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Localization of 5-HT2A, 5-HT3, 5-HT5A and 5-HT7 receptor-like immunoreactivity in the rat cerebellum

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Abstract

Although serotonin (5-hydroxytryptamine, 5-HT) is known to exert a modulatory action on cerebellar function, our current knowledge of the nature of receptor subtypes mediating serotonergic activity in this part of the brain remains fragmentary. In this study, we report the presence and distribution of 5-HT3, 5-HT5A and 5-HT7 receptor-like immunoreactivity in the rat cerebellum using immunofluorescence histochemistry. 5-HT3 immunoreactivity was found in fibers sparsely distributed throughout the cerebellum. Most of them were seen in the cerebellar cortex as fine varicose 5-HT3-positive axonal processes. 5-HT5A immunoreactivity, on the other hand, was observed in neuronal somata of the cerebellar cortex and deep cerebellar nuclei. Based upon cell morphology and the use of cell-specific markers, Purkinje cells, molecular layer interneurons and Golgi cells were found to be 5-HT5A immunopositive. In addition, the use of cell-specific markers allowed us to identify previously reported large 5-HT2A-positive cells in the granular layer as being Golgi cells. Finally, 5-HT7 immunoreactivity was observed only in Purkinje cells. Corroborating previous radioligand-binding, in situ hybridization and immunohistochemical studies, our data relate serotonin receptor subtypes to specific cerebellar cell types and may consequently contribute to the elucidation of serotonergic actions in the cerebellum.

Keywords: Serotonin (5-HT) receptor; Cerebellum; Immunohistochemistry; Purkinje cell; Golgi cell

1. Introduction

The cerebellum is innervated by a plexus of fine varicose serotonin (5-HT)-containing fibers, arising from raphe and reticular nuclei in the brainstem (Takeuchi et al., 1982; Bishop and Ho, 1985). Serotonin exerts its diverse physiological actions through a variety of receptor subtypes, pharmacologically classified into seven groups: 5-HT1–5-HT7 (Hoyer and Martin, 1996; Barnes and Sharp, 1999). Most of these receptors belong to the G-protein-coupled receptor family, with the exception of 5-HT3 (a ligand-gated ion-channel) (Maricq et al., 1991). At present, however, little is known about which receptor subtypes mediate serotonergic activity in the cerebellum. The purpose of the present immunohistochemical study was to examine the presence and distribution of the serotonin receptor subtypes 5-HT3, 5-HT5A and 5-HT7, which may be involved in the modulatory actions of serotonin on cerebellar function (Ruat et al., 1993; Nayak et al., 1999; Oliver et al., 2000).

Data on the expression of 5-HT3 receptors in the cerebellum are inconsistent. Although 5-HT3-selective radioligand-binding studies (Kilpatrick et al., 1987) and mRNA detection studies (Maricq et al., 1991) have indicated a complete lack of 5-HT3 receptors in the cerebellum, the presence of 5-HT3 receptors has recently been demonstrated in cerebellar synaptosomes (Nayak et al., 1999). 5-HT5A mRNA expression, on the other hand, has been demonstrated in the cerebellum of both rat (Erlander et al., 1993) and human (Pasqualetti et al., 1998). Weak cerebellar immunoreactivity for 5-HT5A has been reported as well (Oliver et al., 2000). Unfortunately, the latter study was not conducted at the cellular level in the cerebellum. Furthermore, while the functional coupling of 5-HT5A receptors to G-proteins has

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recently been described (Francken et al., 2000), its physiological actions remain to be elucidated. In situ hybridization (Ruat et al., 1993) and pharmacological (Ying and Rusak, 1997) experiments also reported the presence of 5-HT7 receptors in the cerebellum. These findings, however, were not confirmed in other studies (Gustafson et al., 1996).

