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Possible coexistence of amino acid (γ-aminobutyric acid), amine (dopamine) and peptide (substance P); neurons containing immunoreactivities for glutamic acid decarboxylase, tyrosine hydroxylase and substance P in the hamster main olfactory bulb

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Summary. The coexistence of immunoreactivities for glutamic acid decarboxylase (GAD), tyrosine hydroxylase (TH) and substance P (SP) was revealed in the hamster main olfactory bulb, using the peroxidase-antiperoxidase immunohistochemical method. Adjacent 40 μm thick Vibratome sections were incubated in different antisera and those cells which were bisected by the plane of sectioning were identified at the paired surfaces of two consecutive sections. The coexistence of the immunoreactivities for 1) TH and GAD, 2) TH and SP and 3) GAD and SP in the same cells could thus be determined by observing the immunoreactivity of the two halves of the cell incubated in two different antisera. About 70% of TH-like immunoreactive (TH-LI) neurons in the periglomerular region also contained GAD-like immunoreactivity, whereas about 45% of GAD-LI ones were also TH-like immunoreactive. Furthermore, almost all (more than 95%) of SP-LI neurons contained both GAD-like and TH-like immunoreactivities. These observations indicate that in the periglomerular region of the hamster main olfactory bulb, some neurons (about 9% of all neurons containing TH-like and/or GAD-like immunoreactivities) may contain three different categories of neuroactive substances, that is, amino acid (GABA), amine (dopamine) and peptide (SP).

Key words: Coexistence – GABA – Catecholamine – Substance P – Olfactory bulb

Introduction

The olfactory bulb is a particularly favorable region for analysing chemical organization because of its highly laminated structure and its enormous richness in neuroactive substances in amounts and in a variety that rival any other regions of the brain (Halász and Shepherd 1983).

In our previous studies, we showed the laminar distribution of some chemically-defined intrinsic neurons in the rat olfactory bulb focusing on subpopulations of GABAergic neurons (Kosaka et al. 1985a, 1987a, c). Of particular interest is the coexistence of immunoreactivities for tyrosine hydroxylase (TH), a key enzyme of the catecholamine synthesis, and γ-aminobutyric acid (GABA) and/or glutamic acid decarboxylase (GAD), a specific GABA synthesizing enzyme, in some neurons of the rat main olfactory bulb (Kosaka et al. 1985a, 1987a, b). In adult rats, about 65% and 88% of TH-like immunoreactive (TH-LI) neurons also contain GAD-like and GABA-like immunoreactivities, respectively (Kosaka et al. 1987a). This indicates that TH-LI neurons might be considered to consist of two chemically-defined subpopulations, one with, and one without, GABAergic properties.

On the other hand, based on the light microscopic and ultrastructural analyses (Halász et al. 1977, 1981), the presumable dopaminergic TH-LI neurons (Halász et al. 1977) are traditionally classified into two subgroups, that is, periglomerular and tufted cells. However, the proportion of each of these two morphologically different neuronal groups in the TH-LI neurons is not known in the rat olfactory bulb. At present we cannot directly correlate the chemically-defined subpopulations of TH-LI neurons to the morphologically-defined ones, although we can tentatively assume that TH-LI neurons with GABAergic traits are periglomerular cells and those without GABAergic traits are tufted cells.

In the hamster olfactory bulb, Davis and Macrides (1983) reported that more than 80% of TH-LI neurons in the periglomerular region are external

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tufted cells, based on light microscopic observations such as somal sizes and dendritic features. As external tufted cells are considered not to contain GABAergic traits (Halász and Shepherd 1983), we can expect that the majority of TH-LI cells in the periglomerular region of the hamster main olfactory bulb might not contain GAD-like immoreactivity. If this is the case, it is highly probable that TH-LI neurons without GAD-like immunoreactivity are morphologically-defined tufted cells in the hamster olfactory bulb. Thus, the hamster olfactory bulb might be more favorable for revealing the correlation between chemically-defined subpopulations of TH-LI neurons and morphologically-defined ones than the rat olfactory bulb. Furthermore, in the hamster olfactory bulb, substance-P (SP) is reported to be contained in some external tufted cells (Davis et al. 1982; Burd et al. 1982; Kream et al. 1984; Baker 1986a). In the present study, we examined the coexistence of immunoreactivities for TH, GAD and SP in the hamser olfactory bulb using an adjacent section technique and attempted to classify neurons in the periglomerular region based on their chemical nature.

Material and methods

Twelve male syrian hamster were used. As specific-pathogen free (SPF) hamsters were not available, we used conventional hamsters in the present study. They were 5 weeks (7 hamsters) and 10–12 weeks old (5 hamsters) and weighed about 60–90 g and 100–150 g, respectively.

