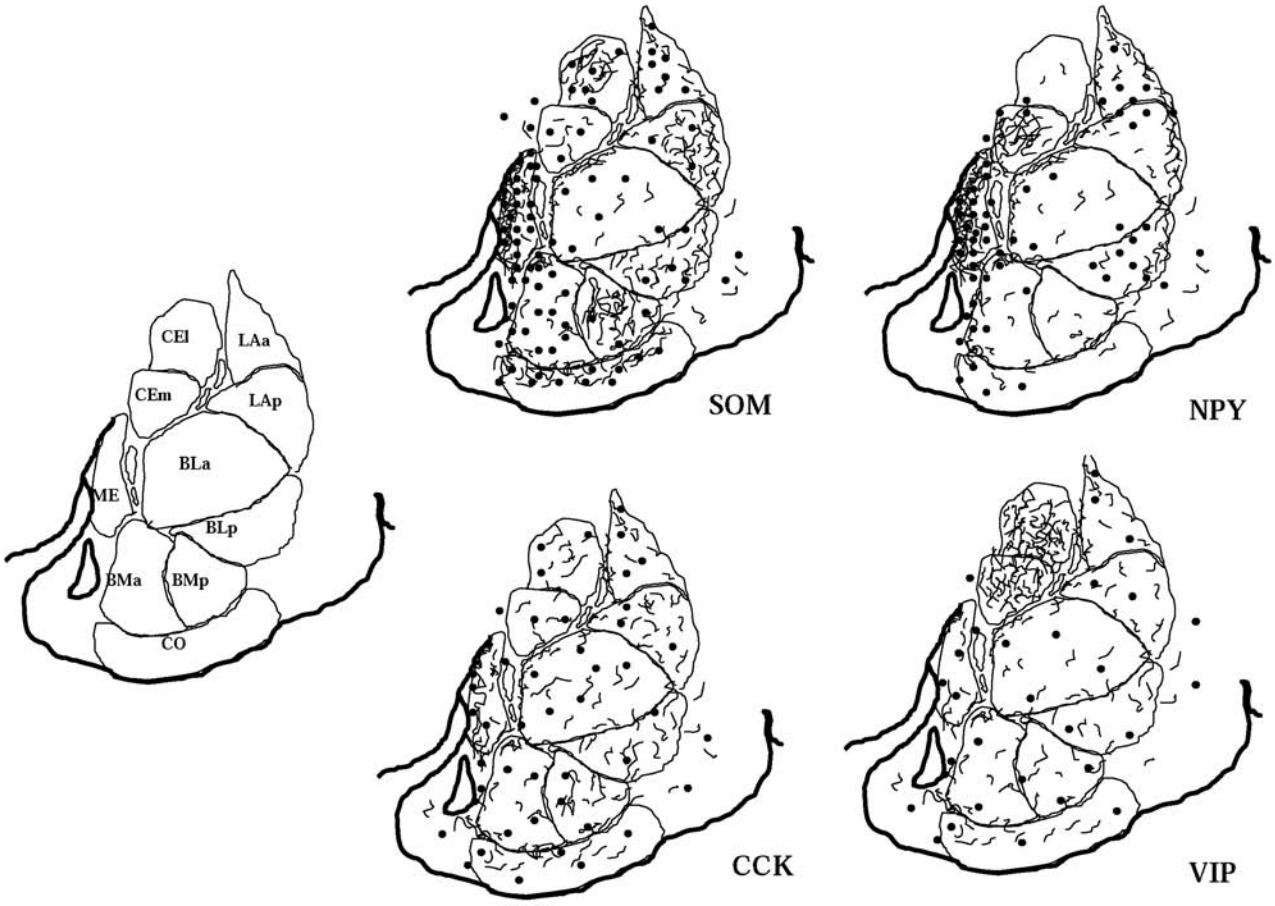
