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Types of Cholecystokinin-Containing Periglomerular Cells in the Mouse Olfactory Bulb

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The periglomerular cells (PG) of the olfactory bulb (OB) are involved in the primary processing and the refinement of sensory information from the olfactory epithelium. The neurochemical composition of these neurons has been studied in depth in many species, and over the last decades such studies have focused mainly on the rat. The increasing use of genetic models for research into olfactory function demands a profound characterization of the mouse olfactory bulb, including the chemical composition of bulbar interneurons. Regarding both their connectivity with the olfactory nerve and their neurochemical fate, recently, two different types of PG have been identfied in the mouse. In the present report, we analyze both the synaptology and the chemical composition of specific PG populations in the murine olfactory bulb, in particular, those containing the neuropeptide cholecystokinin. Our results demonstrate the existence in the mouse of non-GABAergic PG and that these establish synaptic contacts with the olfactory nerve within the glomeruli. Based on previous classifications, we propose that this population would constitute a new subtype of type 1 mouse PG. In addition, we demonstrate the partial coexistence of cholecystokinin with the calcium-binding proteins neurocalcin and parvalbumin. All these findings add further data to our knowledge of the synaptology and neurochemistry of mouse PG. The differences observed from other rodents reflect the neurochemical heterogeneity of PG in the mammalian OB.

Key words: cholecystokinin; juxtaglomerular neurons; neurocalcin; olfactory glomerulus

One of the most distinctive structures of the olfactory bulb (OB) is the olfactory glomerulus (Pinching and Powell, 1971). Inside the olfactory glomeruli, the olfactory nerve (ON) axons establish synapses with the dendrites of the OB projecting neurons or with interneurons (Mori et al., 1999). Glomeruli are involved in the processing and transmission of olfactory information. Each glomerulus is divided into two zones: the ''sensory compartment'' and the ''synaptic compartment,'' (Gutièrrez-Mecinas et al., 2005). The sensory compartment comprises preterminals and terminals of ON axons, which establish excitatory synapses with the dendrites of both projecting neurons and interneurons (Kosaka et al., 1998; Gutièrrez-Mecinas et al., 2005). The synaptic compartment comprises the dendritic processes of interneurons that establish inhibitory synapses with mitral/tufted cells and interneurons.

Glomeruli are surrounded by different interneurons known as juxtaglomerular neurons, periglomerular cells (PG) being the most widely represented (Pinching and Powell, 1971), which modulate the sensory information into the glomeruli. The neurochemical composition of PG has been studied in depth in the rat. It was originally reported that the entire population of PG used GABA as a neurotransmitter (Shepherd and Greer, 1998), but later studies revealed the existence of both GABAergic and non-GABAergic PG subpopulations (Kosaka et al., 1998; Toida et al., 1998; Briñón et al., 1999; Crespo et al., 2003; Gutièrrez-Mecinas et al., 2005). According to their synaptic contacts with the ON axons, rat PG are

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classified into two types (Kosaka et al., 1998). Type 1 PG form synapses with ON axons into the sensory compartment, whereas type 2 PG establish few or no synapses with them, and their dendrites are located in the synaptic zone (Gutièrrez-Mecinas et al., 2005). Based on its neurochemical profile, each type is divided into different subtypes. Type 1 PG are GABAergic, expressing TH (and hence dopaminergic) or neuronal nitric oxide synthase (Crespo et al., 2003). A small group of rat type 1 PG is non-GABAergic and contains cholecystokinin (CCK) or somatostatin (Gutièrrez-Mecinas et al., 2005). Type 2 are non-GABAergic and express calbindin (CB) or calretinin (CR), being differentiated into two subtypes (Kosaka et al., 1995; Toida et al., 1998).

This compartmentalized organization of glomeruli and the division of PG into different types has been described for different mammalian species (Kosaka and Kosaka, 2001, 2004; Baltanás et al., 2007). However, the neurochemical composition of PG reported for the rat is not the same as that in other mammal species (López-Mascaraque et al., 1989; Alonso et al., 1995; Briñón et al., 2001; Crespo et al., 2002).

Currently, the neurochemical characterization of PG is focused on the mouse. Indeed, two different types of PG have been demonstrated (Kosaka and Kosaka, 2007; Panzanelli et al., 2007; Parrish-Aungst et al., 2007; Kiyokage et al., 2010). As in the rat, type 1 PG send their dendrites into the sensory compartment, whereas type 2 confine them within the synaptic cleft (Kosaka and Kosaka, 2007). Additionally, type 1 PG are GABAergic, containing glutamic acid decarboxylase (GAD) of 65 kDa or 67 kDa, or both (Parrish-Aungst et al., 2007; Kiyokage et al., 2010). Most type 1 PG are GAD67-positive, whereas only 30% of GAD65-reactive PG receive inputs via ON axons (Kiyokage et al., 2010). Additionally, a subset of type 1 PG that are dopaminergic has been described (Parrish-Aungst et al., 2007; Kiyokage et al., 2010).