Hamsters were perfused through the heart with lactated Ringer's solution followed by a mixture of 4% paraformaldehyde with 0.05–0.1% glutaraldehyde and 0.2% picric acid in 0.1M Millonig's phosphate buffer. The brains were left in situ for 1 h at room temperature, then removed from the skull and immersed in 4% paraformaldehyde in 0.1M Millonig's phosphate buffer. Olfactory bulbs were dissected out, rinsed several times in 0.1M phosphate buffer, and cut into 40 μm thick serial sections coronally or horizontally on a Vibratome (Oxford instruments). Then the sections were processed for immunohistochemistry following the procedure of the peroxidase-antiperoxidase (PAP) method (Sternberger 1979) as described previously (Kosaka et al. 1985a, b, 1987a, b). Briefly, this involved; preincubation with 20% normal goat serum in phosphate buffered saline (PBS); incubation in rabbit primary antisera for 24–48 h at room temperature; washing in 1% normal goat serum in PBS three times each for 20–30 min; incubation in goat anti-rabbit IgG serum (MBL) diluted 1 : 50 for 1.5–2 h at room temperature; washing in PBS three times each for 20–30 min; incubation in rabbit PAP (DAKO) diluted 1 : 100 for 1.5–3 h at room temperature; washing in 0.05M Tris buffer (pH 7.6); incubation in 0.05% diaminobenzidine tetrahydrochloride (DAB) containing 0.01% H_2O_2 for 5–10 min at room temperature; washing in 0.05M Tris buffer; washing in 0.1M phosphate buffer. After post-fixation in 1–1.3% O_sO_4 in 0.07M phosphate buffer, sections were dehydrated in a graded series of ethanol, infiltrated in propylene oxide and flat-embedded in Epon 812 between Teflon-coated glass slides and cover glasses (Wilson and Groves 1979). Triton X100 was sometimes added to primary antisera at the final concentration of 0.1%.

Antiserum to GAD (Wu et al. 1982) was used at dilutions of 1 : 1,000 to 1 : 2,000. Antiserum to TH (Nagatsu 1983) were used at a dilution of 1 : 20,000. Three antisera to SP were used. Two were donated by Dr. Yanaihara (R2404) and Dr. Tohyama and another one was purchased from Immunonuclear Corp. These S-P antisera were used at dilutions of 1 : 3,000 to 1 : 5,000 and they gave the same results.

For controls, primary antisera were replaced by 1) normal rabbit serum or 2) antisera pretreated with synthetic SP. For producing antisera pretreated with SP, 0.1, 1 or 10 nmol of SP (Peptide Institute, Inc. Japan) was added to 1 ml of 1 : 5,000 diluted anti-SP sera, to 1 ml of 1 : 1,000 diluted anti-GAD serum and to 1 ml of 1 : 20,000 diluted anti-TH serum and incubated for 24 h at 4° C. The pretreatment of anti-SP sera with even 0.1 nmol of SP nearly completely abolished their specific immunostaining, whereas the addition of 10 nmol of SP had no effects on specific immunostaining with anti-GAD or anti-TH serum.

To demonstrate the colocalization of two different antigens in the same cell, we used the method of comparing the paired surfaces of adjacent sections incubated in different antisera and identifying the same perikarya bisected into two consecutive sections (Kosaka et al. 1985a, b). Sections were examined with a X10 ocular and X100 oil-immersion objective with a light microscope equipped with a camera lucida apparatus. Sizes of immunoreactive soma were measured under a light microscope (ocular X10, objective X100 oil-immersion) using an ocular scale. For the measurement of somal sizes, we used sections which were simultaneously processed after DAB reactions to reduce the difference in shrinkage among sections during processing such as dehydration.

Results

1. General description

GAD-like immunoreactive (GAD-LI) perikarya were clustered in the periglomerular region and in the granule cell layer, and some were also scattered in the external plexiform layer and inner plexiform layer. In the mitral cell layer, GAD-LI perikarya appeared to wedge themselves among far larger unstained mitral cell somata (Fig. 1A).

TH-like immunoreactive (TH-LI) perikarya were clustered in the periglomerular region (Fig. 1B). In the external plexiform layer, especially in its outer half, TH-LI perikarya somewhat larger than those in the periglomerular region were scattered and extended their dendrites into the glomerular layer. A few relatively small TH-LI perikarya were also scattered in the external plexiform layer, mitral cell layer, inner plexiform layer and granule cell layer.

SP-like immunoreactive (SP-LI) perikarya were clustered in the periglomerular region, and appeared to extend their dendritic processes into glomeruli (Fig. 1C). In the periglomerular region, the number of SP-LI perikarya was apparently far smaller than those of GAD-LI perikarya and TH-LI perikarya. Furthermore, comparing to GAD-LI or TH-LI ones, the number of SP-LI perikarya and intraglomerular elements appeared to vary considerably among ani-