Therefore, a detailed immunohistochemical study of 5-HT3, 5-HT5A and 5-HT7 receptors was performed in the rat cerebellum. In addition, the identity of large neuronal cells in the granular layer recently reported to be 5-HT2A immunopositive (Maeshima et al., 1998; Cornea-Hebert et al., 1999) was determined. To allow immunolocalization of serotonin receptors at a cellular level and to relate them to specific cerebellar cell types, the following neurochemical markers were included: parvalbumin (Schneeberger et al., 1985), the metabotropic glutamate receptor subtype 2 (mGluR2) (Neki et al., 1996; Geurts et al., 2001), somatostatin (Johansson et al., 1984; Geurts et al., 2001), the unidentified cytoplasmic antigen Rat-303 (Hockfield, 1987; Geurts et al., 2001) and calretinin (Rogers, 1989; Geurts et al., 2001).

2. Materials and methods

2.1. Tissue preparation

Six adult Wistar rats (235–345 g; Iffa Credo, Brussels, Belgium) were sacrificed with an overdose of sodium pentobarbital i.p. (Nembutal; Sanofi Animal Health, Brussels, Belgium) and transcardially perfused with heparinized physiological saline, followed by a fixative consisting of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The cerebellum was removed, post-fixed in the same fixative for another 2 h and divided into vermis and hemispheres. Tissue parts were cryoprotected in 10 mM phosphate-buffered saline (PBS; pH 7.4) containing 30% sucrose, frozen in Tissue Tek (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and cut on a cryostat into 16-μm-thick sagittal sections. Cryosections were mounted onto poly-L-lysine-coated glasses and immediately processed for immunohistochemistry. National and international principles of laboratory animal care were followed and the experiments were approved by the local ethics committee of the University of Antwerp.

2.2. Immunohistochemistry

After rinsing in PBS and preincubation for 30 min with 10% normal goat serum in PBS containing 0.1% bovine serum albumin, 0.05% thimerosal, 0.01% NaN_3 and 1% Triton X-100 to block non-specific binding of antibodies, immunofluorescence histochemistry was performed. The primary antibodies used in this study are summarized in Table 1. When the goat polyclonal anti-calretinin antibody was used, preincubation was performed with 10% normal donkey serum instead of normal goat serum. All incubations were carried out at room temperature in a humid atmosphere. Cryosections were incubated overnight for the following combinations or single labeling: (1) 5-HT3, (2) 5-HT5A and parvalbumin, (3) 5-HT5A and mGluR2, (4) 5-HT5A and somatostatin (S10 Ab), (5) 5-HT5A and calretinin (goat polyclonal antibody), (6) 5-HT5A and Rat-303, (7) 5-HT7, (8) 5-HT2A and somatostatin, and (9) 5-HT2A and calretinin (rabbit polyclonal antibody). Bound primary antibodies were detected the following day by incubating sections with the appropriate secondary antibodies for 1.5 h: for combination (1) a fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin (Ig) (1:100), for combinations (2,3,4,6,8,9) a Cy3-conjugated goat anti-rabbit Ig (1:200) and an FITC-conjugated goat anti-mouse Ig (1:100), for

Table 1
Characteristics of primary antibodies used

Antigen	Immunogen	Species	Dilution	Source
5-HT2A	Amino acids 1–72 of human 5-HT2A	Mouse	3 μg/ml	BD PharMingen (San Diego, USA)
5-HT3	Amino acids 444–457 of rat 5-HT3	Rabbit	1:500	Sigma (St. Louis, USA)
5-HT5A	Amino acids 17–34 of rat 5-HT5A	Rabbit	1:100	Oncogene Research Products (Boston, USA)
5-HT7	Amino acids 8–23 of rat 5-HT7	Rabbit	1:50	Oncogene Research Products (Boston, USA)
Parvalbumin	Purified parvalbumin from carp muscle	Mouse	1:1000	Sigma (St. Louis, USA)
mGluR2	Amino acids 87–134 of rat mGluR2	Mouse	1:500	Dr. R. Shigemoto (MAb mG2Na-5)[a]
Rat-303	Homogenized rat spinal cord	Mouse	1:5	Dr. S. Hockfield[b]
Somatostatin	Somatostatin 14, whole molecule	Mouse	1:500	Dr. A.M.J. Buchan (S10 Ab)[c]
Somatostatin	Human somatostatin	Rabbit	1:50	Euro-Diagnostica (Arnhem, The Netherlands)
Calretinin	Recombinant human calretinin	Rabbit	1:2500	SWant (Bellinzona, Switzerland)
Calretinin	Recombinant human calretinin	Goat	1:500	SWant (Bellinzona, Switzerland)