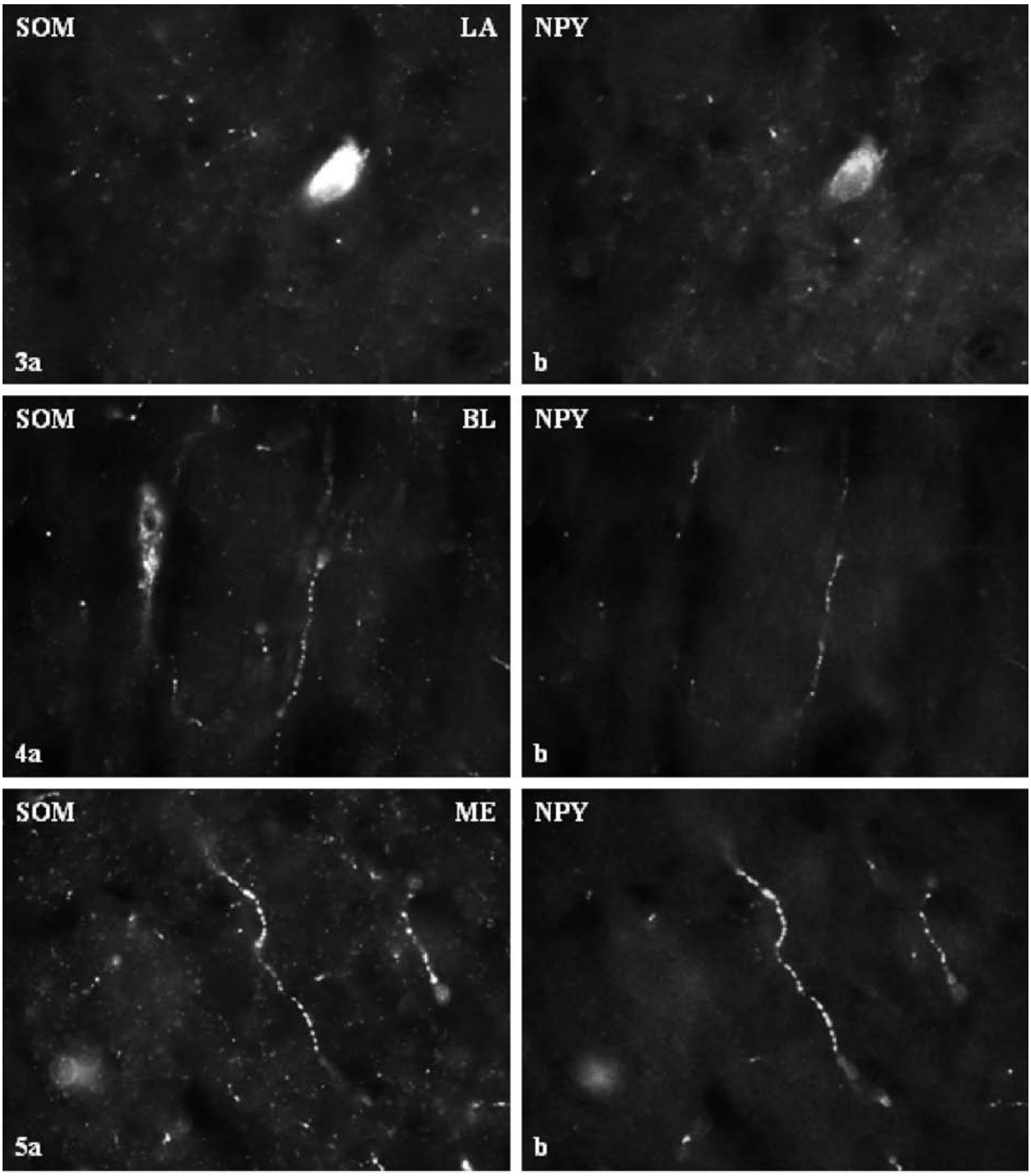