Type 2 PG are GABAergic, containing CB or CR (Kosaka and Kosaka, 2007; Panzanelli et al., 2007; Parrish-Aungst et al., 2007). Most of them contain the GAD67 rather than the GAD65 (Kiyokage et al., 2010). These data indicate that the compartmentalization of glomeruli is maintained in the mouse OB and also that the neurochemical composition of PG shows divergence compared with the rat. Here, both the synaptology and the neurochemical features of PG containing CCK, which label a subtype of the *rat* type 1 PG (Gutiérrez-Mecinas et al., 2005), were analyzed. Our results support the characterization of a new subpopulation of PG in the mouse OB. Additionally, our study reveals that the chemical composition of mouse PG exhibits evident differences from the compositions described previously for other rodents.

MATERIALS AND METHODS

Animals and Tissue Preparations

Adult (P90) male mice (n = 9) from B6EiC3Sn-a littermates were used. The animals were kept, handled and sacrificed following the animal care rules of the Council of the European Communities (86/609/EEC) and current Spanish legislation (RD 1201/2005), and the experiments were approved by the Bioethical Committee of the University of Salamanca. The generation of transgenic mice expressing enhanced green fluorescent protein (GFP) under control of the regulatory region of the mouse GAD65 gene has been described previously (Parrish-Aungst et al., 2007; Kiyokage et al., 2010). Briefly, heterozygous transgenic mice were generated, expressing GFP under the control of the 65-kDa glutamic acid decarboxylase promoter (GAD65-GFP from the GAD65_3e/gfp5.5 No. 30 line in a genetic background of C57BL6 with an F1 backcross to B6CBAF1/J wild-type mice, yielding mice heterozygous for the transgene). In this line, a 6.5-kb segment of the GAD65 gene including 5.5 kb of the 5′-upstream region, the first two exons, and a portion of the third exon with the corresponding introns drives GFP expression in GABAergic neurons in most brain regions.

After anesthesia with a mixture of xylazine (Rompum; Bayer, Kiel, Germany) and ketamine hydrochloride (Imalgene, Merial, Lyon, France), mice were perfused intraortically with heparinized saline for 1 min and fixative solution containing 4% (w/v) paraformaldehyde and 0.2% (w/v) saturated picric acid in 0.1 M phosphate buffer (PB), pH 7.4, for 15 min. After perfusion, the OB were dissected out and postfixed in the same solution for 2 hr at room temperature. Then, they were washed in PB and cryoprotected with 30% (w/v) sucrose overnight at 4°C until they sank. Thirty-micrometer-thick coronal sections were cut using a freezing-sliding microtome (Leica Frigomobil, Jung SM 2000, Nussloch, Germany); the slices were collected in PB, pH 7.4, and stored at –20°C in a freezing mixture containing 30% (v/v) glycerol and 30% (v/v) polyethylene glycol in 0.1 M PB, pH 7.4.

The OB destined for electron microscopic analyses were obtained by prior perfusion with a fixative solution composed of 4% (w/v) paraformaldehyde, 0.5% (w/v) glutaraldehyde, and 0.2% saturated picric acid (w/v) in 0.1 M PB, pH 7.4. The OB were dissected and postfixed in the same solution (without glutaraldehyde) for 2 hr. Fifty-micrometer-thick sections were cut on a vibratome and stored at 4°C in 0.1 M PB containing 0.05% sodium azide.

Immunofluorescence

To check the nature of CCK-positive PG, we performed double immunostaining for this neuropeptide and GAD67 or TH (to identify type 1 PG) and CB or CR (to recognize type 2 PG). In addition, to complete the study of the neurochemical composition of these neurons, we investigated whether they contained the calcium-binding proteins NC and PV, which have previously been employed for characterization of the PG of many other species. Coronal sections were washed in phosphate-buffered saline (PBS), treated with 1% (w/v) $NaBH_4$ in 0.1 M PBS for 20 min, and rinsed in PBS (Weruaga-Prieto et al., 1996). Then, the sections were incubated for 1 hr at room temperature in blocking serum containing 5% (v/v) normal goat serum (NGS) and 0.2% (v/v) Triton X-100 in PBS. Primary antibodies (Table I) were diluted in the same solution and incubated for 72 hr at 4°C.

TABLE I. Primary Antibodies

Primary antibody	Reference or source	Dilution
Rabbit anticalbindin D-28k	Swant, Bellinzona, Switzerland (CB-38)	1:7,000
Rabbit anticalretinin	Swant (7699/3H)	1:2,000
Rabbit antineurocalcin	Nakano et al., 1992	1:3,000
Rabbit antiparvalbumin	Swant (PV 25)	1:2,000
Mouse antiparvalbumin	Swant (PV235)	1:1,000
Rabbit antityrosine hydroxylase	Institute Jacques Boy, Reims, France (No. 208020234)	1:10,000
Mouse antityrosine hydroxylase	Chemicon, Temecula, CA (clone LNC1 No. MAB5406)	1:7,000
Mouse anti-GAD67	Chemicon (clone 1G10.2 No. MAB318)	1:1,000
Goat anti-GFP	Abcam, Cambridge, United Kingdom (ab290)	1:1,000
Mouse anticholecystokinin	Cure Digestive Diseases Research Center, University of California	1:800

After washing in PBS, the Cy3- and Cy2-conjugated secondary antibodies (1:500; Jackson Immunoresearch, West Grove, PA) were applied together for 2 hr. Finally, after a rinse in PBS, sections were mounted on gelatin-coated slides and coverslipped with antifade solution. Controls were carried out by incubating the sections in the absence of primary or secondary antibodies, incubating only one primary antibody with no corresponding secondary antibody. In these experiments, we failed to detect nonspecific binding or cross-reactions. The resulting material was examined with a confocal microscope (Leica TCS SP2, Mannheim, Germany).