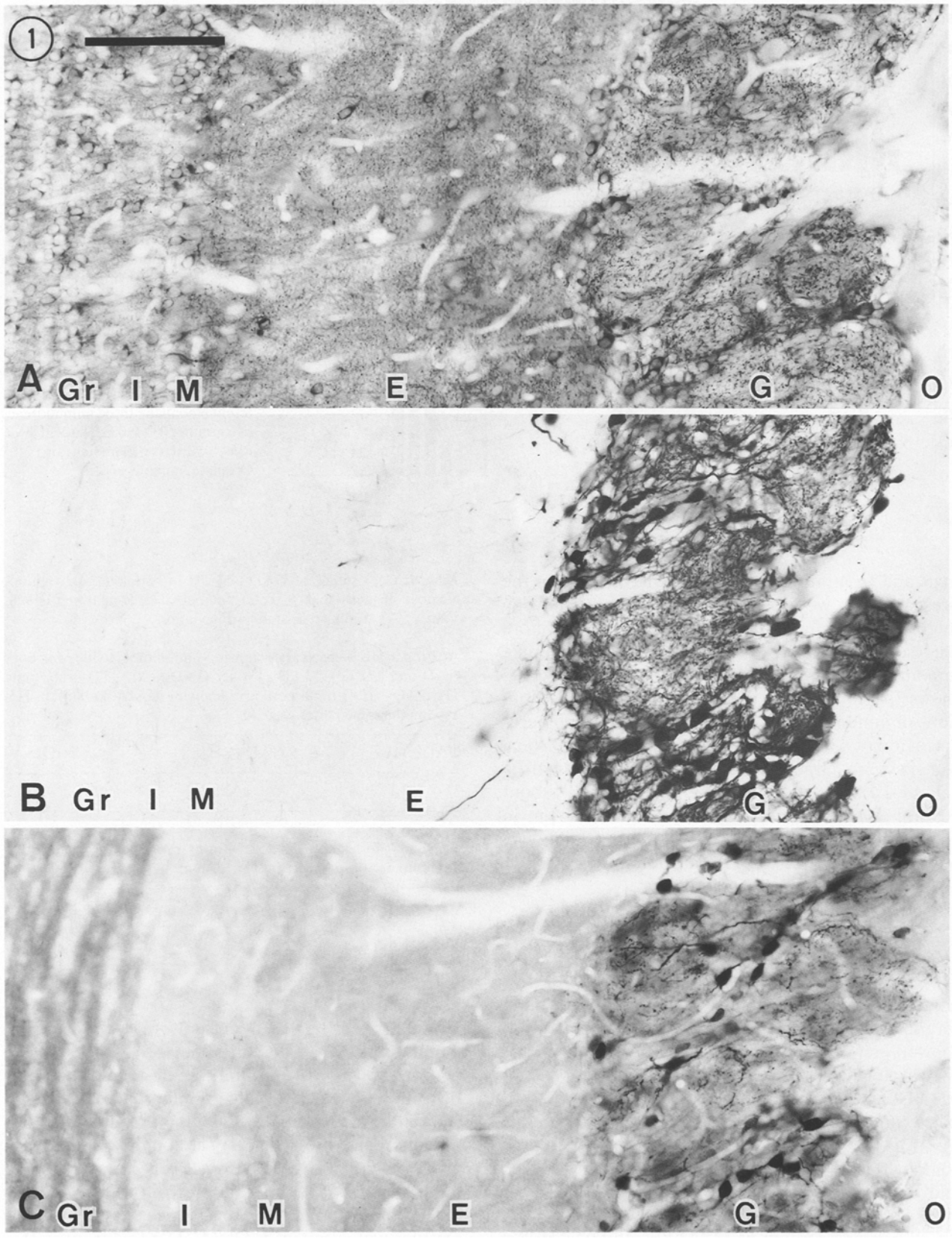


Fig. 1A–C. Low magnification photomicrographs of three consecutive 40 μm thick sections incubated with anti-GAD serum (**A**), anti-TH serum (**B**) and anti-SP serum (**C**). O, olfactory nerve layer; G, glomerular layer; E, external plexiform layer; M, mitral cell layer; I, inner plexiform layer; Gr, granule cell layer. Scale bar = 100 μm