Fig. 2. Schematic drawing at the level of the middle portion of the porcine amygdala showing the distribution pattern of SOM, NPY, CCK and VIP immunoreactivity.

LA. Moderate to high numbers of SOM-IR cells were also observed in the medial and basolateral nuclei. Many labeled cells were also consistently observed in the lateral part of the central nucleus, while in the remaining CA regions the density of SOM-positive cell bodies ranged from moderate to low. In each CA nucleus SOM-IR neurons were outnumbered by the SOM-immunonegative somata of pyramidal neurons. Most SOM-IR neurons were smaller than the somata of pyramidal cells. Neurons exhibiting SOM-immunoreactivity formed, in each nucleus studied, a morphologically heterogeneous population of non-pyramidal Golgi type II cells with mostly rounded or slightly elongated somata and several poorly arborised dendrites (Fig. 3). Their somata measured 15-25 μm in diameter. Triangular and spindle shaped cells, however also present, were very rarely observed in the porcine CA (Fig. 4). The density of SOM-IR fibers also varied substantially from region to region in the pig CA (Fig. 2). The highest density of SOM-IR fibers was observed in the cortical and medial nuclei, in the lateral part of the central nucleus, in the caudal (small-celled) parts of the basomedial and basolateral nuclei and in the rostral (parvicellular) division of the lateral nucleus. In the basolateral nuclei, the density of SOM-IR fibers was often inversely related to the density of SOM-IR cells within the nucleus: e.g., while the highest density of SOM-IR cells was found in the anterior (magnocllular) part of the basomedial nucleus, the SOM-IR fibers were mostly concentrated at the lateral aspects (parvicellular and caudal subdivision) of BM (Fig. 2). The morphology of SOM-IR fibers, like that of the somata, was similar in all CA nuclei studied.

ii. The pattern of NPY-immunoreactivity was overlapping with that found for SOM, however, there were some differences as well (Fig. 2). For example, on the rostral sections, NPY-IR structures were relatively scarce. Their number and density increased to the level of the ventral horn of lateral ventricle and from that level remained similar and stable to the end of CA. The highest density of NPY-IR somata were found in the medial nucleus and at the base of the stria terminalis (i.e. within the area located between the medial and the central nucleus). Moderate to high numbers of NPY-IR cells were also observed in the lateral and basolateral



Figs. 3-5. Examples of the distribution and co-incidence pattern of SOM and NPY within porcine amygdala (magnification ×240). **Fig. 3.** A double-labeled SOM /NPY-IR neuron in the LA. Note a low number of either SOM-IR (**a**) and/or NPY-IR (**b**) nerve fibers in this area. **Fig. 4**. Co-localization pattern of SOM (**a**) and NPY (**b**) within the BL. In this nucleus, several spindle-shaped SOM-IR, but simultaneously NPY-immunonegative neurons were observed. Co-localization of both antigens was often observed in the varicose nerve fibers. **Fig. 5.** Double-labeling towards SOM (**a**) and NPY (**b**) revealed a high number of varicose nerve fibers in the ME.

nuclei. In contrast to the distribution pattern of SOM-IR cell bodies, only a few NPY-IR neurons were found in the basomedial, cortical and central nuclei. The density of NPY-IR fibers was highest in the medial and central nuclei, while a moderately to dense plexuses of NPY-IR terminals were also seen in the lateral and basolateral nuclei (Fig. 2). A relatively low number of fibers exhibiting this peptide was evenly distributed in all remaining regions of the porcine CA. It is worth mentioning that the morphology of NPY-IR cell bodies and fibers was very similar to those of SOM-IR ones (Figs. 3-5).