Electron Microscopy

Free-floating sections were treated with $NaBH_4$ 1% in PB for 30 min and extensively rinsed in 0.1 M PB. Once the sections had been cryoprotected by immersing them for 30 min in 25% (w/v) sucrose and 10% (v/v) glycerol in 0.05 M PB, they were freeze-thawed three times in liquid nitrogen to enhance antibody penetration. The avidin-biotin-peroxidase method (Hsu et al., 1981) was used for immunostaining. Sections were sequentially incubated in 1) blocking solution, containing 10% (v/v) of NGS and 2% (w/v) bovine serum albumin (BSA; Sigma, St. Louis, MO; A9647) in PB for 45 min at room temperature; 2) 1:800 monoclonal mouse anti-CCK in PB containing 5% NGS for 72 hr at 4°C; 3) 1:200 biotinated goat anti-mouse IgG in PB (Vector, Burlingame, CA) for 2 hr at room temperature; and 4) 1:200 avidin-biotinylated horseradish peroxidase complex in PB (Vector) for 2 hr at room temperature. After each step, sections were carefully rinsed in PB (3 × 10 min).

Finally, the peroxidase reaction was developed, using 0.05% (v/v) diaminobenzidine tetrahydrochloride (Sigma; D5637) as chromogen and 0.003% hydrogen peroxide in PB, until the labeling could be clearly visualized under the microscope. Sections were then carefully rinsed in PB (3 × 10 min), treated with 1% (v/v) osmium tetroxide (Electron Microscopy Sciences, Hatfield, PA; 19150) for 1 hr at room temperature, and washed in PB. They were then stained with 1% (w/v) uranyl acetate for 90 min, dehydrated through graded ethanol series, cleared in propylene oxide, and flat embedded in Durcupan (ACM; Fluka AG). Durcupan was polymerized overnight at 60°C.

Flat-embedded sections were examined under light microscopy, and after analysis selected sections containing CCK-positive PGs were reembedded in Durcupan. Ultrathin serial sections were obtained with an ultramicrotome (Leica) and mounted on Formvar-coated single-slot nickel grids. Finally,

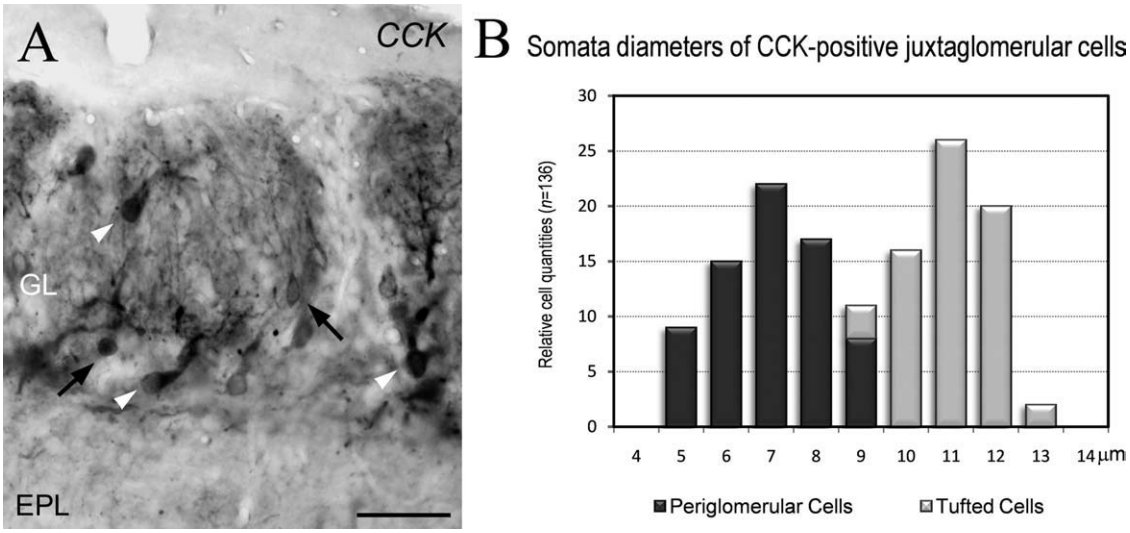

Fig. 1. CCK-positive juxtaglomerular cells conform two neuron populations. **A:** CCK-immunoreactive juxtaglomerular neurons located in the GL of the mouse OB, as seen with light microscopy. After diaminobenzidine staining, both external tufted cells (arrowheads) and PG (arrows) can be observed. Note the differences in both the morphological features and the degree of immunoreactivity for this neuropeptide between both types of interneurons. Scale bar = 40 µm. **B:** According to the size distribution of their somata, CCK-positive cells around glomeruli can be divided into two classes that match PG cells (7.5 ± 1.2 µm; mean ± SD) and external tufted cells (11.6 ± 1.0 µm) according to other morphological considerations.

the ultrathin sections were stained with lead citrate and analyzed under an electron microscope at 100 KV (Jeol, JEM-1010).