[a] Neki et al., 1996.
[b] Hockfield, 1987.
[c] Buchan et al., 1985.

combination (5) a lissamine rhodamine sulfonyl chloride (LRSC)-conjugated donkey anti-rabbit Ig (1:50) and a biotinylated donkey anti-goat Ig (1:100), and for (7) an FITC-conjugated goat anti-rabbit Ig (1:100). Secondary antibodies were obtained from Jackson (West Grove, PA, USA), except for the biotinylated donkey anti-goat Ig which was obtained from Amersham (Buckinghamshire, UK). The latter antibody was visualized with FITC-conjugated Streptavidin (1:1000, 1 h, Jackson). Between subsequent steps, sections were washed with PBS. Finally, sections were mounted in Vectashield (Vector Laboratories, Burlingame, CA, USA). To test method specificity, cerebellar sections were incubated as described above, but the primary antibodies were omitted. This resulted in a complete absence of immunostaining. The specificity of 5-HT receptor antisera was guaranteed by the manufacturers by 5-HT receptor preabsorption tests performed on rat brain sections and western blot analysis. In addition, specificity of 5-HT receptor antisera for the respective epitopes of 5-HT2A, 5-HT3, 5-HT5A and 5-HT7 was characterized previously (Oliver et al., 2000; Fu et al., 2001; Jansson et al., 2001; Neumaier et al., 2001).

2.3. Microscopic analysis

Fluorescent signals were imaged by a confocal laser scanning microscope (Zeiss LSM 410) and attached image reconstruction facilities (Imaris 2.7 software, Fairfield Imaging, Kent, UK) on a Silicon Graphics Indigo 2 workstation. FITC labeled antibodies were excited at 488 nm with an argon laser while Cy3-labeled or LRSC-labeled antibodies were excited at 543 nm with a helium-neon laser. Dual labeled sections were examined with sequential scans during which the second laser was turned off in order to prevent possible bleed-through.

3. Results

3.1. 5-HT3 receptor

Immunofluorescence histochemistry against 5-HT3 clearly demonstrated 5-HT3 immunoreactivity (IR) in the cerebellar cortex. 5-HT3-positive fibers were found at low density in both the molecular (Fig. 1A) and