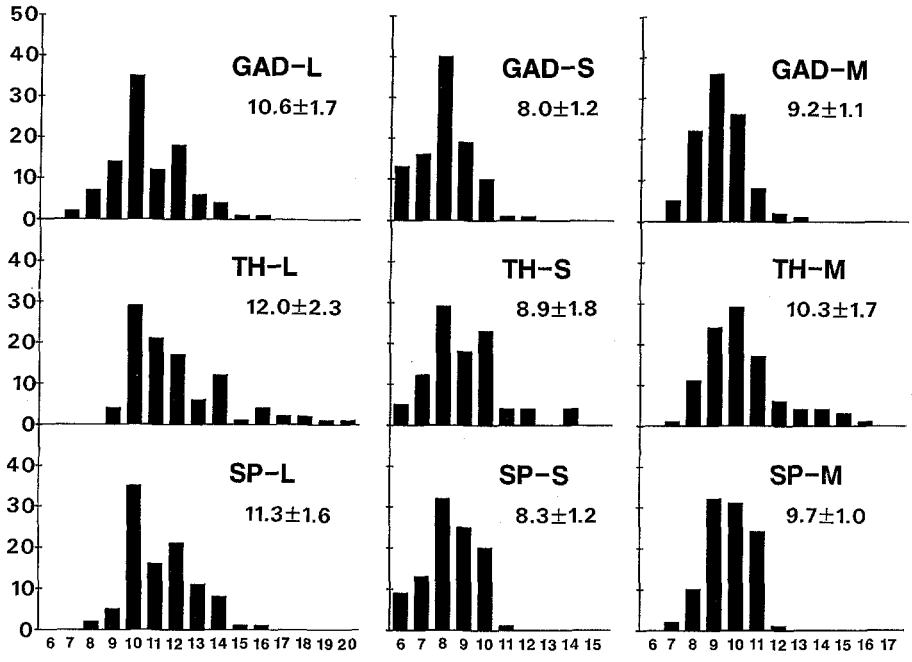


Fig. 2. Frequency distribution of the major(-L), minor(-S) and mean(-M) diameters of GAD-like or TH-like or SP-like immunoreactive somata (100 somata each) in the periglomerular region of a hamster. Mean diameter = $\sqrt{\text{major diameter} \times \text{minor diameter}}$. Numbers in each histogram are means ± standard deviations (μm). Major, minor and mean diameters are significantly different among the three immunoreactive groups ($P < 0.025$, Student's t-test). Abscissa, diameter (μm). Ordinate, number of cells

mals and from area to area (see Discussion). In the present study, we used only olfactory bulbs showing intense SP-like immunoreactivity and furthermore analysed the areas showing intense SP-like immunoreactivity of these olfactory bulbs. Figure 2 shows the somal sizes and their frequency distributions of immunoreactive neurons in the periglomerular region of a hamster. On average, TH-LI neuronal somata were largest, GAD-LI ones were smallest and SP-LI ones were intermediate in size. About 10% of TH-LI neurons were larger than 13 μm in mean diameter, whereas all SP-LI neurons and 99% of GAD-LI neurons were smaller than that.

2. Coexistence of immunoreactivities for TH and GAD

Close comparison of neighboring surfaces of two adjacent sections, one incubated with anti-GAD serum and the other incubated with anti-TH serum showed many neurons containing both immunoreactivities (Fig. 3). Although GAD-LI neurons without TH-like immunoreactivity appeared to outnumber those containing TH-like immunoreactivity, a majority of TH-LI neurons in the periglomerular region contained the GAD-like immunoreactivity. Table 1A and B show the quantitative analysis of the coexistence of TH-like and GAD-like immunoreactivities. Of all neurons containing GAD-like and/or TH-like immunoreactivities in the periglomerular region, about 40% contained both immunoreactivities.

Table 1. Coexistence of GAD and TH in periglomerular region. Number of hamster is 4. Total number of cells analysed is 808. Values (%) are mean ± standard deviation

A: Proportion of perikarya showing immunoreactivities for both GAD and TH(GAD+TH+), only GAD(GAD+TH–) and only TH(GAD–TH+) in all perikarya showing GAD-like and/or TH-like immunoreactivities (100%)

GAD+TH+	GAD+TH–	GAD–TH+
37.6 ± 3.1	47.0 ± 3.3	15.4 ± 4.6

B: Number of double-labeled neurons expressed as per cent of total number of GAD-LI or TH-LI neurons

GAD+TH+/GAD+	GAD+TH+/TH+
44.5 ± 2.6	71.1 ± 7.8

About 70% of TH-LI neurons were also GAD-like immunoreactive, whereas about 45% of GAD-LI neurons were also TH-like immunoreactive. TH-LI neurons without GAD-like immunoreactivity usually showed larger somal sizes than those containing both immunoreactivities (Fig. 4), although we could not always predict the coexistence of TH and GAD immunoreactivities in a neuron from its somal size.

In the external plexiform layer, a few TH-LI neurons were scattered. Of 34 TH-LI neurons in this layer, only 7 (20.6%) were also GAD-like immunoreactive, whereas others were recognized as

Table 2. Coexistence of GAD and SP in periglomerular region. Number of hamster is 5. Total number of cells analysed is 1917. Values (%) are mean ± standard deviation

A: Proportion of perikarya showing immunoreactivities for both SP and GAD(SP+GAD+), only SP(SP+GAD–) and only GAD(SP–GAD+) in all perikarya showing SP-like and/or GAD-like immunoreactivities (100%)

SP+GAD+	SP+GAD–	SP–GAD+
11.4 ± 2.2	0.5 ± 0.5	88.1 ± 2.1

B: Number of double-labeled neurons expressed as per cent of total number of SP-LI or GAD-LI neurons

SP+GAD+/SP+	SP+GAD+/GAD+
95.7 ± 4.7	11.4 ± 2.2

Table 3. Coexistence of TH and SP in periglomerular region. Number of hamster is 4. Total number of cells analysed is 930. Values (%) are mean ± standard deviation

A: Proportion of perikarya showing immunoreactivities for both SP and TH(SP+TH+), only SP(SP+TH–) and only TH(SP–TH+) in all perikarya showing SP-like and/or TH-like immunoreactivities (100%)

SP+TH+	SP+TH–	SP–TH+
27.2 ± 4.3	0.9 ± 0.6	71.9 ± 4.8

B: Number of double-labeled neurons expressed as per cent of total number of SP-LI or TH-LI neurons

SP+TH+/SP+	SP+TH+/TH+
97.1 ± 2.0	27.2 ± 4.6

unstained profiles in sections incubated with anti-GAD serum.