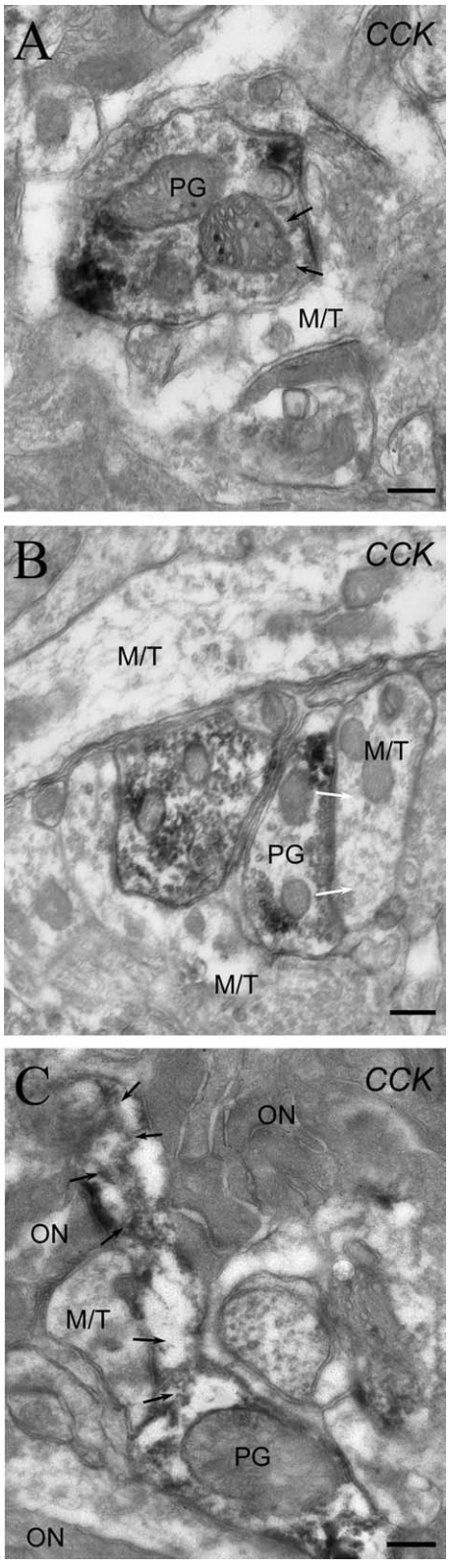

Image Analysis

A quantitative analysis of double-immunostained PG was performed. Five equidistant levels throughout the rostrocaudal axis of the two OBs of each mouse were established, as previously described (Weruaga et al., 1996; Murias et al., 2007). Then, coronal sections were divided into four quadrants: dorsal, lateral, ventral, and medial. The positive neurons included in all these divisions were counted for each marker in ImageJ software (v.1.38x; National Institutes of Health). To measure the maximum diameter of CCK-positive juxtaglomerular neurons, ImageJ was used, analyzing the whole glomerular layer of the medial quadrants in central levels of the OB.

RESULTS

Distribution and Size of CCK-Positive Mouse PG

The immunohistochemical studies performed for the detection of CCK revealed positive neurons in all bulbar layers but not in the olfactory nerve or in the mitral cell layers. They were mainly confined to the GL of the mouse OB (Fig. 1A). The diameter measurements of the juxtaglomerular cells of a central section (medial quadrant) pointed to a typical bimodal size distribution with two peaks (n = 136; Fig. 1B), suggesting two neuronal types. CCK-positive neurons were identified as both external tufted cells and PG. Both types of neuron could be discerned from their morphologies: the PG were smaller (7.5 ± 1.2 μm; mean ± SD) than the external tufted cells (11.6 ± 1.0 μm), and the PG soma was round, with one or two very thin primary dendrites, whereas the external tufted cells had a more spindle-like shape and a thick primary dendrite. In addition, the PG exhibited a lower degree of CCK immunoreactivity than the external tufted cells.

CCK-Reactive PG Belong to Type 1 Mouse PG

As described in the introductory paragraphs, the classification of the PG is based mainly on the establishment of synaptic contacts with the olfactory nerve within the glomeruli. Accordingly, we first analyzed the synaptology of CCK-immunoreactive mouse PG using electron microscopy. Gemmules of CCK-positive PG were distributed in both the sensory and the synaptic compartments of the glomerular neuropil, but they showed the synaptic features of type 1 PG; i.e., they

Fig. 2. CCK immunoreactivity in the mouse GL: electron microscopy. CCK-immunoreactive profiles located in the GL of the mouse OB, in ultrathin sections for electron microscopy after free-floating CCK immunodetection. **A:** A mitral/tufted neuron forms an asymmetric contact with a CCK-positive PG profile (black arrows). **B:** A CCK-positive profile forms a typical PG symmetric synapsis onto a mitral/tufted dendrite (white arrows). **C:** Two olfactory nerve terminals (ON) form asymmetric contacts (pairs of black arrows) with a CCK-positive PG gemmule, which also receives another asymmetric synapsis from a mitral/tufted dendrite; together, these cues demonstrate that CCK-positive PG innervate the sensory compartment. Scale bars = 200 nm.