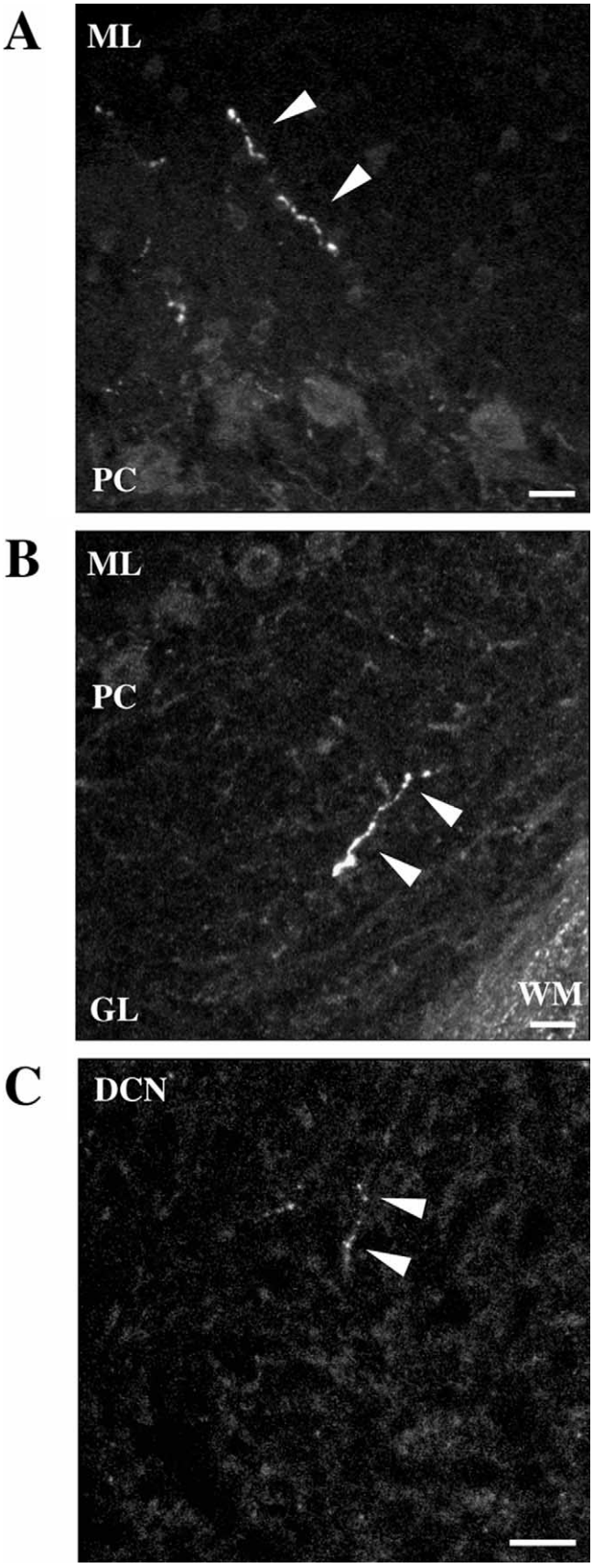

Fig. 1

Fig. 1. Localization of 5-HT3 IR in rat cerebellum. Fluorescence immunohistochemistry for 5-HT3 (visualized with FITC) shows fine varicose 5-HT3-positive fibers (indicated by arrowheads) in the molecular layer (**A**) and granular layer (**B**) of the cerebellar cortex. Some faint 5-HT3-positive processes (arrowheads) are also seen in the deep cerebellar nuclei (**C**). Abbreviations: DCN, deep cerebellar nuclei; GL, granular layer; ML, molecular layer; PC, Purkinje cell layer; WM, white matter. Scale bars = 20 μm.