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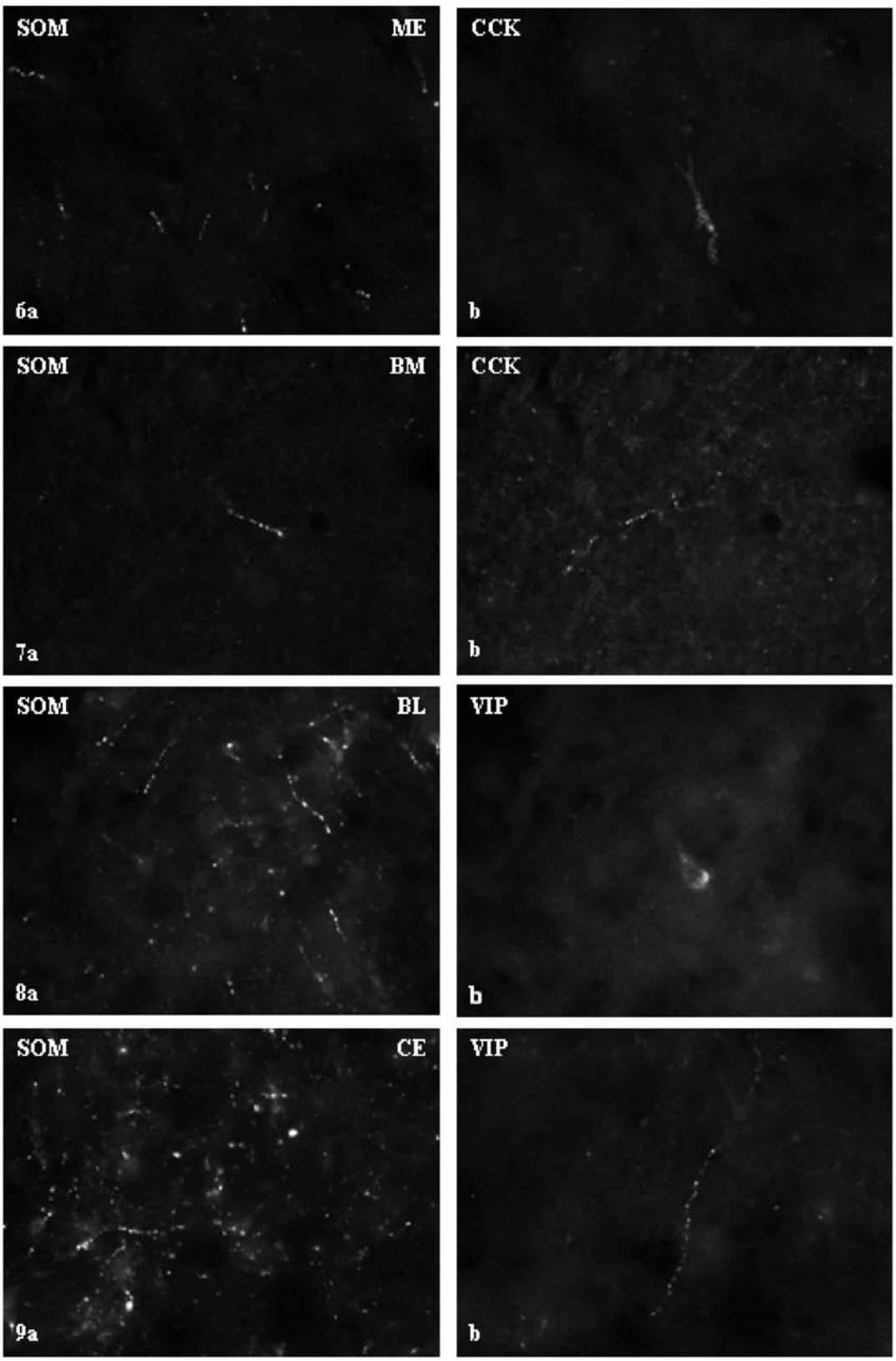

Figs. 6-9. Examples of the distribution and co-incidence pattern of SOM and CCK (Figs. 6, 7) and SOM and VIP (Figs. 8, 9) within porcine amygdala(magnification ×220). **Fig. 6**. Co-localization pattern of SOM and CCK in the porcine ME. Note that SOM-IR nerve terminals were lacking CCK (**a**), and that solitary CCK-IR neurons were simultaneously SOM-immunonegative (**b**). **Fig. 7**. Solitary SOM-IR (**a**) and CCK-IR fibers (**b**) in the neuropil of porcine BM. Note a lack of co-localization of both antigens in nerve fibers. **Fig. 8**. Dense SOM-IR (**a**) varicose nerve fibers in BL were lacking VIP-immunoreactivity (**b**). Note, that the VIP-IR neuron is simultaneously devoid of any SOM-IR input. **Fig. 9**. Patchily distributed SOM-IR nerve terminals (**a**) and VIP-IR terminal (**b**) within the CE. Note a lack of co-localization of both antigens in nerve fibers.