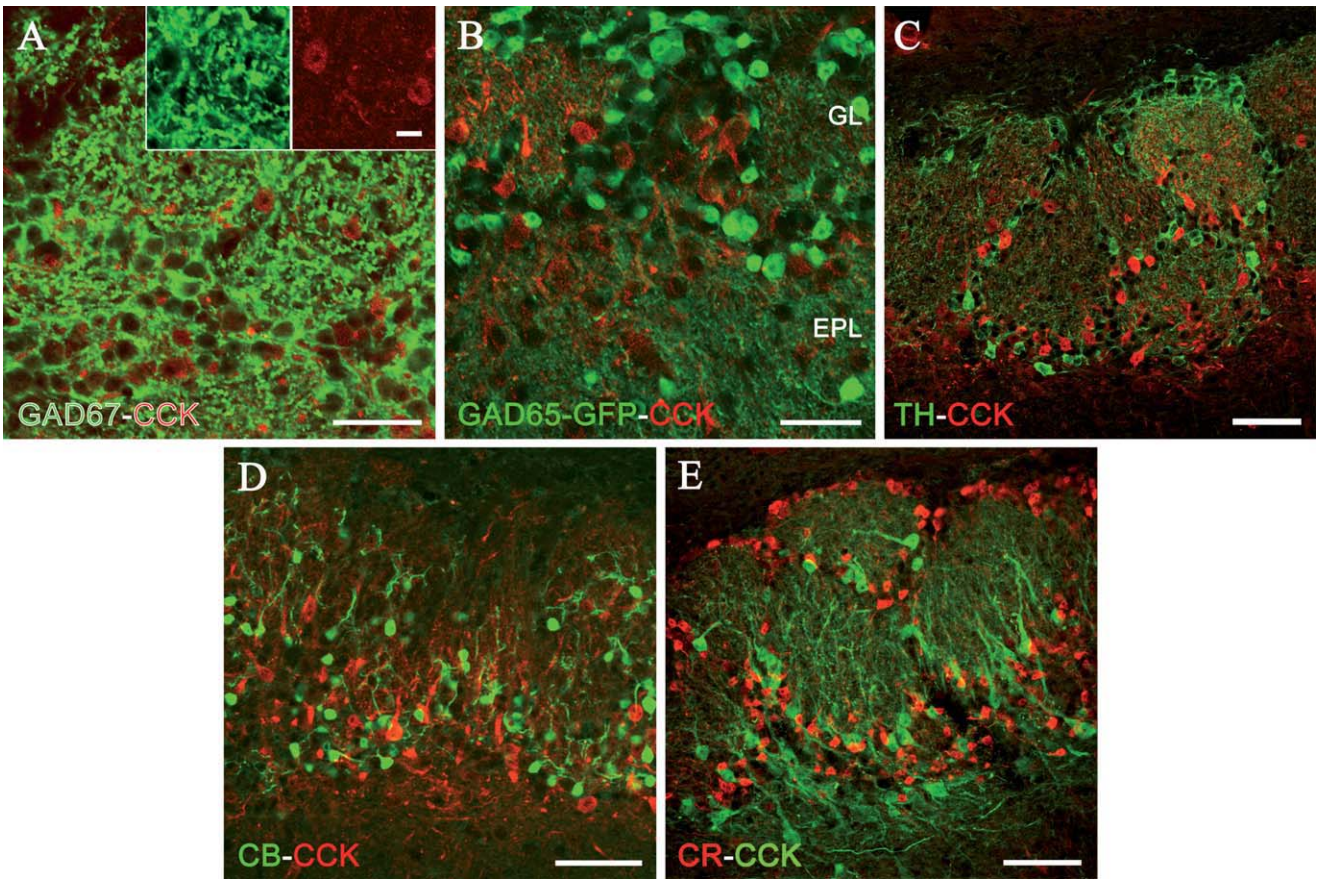

Fig. 3. Analysis of the coexistence for CCK and GAD67/GAD65/TH/CB/CR. A,C,D,E are from wild-type mice; B is from a GAD65-GFP knock-in mouse. **A–C:** Double immunofluorescence for CCK (red) and GAD67, GAD65, or TH (green) reveals that neither GABAergic nor dopaminergic mouse PG contain CCK. **D,E:** Costaining for CCK (red) and CB (D) or CR (E) shows that CCK does not contain these calcium-binding proteins in the PG of the mouse OB. Scale bars = 40 μm in A–E; 10 μm in insets. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

made synaptic contacts with ON axons. Gemmules of CCK-positive PG were postsynaptic in asymmetric contacts from the ON and projection neurons (mitral and tufted cells) and presynaptic in symmetric synapses to projection neurons (Fig. 2A–C).