3. Coexistence of GAD-like and SP-like immunoreactivities

As previously described, SP-LI neurons varied rather considerably in number from region to region. However, when we compared two paired surfaces of adjacent sections incubated with anti-GAD and anti-SP sera, respectively, almost all SP-LI neurons also contained GAD-like immunoreactivity regardless of their numerical densities (Fig. 5).

Table 2A and B show the quantitative analysis of the coexistence of GAD-like and SP-like immunoreactivities in areas showing many SP-LI neurons (see Discussion). In these areas, about 10% of GAD-LI neurons appeared to also contain SP-like immunoreactivity.

4. Coexistence of TH-like and SP-like immunoreactivities

Similarly to the coexistence of GAD-like and SP-like immunoreactivities, almost all SP-LI neurons were also TH-like immunoreactive (Fig. 6). Table 3A and B show that in the areas where many SP-LI neurons were seen, about 27% of TH-LI neurons also contained SP-like immunoreactivity.

As almost all SP-LI neurons were GAD-like immunoreactive and TH-like immmunoreactive, we can conclude that some neurons in the periglomerular region of the hamster main olfactory bulb contain immunoreactivities for three antigens, that is, GAD, TH and SP.

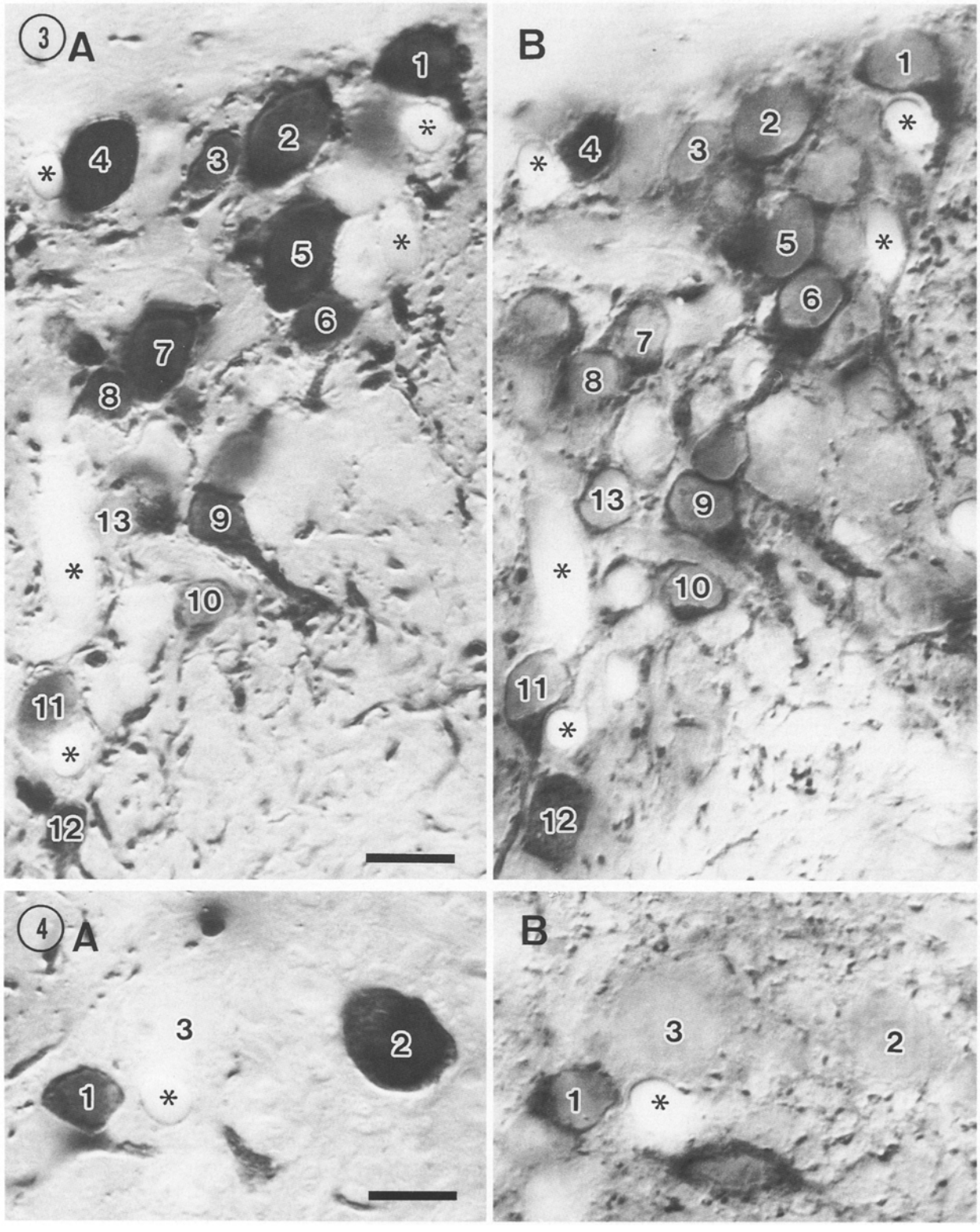
Discussion

In the present paper, we have shown in the hamster main olfactory bulb 1) that immunoreactivities for TH and GAD colocalize in some neurons in the periglomerular region, 2) that almost all SP-LI neurons contain GAD-like immunoreactivity and 3) that almost all SP-LI neurons contain TH-like immunoreactivity. From the latter two findings, we can safely conclude that in the hamster main olfactory bulb, almost all SP-LI neurons also contain immunoreactivities for both TH and GAD, that is, these neurons contain immunoreactivities related to three different categories of neuroactive substances, amino acid, amine and peptide.

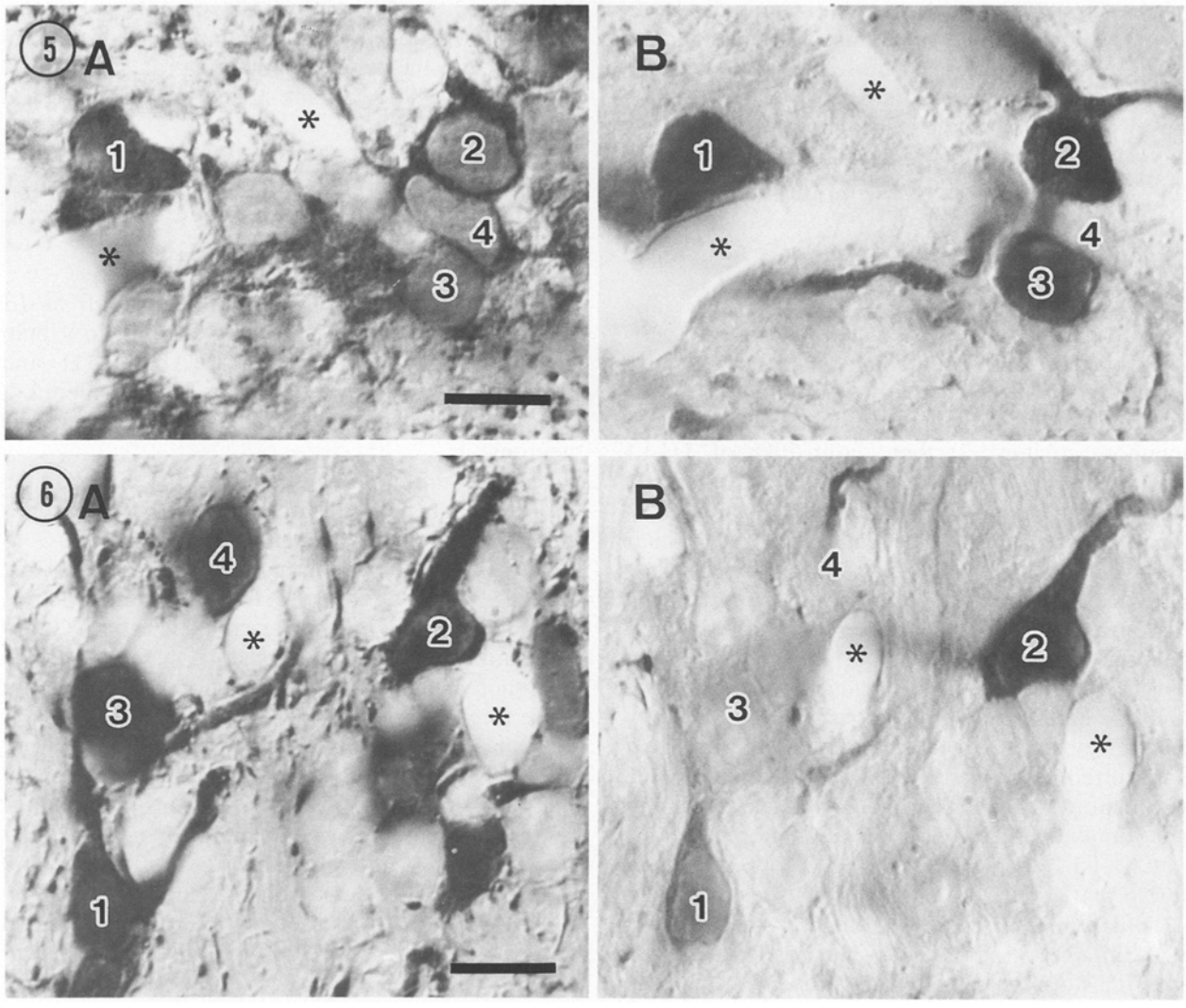
In this section, we would like to discuss the following three points; 1) materials, that is, the problem of possible rhinitis, 2) coexistence of TH and GAD and the identification of cell types, and 3) the coexistence of three antigens, GAD, TH, and SP.