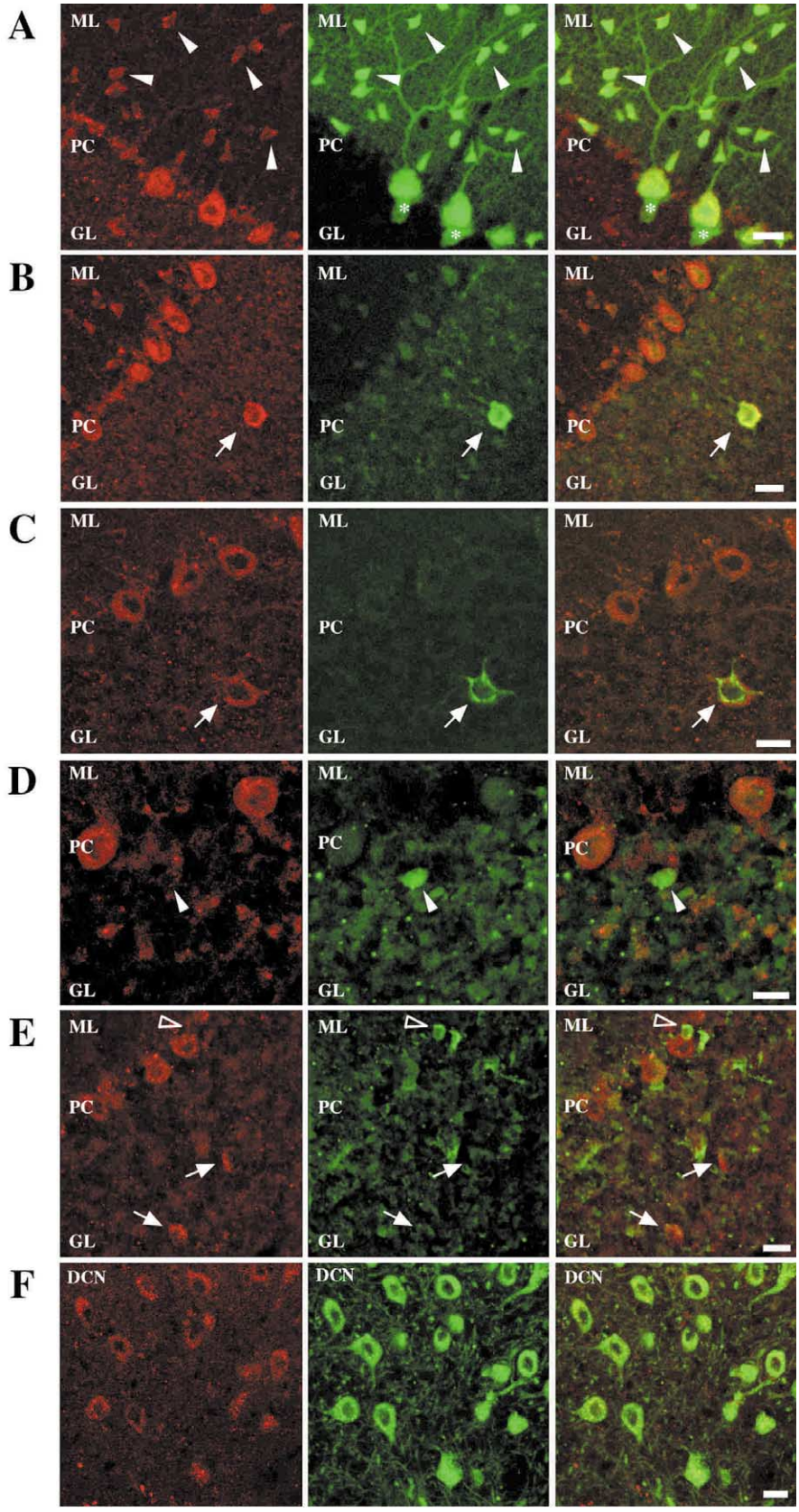

Fig. 2

granular layer (Fig. 1B). In the molecular layer, varicose 5-HT3-positive fibers were mostly vertically oriented. The orientation of 5-HT3-immunolabeled processes in the granular layer, on the other hand, appeared random. In the deep cerebellar nuclei some faint 5-HT3-positive fibers were observed as well (Fig. 1C).

3.2. 5-HT5A receptor

5-HT5A IR was found in molecular, granular and Purkinje cell layers of the cerebellar cortex (Fig. 2). Purkinje cells, evident as a discrete monolayer between granular and molecular layers, clearly displayed 5-HT5A immunostaining (Fig. 2A–E). In addition, the 5-HT5A antibody labeled numerous small cells in the molecular layer (Fig. 2A, arrowheads) and large cells dispersed throughout the granular layer (Fig. 2B, arrow). The identity of these cells was determined by double immunofluorescence histochemistry. Small 5-HT5A-positive cells in the molecular layer expressed parvalbumin and were consequently considered to be stellate/basket cells (Fig. 2A). Parvalbumin immunolabeling of basket cells resulted in the staining of 'pinceau' structures, i.e. basket cell endings contacting the initial segment of a Purkinje cell axon (Fig. 2A, asterisks). Purkinje cells also expressed both 5-HT5A and parvalbumin IR (Fig. 2A). Large 5-HT5A-positive cells in the granular layer, on the other hand, were identified as Golgi cells based on their morphology, rounded or polygonal cell bodies with radiating dendritic arborizations (Fig. 2C), and the co-expression of mGluR2 IR (Fig. 2B). The identity of 5-HT5A-positive Golgi cells was confirmed as well by their immunolabeling for somatostatin (Fig. 2C). Lugaro cells and unipolar brush cells (UBCs), two other types of large granular layer interneurons, did not appear to be 5-HT5A positive (Fig. 2D and E, respectively). Indeed, no double-labeled cells were found in sections immunostained for 5-HT5A and calretinin, a neurochemical marker for both Lugaro cells (Fig. 2D, arrowhead) and UBCs (Fig. 2E, open arrowhead). In the deep cerebellar nuclei, neuronal cells in the lateral nucleus (nucleus dentatus) visualized with the Rat-303 antiserum showed 5-HT5A IR (Fig. 2F). Some 5-HT5A immunostaining was also seen in neuronal cells of the nucleus interpositus. In the medial nucleus, however, no labeling for 5-HT5A was observed.