CCK-Positive Periglomerular Cells Constitute a New Non-GABAergic Subtype of Mouse Type 1 Periglomerular Cells

The electron microscopic study revealed that CCK-positive mouse PG should be included in type 1. A previous report (Kosaka and Kosaka, 2007) has described how type 1 mouse PG are GABAergic cells that also express TH (a specific marker for dopaminergic neurons). We therefore examined whether CCK-positive PG belong to the GABAergic and dopaminergic phenotype. Thus, double immunofluorescence for CCK and GAD67, GAD65, or TH was performed (Fig. 3). Our analysis revealed that CCK-reactive mouse PG were neither GABAergic nor dopaminergic (Fig. 3A–C). Accordingly, CCK-reactive mouse PG should be included within a new subtype of type 1 mouse PG.

Type 2 mouse PG are GABAergic PG that contain CB or CR (Kosaka and Kosaka, 2007). We therefore checked that CCK-positive PG did not display the neurochemical features of type 2 mouse PG (Fig. 3D,E). Our results clearly demonstrated that, as in the rat (Gutièrrez-Mecinas et al., 2005), CCK-immunoreactive PG did not share the neurochemical features of type 2 PG (Fig. 3D,E).

CCK-Positive Periglomerular Cells Contain Neurocalcin and Parvalbumin

We also analyzed whether CCK-reactive PG might have additional neurochemical features. NC or PV are markers of PG subpopulations (Briñón et al., 1999; Murias et al., 2007). Accordingly, we performed an additional study to determine whether CCK-reactive mouse PG might express these calcium-binding proteins. Our results clearly revealed that CCK-positive PG expressed both NC and PV. In fact, quantitative analyses revealed that a high percentage of CCK-immunolabelled PG expressed