1. Materials

In the present study, we used so-called conventional syrian hamsters because specific pathogen free (SPF) hamsters were not available to us. As is well known, many rodents are assumed to be affected by rhinitis which is supposed to cause to some extent degeneration of olfactory receptors and thus deafferentation of primary olfactory nerves from the olfactory bulb. Deafferentation and functional olfactory deprivation are known to cause a considerable reduction of the TH-like and SP-like immunoreactivities in the olfac-



tory bulb (Baker et al. 1983; Kawano and Margolis 1982; Kream et al. 1984; Brunjes et al. 1985; Kosaka et al. 1987d), whereas at least functional olfactory deprivation appears not to cause such drastic effects on the GAD-like immunoreactivity (Kosaka et al. 1987d). As SP-LI neurons might be far smaller in



Figs. 3–6. Nomarski optics photomicrographs of paired surfaces of two consecutive 40 μm sections incubated with two different antisera. Scale bars = 10 μm

Fig. 3A, B. Periglomerular region. Two adjacent sections incubated with anti-TH serum (**A**) and anti-GAD serum (**B**). Many cells (1–12) show both immunoreactivities. Cell 13 is immunoreactive for GAD but is recognized as an unstained profile in **A.** Asterisks indicate profiles of blood vessels as landmarks

Fig. 4A, B. Periglomerular region at the border of the superficial part of external plexiform layer. Two adjacent sections incubated with anti-TH serum (**A**) and anti-GAD serum (**B**). Cell 1 is immunoreactive for both TH and GAD, whereas cell 2 shows only TH-like immunoreactivity. Cell 3 shows neither TH-like nor GAD-like immunoreactivity. Asterisk indicates an profile of a blood vessel as landmark

Fig. 5A, B. Periglomerular region. Two adjacent sections incubated with anti-GAD serum (**A**) and anti-SP serum (**B**). Cells 1–3 show both immunoreactivities, whereas cell 4 shows only GAD-like immunoreactivity. Asterisks indicate blood vessels as landmarks

Fig. 6A, B. Periglomerular region. Two adjacent sections incubated with anti-TH serum (**A**) and anti-SP serum (**B**). Cells 1 and 2 show both TH-like and SP-like immunoreactivities, whereas cells 3 and 4 show only TH-like immunoreactivity. Asterisks indicate blood vessels as landmarks

number than TH-LI and GAD-LI ones and might lose their immunoreactivity more easily than at least GAD-LI ones, SP-LI neurons were assumed to unmask easily the effects of rhinitis in some forms such as variations in the number of immunoreactive neurons among animals and/or regions.

Taking these into consideration, we analysed the areas containing relatively large numbers of SP-LI elements on the assumption that those areas might be less affected by rhinitis, although not perfectly intact. This assumption may be supported to some extent by our quantitative analysis on the coexistence of TH

and GAD immunoreactivities shown in Table 1, where the mean values and especially the standard deviations are very close to those of 5–12 weeks old SPF rats shown in Table 2 in Kosaka et al. (1987a). Thus, we can consider the values shown in Tables 1–3 as good approximations in hamsters unaffected by rhinitis.

2. Coexistence of TH-like and GAD-like immunoreactivities

The present study revealed 1) that in the periglomerular region of the hamster main olfactory bulb about 40% of all neurons containing TH-like and/or GAD-like immunoreactivities contain both immunoreactivities, 2) that about 70% of TH-LI neurons contain GAD-like immunoreactivity and 3) that about 45% of GAD-LI neurons also contain TH-like immunoreactivity. These values are very similar to those of rat main olfactory bulb reported previously (Kosaka et al. 1987a, b). Thus, regarding the relationship between GABAergic and catecholaminergic properties in the periglomerular region, hamster and rat appear to be similar to each other.

In the hamster main olfactory bulb, the majority of TH-LI neurons (more than 80%) were proposed to be tufted cells on the basis of light microscopic examinations of immunohistochemical materials and their comparison with Golgi-impregnation materials (Davis and Macrides 1983). Our present study showed that about 70% of TH-LI neurons contained GAD-like immunoreactivity, which is supposed not to be contained in tufted cells (Halász and Shepherd 1983). This discrepancy between these two studies is apparently due to the definition or identification of cell types. In immunohistochemical studies, the identifications of cell types are only tentatively done by comparing sizes and shapes of immunoreactive perikarya and dendrites to those of Golgi-impregnated neurons, but it seems occasionally very difficult to identify cell types in this way. Furthermore, morphologically indistinguishable neurons occasionally appear to be different in chemical nature and vice versa. Presumably it might now be necessary to define a cell type based on both morphological and chemical features. The first step for us to try to solve cell identification problems might be the combined immunocytochemical and Golgi-electron microscopic analysis.