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Table 1. Cell counts and percentages of single- and double-labeled neurons in different nuclei of the porcine amygdala.

Nucleus	Total neurons counted	Double-labeled cells (SOM-IR/NPY-IR)	Single-labelled cells		% of SOM-IR cells with NPY	% of NPY-IR cells with SOM
			SOM	NPY		
LA	743	571	161	11	78,1	98,1
BL	639	306	326	7	48,4	97,7
BM	628	118	506	4	18,9	96,7
CE	588	64	522	2	10,9	96,9
CO	537	106	429	2	19,8	98,1
ME	641	358	249	34	58,9	91,3
CA	3776	1523	2193	60	39,2	96,5

iii. CCK was found to be distributed in a pattern quite different from that presented by SOM- or NPY-IR structures (Fig. 2). Both, cell bodies and fibers expressing CCK were significantly less numerous than SOM- or NPY-IR ones, being homogenously scattered in all CA subdivisions studied. This pattern of scattered and homogenous distribution was observed throughout the whole rostro-caudal extent of the pig CA. The morphology of CCK-IR cell bodies and fibers was different from that of the respective structures containing SOM or NPY (Fig. 6-7). CCK-IR cell bodies were heterogeneous in size, but were generally smaller than these containing SOM or NPY (Fig. 6). CCK-IR fibers had regularly dispersed small varicosities and were thinner than these of SOM- or NPY-IR subsets (Fig. 7).

iv. VIP-IR structures were found to be scattered in a quite similar manner to that of CCK-IR ones, however with two exceptions (Fig. 2): the central nucleus was the only region in CA that was completely devoid of any VIP-IR cell bodies. Instead of that, the particularly dense plexuses of VIP-IR fibers were found within this CA area. It is worth mentioning that many of VIP-IR and CCK-IR somata and fibers were very similar to each other in the size and morphology (Figs. 8-9).

The coexistence pattern of SOM and NPY, VIP or CCK in the porcine CA

All details concerning the coexistence of SOM with NPY, VIP and CCK in the somata and fibers of the porcine CA were demonstrated in the Table 1. The morphology and characteristic features of single and double labelled immunoreactive elements were visualized in Figs. 3-9.

The most important observations were:

i. In all double-labellig stainings SOM-IR neurons and fibers always outnumbered neurons and fibers exhibiting immunoreactivity towards any of other neuropeptides studied;

ii. The extensive colocalization of SOM with NPY was observed in amygdala taken from all the brains used in this investigation (Figs.3-5 and Table1). In contrast, no double-labeled cells and fibers were found in the case of SOM and CCK or SOM and VIP labelings (Figs.6-9);

iii. Virtually all NPY-IR non-pyramidal neurons in LA, BL, BM and CO were simultaneously SOM-IR (Table 1). These cells constituted almost 80% of the SOM-IR population in both divisions of the lateral nucleus, more than 45% of the SOM-IR population in the basolateral nucleus and less than 20% of the SOM-IR population in the basomedial and cortical nuclei (Table 1). In CE, like in the adjacent striatum, virtually all of NPY-IR neurons exhibited SOM-immunoreactivity (95-100%) (Table 1). However, NPY-IR neurons constituted only about 10% of the SOM-IR neuronal population in this nucleus (Table 1). It should also be noted, that all double-labeled NPY/SOM-IR cells were observed in the medial part of the central nucleus. Lateral part was almost completely devoid of NPY/SOM-IR and solely NPY-IR cells. Ninety to ninety-five percent of NPY-IR neurons were SOM-IR in ME (Table 1). These NPY-IR neurons constituted about 60% of SOM-IR neurons in this nucleus (Table 1).

Discussion

This is the first study aimed at providing a detailed description of the distribution and neurochemical characteristics of SOM-IR structures in the porcine CA. Such analysis seem to be especially valuable in the light of the recent morphological and embryological studies, which indicate that the porcine brain shares many similarities with the human one and so that the pig can be used as a good animal model for understanding the activities of the human organs [29].