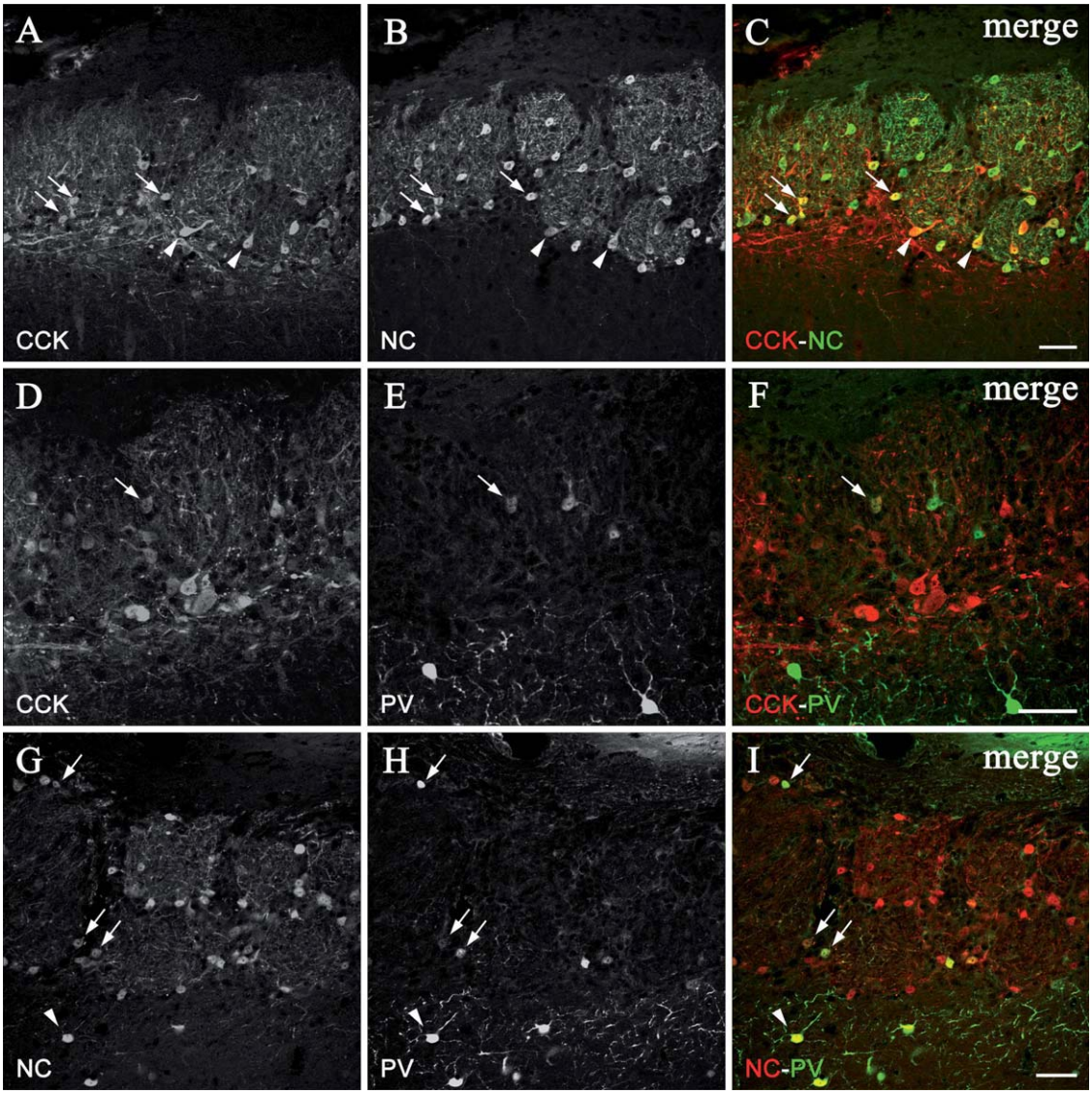

Fig. 4. CCK/NC/PV-reactive PG constitute an overlapping mouse PG population. **A–C:** Double immunostaining for CCK and NC demonstrates that both PG (arrows) and external tufted cells (arrowheads) coexpress both markers. Some juxtaglomerular neurons containing a single marker can be observed. **D–F:** Analysis of the coexpression of CCK and PV reveals the existence of a subpopulation of mouse PG containing both proteins (arrow). **G–I:** Most PV-positive PG coexpress NC in the mouse OB (arrows); in addition, interneurons located in the EPL also contain both markers (arrowhead). Scale bars = 40 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

NC (85.26% ± 7.59%; Fig. 4A–C) and that a certain proportion of NC-reactive PG contained CCK (60.27% ± 8.25%). Most of the scarce PV-immunostained PG present in the GL coexpressed CCK (81.22% ± 13.08%; Fig. 4D–F). CCK-reactive PG were more numerous than those expressing PV, so only about one-third of CCK-positive PG also coexpressed PV (30.10% ± 8.49%). Because the CCK-positive PG contained both calcium-binding proteins, we also checked whether NC-reactive PG expressed PV and vice versa (Fig. 4G–I). This part of the study revealed that almost all PV-reactive PG contained NC (95.79% ± 6.89%), whereas only about 10% of NC-positive PG expressed PV (10.89% ± 2.90%). Moreover, NC- and/or PV-reactive PG did not coexpress the markers that characterize either type 1 (GAD67 and TH; Fig. 5A–D) or type 2 (CB and CR; Fig. 5E–H). However, it should be noted that there were both NC- and PV-positive PG that did not contain CCK.

DISCUSSION

CCK-Positive PG Are a New Mouse Type 1 PG Subpopulation

Recently, in the mouse OB, two major types of PG have been described, based on the previous classifi-