In addition to rat and hamster, the coexistence of immunoreactivities for GABA and/or GAD and TH was very common in olfactory bulbs of other mammals such as cat and mouse (our unpublished observations). Furthermore, this coexistence was also seen in olfactory bulbs of submammals such as shark (chondroichthyes), goldfish (teleost), frog (amphibian) and snake (reptile) (our unpublished observations). Thus, the neurons containing catecholaminergic and GABAergic traits appear to be conserved phylogenetically.

3. Coexistence of immunoreactivities for SP, TH and GAD

The present study showed that almost all SP-LI neurons in the syrian hamster main olfactory bulb contained immunoreactivities for both TH and GAD, and thus that they can be considered a subpopulation of so-called GABA-catecholaminergic neurons.

Furthermore, we can calculate the approximate proportion of chemically defined neuronal subgroups in the periglomerular region from Tables 1–3 as follows: of all neurons immunoreactive for GAD or TH or SP, 47% are only GAD-like immunoreactive, 15% only TH-like immunoreactive, 28% both GAD-like and TH-like immunoreactive without SP-like immunoreactivity, and 9% immunoreactive for all of these three.

Our conclusion in the present study is very different from that of Baker (1986b), who proposed that SP-LI neurons and TH-LI neurons in the hamster olfactory bulb are completely separate populations in spite of their similarity in morphology and distribution. This discrepancy might be caused by differences in methods used for colocalization of two antigens in the same neurons. We used adjacent section technique where immunohistochemical procedures are performed perfectly independently and thus we have no need to consider the interactions between two immunostainings. On the other hand, Baker (1986b) used the two-color double PAP method employing different color chromogens. As previous systematic studies (Sterberger and Joseph 1979; Joseph and Sternberger 1979) clearly showed, without critical adjustments of incubation conditions such as concentrations of primary antisera and those of chromogens, this method occasionally causes no color mixing even when two antigens are really colocalized in the same structures. It is also noteworthy that in one of the successful examples of the application of this two-color double PAP method, Oertel et al. (1983) considered the differential distribution of somatostatin (restricted at perinuclear region) and GAD (distributed diffusely in perikaryal and dendritic cytoplasm) immunoreactivities as the main reason for their simultaneous localization.

Our observations indicate that three different categories of neuroactive substances, that is, peptide

(SP), amine (presumably dopamine) and amino acid (GABA), colocalize in the same neurons. Although many examples of the coexistence of a classical transmitter and peptide(s) (Hökfelt et al. 1986 for review) and several examples of the coexistence of two classical transmitters (Kosaka et al. 1988 for the list of these examples) are reported, the possible coexistence of amino acid, amine and peptide(s) has not been reported previously so far as we know. However, this kind of coexistence may be supposed to be seen in some other regions such as rat cerebral cortex and rat raphe nuclei. In the rat cerebral cortex, almost all intrinsic neurons containing choline acetyltransferase (ChAT)-like immunoreactivity are reported to also contain vasoactive intestinal polypeptide (VIP)-like immunoreactivity (Eckenstein and Baughman 1984). Recently we (Kosaka et al. 1988) found that about 50% of ChAT-like immunoreactive cortical neurons contain GABA-like immunoreactivity, indicating that some intrinsic cortical neurons may contain GABA, acetylcholine and VIP. Milhorn et al. (1987) reported the coexistence of GAD-like and 5-hydroxytryptamine (5-HT)-like immunoreactivities in the rat raphe nuclei where the coexistence of 5-HT-like immunoreactivity with SP-like and/or thyrotropin-releasing hormone (TRH)-like immunoreactivities was also reported (Chan-Palay et al. 1978; Hökfelt et al. 1978; Johansson et al. 1981). Thus, in the rat raphe nuclei GABA, 5-HT and SP (and/or TRH) may be contained in the same neurons.

Kream et al. (1984) reported the simultaneous reduction of SP and catecholaminergic expression in neurons of the hamster olfactory bulb induced by deafferentation of primary olfactory nerves. In conjunction with the present observations, their results might be interpreted as follows; SP and catecholaminergic traits coexisting in the same neuron are coregulated in the same direction by the primary afferent nerves. Our preliminary experiments indicated that SP-like and TH-like immunoreactivities could also be reduced by functional olfactory deprivation without deafferentation, as does the TH-like immunoreactivity in the rat olfactory bulb (Kosaka et al. 1987d), suggesting these two expressions might be dependent on neuronal activities.

On the other hand, SP and catecholaminergic traits are also reported to coexist in sympathetic neurons where these two appear to be coregulated in the opposite directions; that is, deafferentation decreases catecholaminergic traits but increases SP (Black et al. 1987 for review). Regulation of SP expression might be somewhat different between olfactory bulb neurons and sympathetic neurons.

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