The distribution of SOM-IR cell bodies and fibers in the porcine CA is consistent with the previous studies in the rat [12] and monkey [8,24]. All these studies

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indicate that CA regions especially rich in SOM-IR somata were the cortical and large-celled parts of the basomedial and lateral nuclei [present study and 8,12, 24]. Numerous SOM-IR cell bodies can also be found in the basolateral nucleus, medial nucleus and lateral part of the central nucleus [present study and 8,12,24]. The distribution of SOM-IR cell bodies and fibers in the cortical, medial and central nuclei is overlapping [present studies and 8]. The distribution of SOM-IR fibers in the basolateral nuclei is rather inversely related to the distribution of SOM-IR cell bodies i.e. within the individual nuclei, regions rich in SOM-IR somata were often almost devoid of SOM-IR fibers, while areas rich in SOM-IR fibers were devoid of SOM-IR cell bodies [present study and 8]. The lateral and basomedial nuclei are especially good examples of such a phenomenon [present study and 8]. The interesting question arises as to whether SOM-IR fibers, located in the small-celled parts of the basomedial and lateral nuclei, belong to the cell bodies situated far from them in the large-celled parts of both these nuclei.

The morphology of SOM-IR neurons [present study and 8,11] indicate that most of them belong to the class II and III sparsely-spiny non-pyramidal neurons seen in the Golgi studies [3,31,32]. They have small ovoid or slightly elongated cell bodies and relatively few sparsely spiny short dendrites [present study and 8, 11]. These cells closely resembled their counterparts in the cerebral cortex [33]. There is however evidence that some SOM-IR neurons can be different in morphology. McDonald and his co-workers [24] have reported that in the dorsal portion of the lateral nucleus most SOM-positive neurons had spiny dendrites. Similar situation can be observed in the central nucleus. The Golgi studies in the variety of mammals indicated that the lateral part of CE is formed first of all by striatal-like medium-size spiny neurons [3,4,34]. The aspiny interneurons are very rare in this area [34]. Since lateral part of CE contain huge population of SOM-IR cells that suggests that most of them can be of spiny nature [present study and 8,12,24]. The medial nucleus can be another good example. The cytoarchitectonic studies with the use of Golgi impregnation method indicate that this nucleus is almost entirely composed of the small, projecting neurons, with dendrites covered with not numerous spines [3, 4]. The aspiny interneurons, like these in the basolateral amygdala, striatum and cerebral cortex, are even less numerous than in the central nucleus [4]. Since ME contains a huge population of SOM-IR and SOM/NPY-IR cells, this may be indicative that many of them can be of spiny nature [present study and 8, 12, 24]. The finding of neurons with spiny dendrites in the dorsal part of the lateral nucleus, lateral part of the central nucleus and in the medial nucleus suggests that these cells may be functionally different from SOM-positive neurons in other portions of the amygdala.

The present studies indicate that many of SOM-IR neurons in the amygdala of the pig contain NPY-IR as well. About 40-80% of SOM-IR neurons in the lateral, basolatral and medial nucleus contain NPY depending on the nucleus studied. It should be noted however that in the cortical, basomedial and central nuclei the extent of SOM and NPY co-localization is much smaller since only 10-20% of SOM-IR neurons contain NPY (present study). The extensive co-localization of SOM with NPY was reported in the amygdala of the rat [12] and monkey [24] as well as in many areas of the hippocampus [35] and cerebral cortex [33,36]. The double-labeling studies performed in the rat [13] demonstrated that the vast majority of SOM-IR neurons in the basolateral nuclei exhibited simultaneously GABA-immunoreactivity (66-82% depending on the nucleus). These SOM-IR neurons constituted 11-18% of the GABA-IR population [13]. There is also extensive colocalization of SOM with calbindin (CB) in the amygdala [13]. In the basolateral nucleus of the rat more than 90% of SOM-IR neurons also exhibited CB-immunoreactivity, whereas in the lateral nucleus about two-thirds of SOM-IR neurons contained significant levels of CB [13]. These SOM/CB neurons constituted about one quarter of the CB-IR population in the rat basolateral nucleus and about one third of the CB-IR population in the lateral nucleus. On the other hand, present study strongly suggests, that there is no colocalization of SOM with CCK or VIP in the porcine CA neurons. This is also in accordance with previous studies in the rat [12] and monkey [24]. Similar observations were also reported in the hippocampus [37] and cerebral cortex [36]. There is evidence as well that there is no coexistence of SOM with parvalbumin [13] and calretinin [13] in the amygdala. It is worth mentioning here, that all these substances coexist with GABA in the CA neurons, forming probably different subpopulations of GABA-ergic interneurons [19,38].