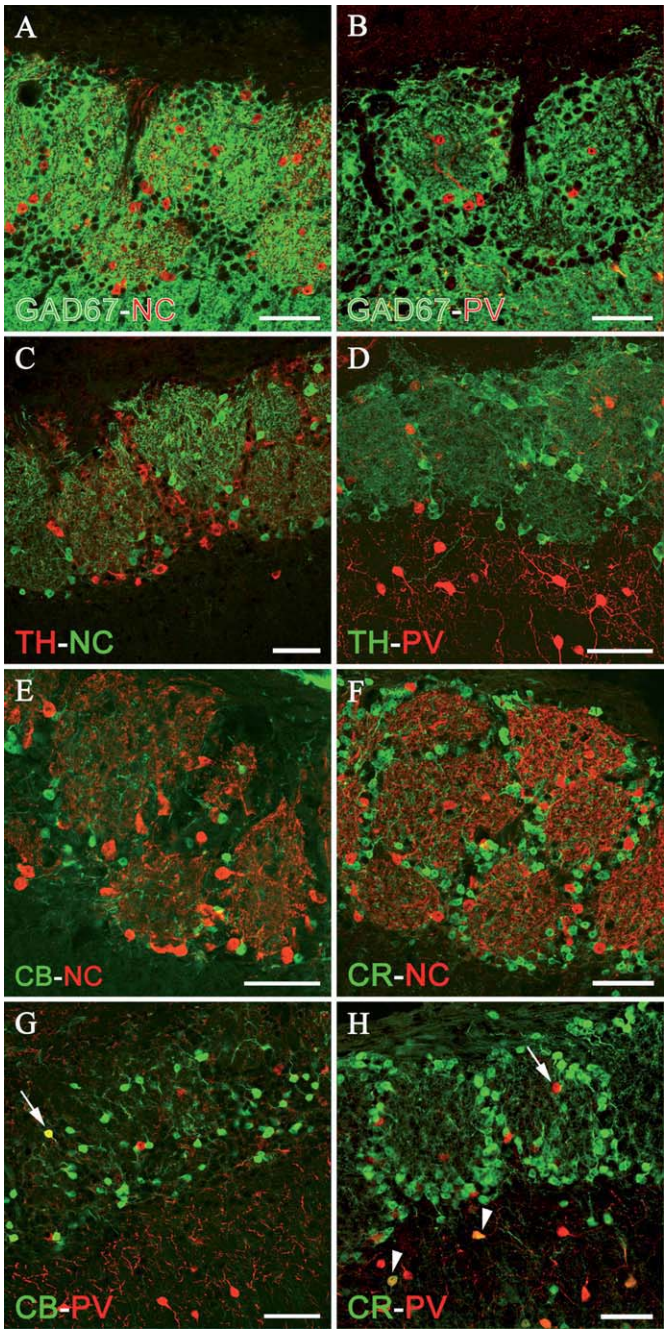

Fig. 5. NC- and PV-positive mouse PG are not included in either type 1 or type 2 PG. **A–D:** NC is not expressed by either the GABAergic or the dopaminergic population located in the GL. **E,F:** PV-positive (red) and GABAergic- or dopaminergic-reactive PG (green) constitute nonoverlapping PG subpopulations in the mouse OB. NC-positive PG do not express CB (E) and rarely exhibit immunoreactivity for CR (F). **G:** Colocalization analysis for PV and CB reveals that very few PG contain both markers (arrow). **H:** Double immunostaining for PV and CR shows that PV is not contained in CR-reactive PG (arrow); however, double-labeled interneurons located in the EPL (arrowheads) can be observed. Scale bars = 40 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cation for the rat olfactory system (Kosaka and Kosaka, 2007). Murine type 1 PG are both GABAergic (mainly GAD67 reative) and dopaminergic, sending their dendrites into the sensory compartment (Kosaka and Kosaka, 2007; Parrish-Aungst et al., 2010; Kiyokage et al., 2010). Type 2 PG also comprise GABAergic (mainly GAD67 positive, but not dopaminergic) neurons that do not form synapses with ON axons and that express CB or CR (Kosaka and Kosaka, 2007; Kiyokage et al., 2010).

We examined whether the CCK-positive population should be included within type 1 or type 2 mouse PG. Electron microscopy analysis revealed that CCK-positive PG form synapses with ON axons in the sensory compartment. Based on the previous classification, we suggest that they should be included in the type 1 mouse PG. Additionally, analysis of the neurochemical composition of CCK-reactive PG revealed that they did not share similarities with the chemical composition of either type 1 or type 2 mouse PG characterized previously (Kosaka and Kosaka, 2007; Parrish-Aungst et al., 2007; Kiyokage et al., 2010). These results are consistent with those previously reported by Parrish-Aungst et al. (2007), who propose the existence of a non-GABAergic PG population, as occurs in the rat OB (Briñón et al., 1999; Gutièrrez-Mecinas et al., 2005). A previous paper reported a technical limitation for the use of GAD67 or GABA to detect all GABAergic PG (Panzanelli et al., 2007). Although this technical limitation cannot be discarded, the present results, together with those reported previously (Parrish-Aungst et al., 2007), strongly suggest the existence of non-GABAergic PG in the mouse OB.

We also observed that a large number of CCK-reactive PG also coexpressed the calcium-binding proteins NC and/or PV. This reflects further differences in the neurochemical composition of the rat PG in comparison with that of the mouse. For example, for the rat, Briñón et al. (1999) reported that about 42% of NC-stained PG colocalized with CR; however, our present results demonstrated that in the mouse these PG constitute segregated populations. The same authors, also with the same animal model, studied the colocalization pattern of NC and PV in the rat OB. They found that small percentages of PG coexpressed both markers (Briñón et al., 1999). In contrast, we show that they constitute an overlapping population in the mouse OB. In sum, although the synaptology of CCK-positive PG is maintained in the mouse OB compared with that of the rat, the neurochemical features of the mouse PG diverge sharply from those described previously for the rat.

Currently, the functions of the neuropeptide CCK in the OB, and in particular in PG, remain uncertain. The possible role of CCK-containing tufted cells in the intrabulbar association system and the bulbar projection system has been documented (Liu and Shipley, 1994; Kosaka and Kosaka, 2007). It is known that by acting as a neuromodulator CCK is involved in inhibitory synaptic transmission through the release of GABA and dopamine by operating on presynaptic $GABA_A$ and $GABA_B$

receptors expressed on neighboring PG (Rakovska, 1995a,b; Kombian et al., 2005; Deng and Lei, 2006). It has also been demonstrated that in the OB the gemmules of CCK-positive PG are in close contact with ON axons containing dopamine D2 receptors (Gutièrrez-Mecinas et al., 2005). Thus, CCK-containing PG might exert an inhibitory modulation of these receptors in a way similar to that described for the nucleus accumbens (Tanganelli et al., 2001). In fact, a modulation of the inhibition carried out by the CCK-reactive tufted cells in the intrabulbar association system has been also proposed (Liu and Shipley, 1994).

Because PG exert their functions through inhibitory synapses, and CCK-positive PG do not contain the main inhibitory neurotransmitter (GABA), other molecules must be invoked to account for the modulation exerted by CCK-containing PG. Substances such as glycine or taurine have been proposed to participate as inhibitory neurotransmitter in the OB (Trombley and Shepherd, 1994; Belluzzi et al., 2004). By contrast, other authors have suggested that CCK may be present in excitatory glutamatergic bulbar neurons (Liu and Shipley, 1994; Parrish-Aungst et al., 2007).

Finally, we have demonstrated the presence of a new subpopulation belonging to type 1 mouse PG. These cells enter into contact with ON axons in the sensory compartment of the glomerulus and do not use GABA or dopamine as neurotransmitters. PG that contact ON axons directly may be more susceptible to olfactory input modifications, such as odor deprivation or peripheral deafferentation (Baker et al., 1993; Weruaga et al., 2000). Thus, it is plausible that CCK-expressing PG could be activity-dependent, insofar as they remain within the sensory compartment. However, whether CCK expression itself is affected by sensorial activity or axon innervation remains to be elucidated. Our findings also reflect the differences between the neurochemical composition of the mouse PG and that of other rodents studied. These differences may reflect dissimilar modulation corridors of sensory information in the periglomerular region.

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