Taking into account morphological and neurochemical heterogeneity of SOM-IR neurons, the question arises what is the putative physiological role of different SOM-IR neuronal subpopulalations within the CA. Available data suggest at last 2 possible functions of SOM-IR neurons in this brain compartment:

i. The most common view is that SOM-IR neurons are interneurons, which modulate the output of the principal projecting neurons in each of the amygdaloid nuclei [3,15,31,32]. There is indirect evidence held from the morphology of SOM-IR cells [present study and 11] that in their vast majority appears to be the class II and III sparsely-spiny non-pyramidal neurons seen in Golgi studies [3,31, 32]. These cells closely resemble their counterparts in the cerebral cortex [31]. There is general agree-

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ment in the literature that non-pyramidal neurons in the cortex and hippocampus are mostly interneurons [33,39]. There is also direct evidence from the studies in the rat that the main postsynaptic targets of SOM-IR terminals in CA are small-caliber dendrites and dendritic spines of the pyramidal cells [15]. Few SOM-IR terminals form synapses with pyramidal cell somata or large-caliber (proximal) dendrites [15]. Since SOM-IR neurons in CA are mostly GABAergic [19], this arrangement suggests that SOM-IR interneurons in the CA can modulate the output of neighboring pyramidal projection neurons by inhibiting these neurons in the different dendritic regions. Since SOM-IR neurons are mainly activated by neighboring pyramidal neurons, rather than by corticofugal fibers [8,12], it appears that feedback inhibition is one of the principal functions of SOM-IR interneurons in CA. It is worth mentioning that 15% of SOM-IR terminals formed synapses with PV-IR, VIP-IR, or even SOM-IR interneurons [15]. That means that SOM-IR interneurons can influence pyramidal cells in second, indirect way by inhibiting or modulating output of other GABA-IR interneurons. The most important in this case seem to be the interactions with PV-IR interneurons which act in the perisomatic region of the pyramidal cells [40] so they can make powerful inhibitory effect on them.

ii. On the other hand, there is fairly substantial evidence in the literature that some of the SOM-IR cells can be also projecting neurons [9,14,41,42, 43]. For example, McDonald [43] demonstrated a somatostatinergic projection from the medial nucleus to the bed nucleus of the stria terminalis in the rat. The same author reported that small number of SOM-IR neurons in the basolateral amygdala were retrogradely labeled by injection of HRP into the striatum and cerebral cortex in the rat [42]. Kawai [44] demonstrated that lesions of the amygdala lead to a reduction of somatostatin immunofluorescence in the brainstem. Higgins [41] and Gray [9] used double-labeling procedures with retrogradely transported fluorescent dyes to demonstrate that cells located in the medial part of central nucleus project to the vagal nuclear complex. Moga [14] also demonstrated that SOM-IR neurons in both divisions of CE project to the parabrachial nuclei. In this context, the existence of neurons with spiny dendrites in the dorsal part of the lateral nucleus, lateral part of the central nucleus and in the medial nucleus supports the idea that at least some SOM-IR cells in the amygdala may be functionally different from SOM-IR interneurons in the other portions of CA i.e. they may be projecting neurons.

In conclusion, the present study provides an evidence that SOM-IR neurons constitute in the porcine CA a huge subpopulation of non-piramidal neurons that often use NPY as a additional neurotransmitter or neuromodulator. In contrast, none of SOM-IR, NPY-IR or SOM-IR/NPY-IR cells exhibited coexistence with CCK or VIP.

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