3.3. 5-HT7 receptor

Sections stained for 5-HT7 displayed only weak immunoreactivity in Purkinje cells (Fig. 3). Apart from 5-HT7-positive Purkinje cell bodies, no 5-HT7 immuno labeling was observed either in the cerebellar cortex or in the deep nuclei.

3.4. 5-HT2A receptor

5-HT2A IR was clearly present in the somata and dendrites of Purkinje cells (Fig. 4A–C). In addition, a population of large neuronal cells in the granular layer displayed moderate 5-HT2A immunostaining (Fig. 4A, arrow). Double immunohistochemistry for 5-HT2A and somatostatin revealed that these large neuronal cells

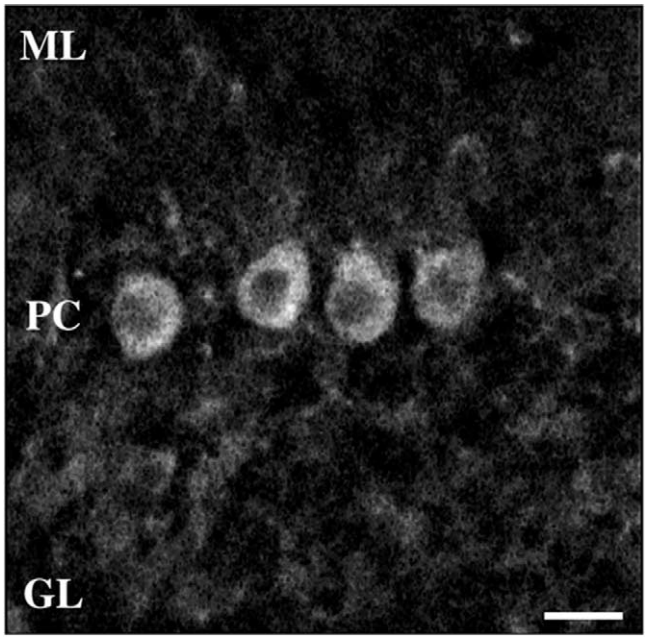

Fig. 3. Localization of 5-HT7 IR in rat cerebellum. Immunostaining for 5-HT7 (visualized with FITC) reveals 5-HT7 IR in Purkinje cells. Apart from 5-HT7-positive Purkinje cell bodies, no immunolabeling was observed. Abbreviations: GL, granular layer; ML, molecular layer; PC, Purkinje cell layer. Scale bar = 20 μm.

Fig. 2. Localization of 5-HT5A IR in rat cerebellum. (**A**) Double immunohistochemistry for 5-HT5A (*red* Cy3-fluorescence) and parvalbumin (*green* FITC-fluorescence) shows 5-HT5A IR in parvalbumin-positive stellate/basket cells (a few of them are indicated by arrowheads) and Purkinje cells. Parvalbumin immunolabeling of basket cells results in staining of 'pinceau' structures (asterisks). (**B**) Double immunostaining for 5-HT5A (*red* Cy3-fluorescence) and mGluR2 (*green* FITC-fluorescence) reveals 5-HT5A IR in mGluR2-positive Golgi cells (arrow). 5-HT5A-positive Purkinje cells and molecular layer interneurons are mGluR2-negative. (**C**) Double staining for 5-HT5A (*red* Cy3-fluorescence) and somatostatin (*green* FITC-fluorescence) confirms 5-HT5A expression in neurochemically identified Golgi cells (arrow). (**D–E**) Double immunohistochemistry for 5-HT5A (*red* LRSC-fluorescence) and calretinin (green FITC-fluorescence) demonstrates the absence of 5-HT5A IR in calretinin-positive Lugaro cells (D, arrowhead) and UBCs (E, open arrowhead). 5-HT5A-positive Golgi cells, on the other hand, do not express calretinin (E, arrows). (**F**) Double staining for 5-HT5A (*red* Cy3-fluorescence) and Rat-303 (*green* FITC-fluorescence) shows 5-HT5A immunolabeling of Rat-303-positive neuronal cells in the nucleus dentatus. Depicted on the right, a third column of images shows merging of the two single-stained images. Abbreviations: DCN, deep cerebellar nuclei; GL, granular layer; ML, molecular layer; PC, Purkinje cell layer. Scale bars = 20 μm.

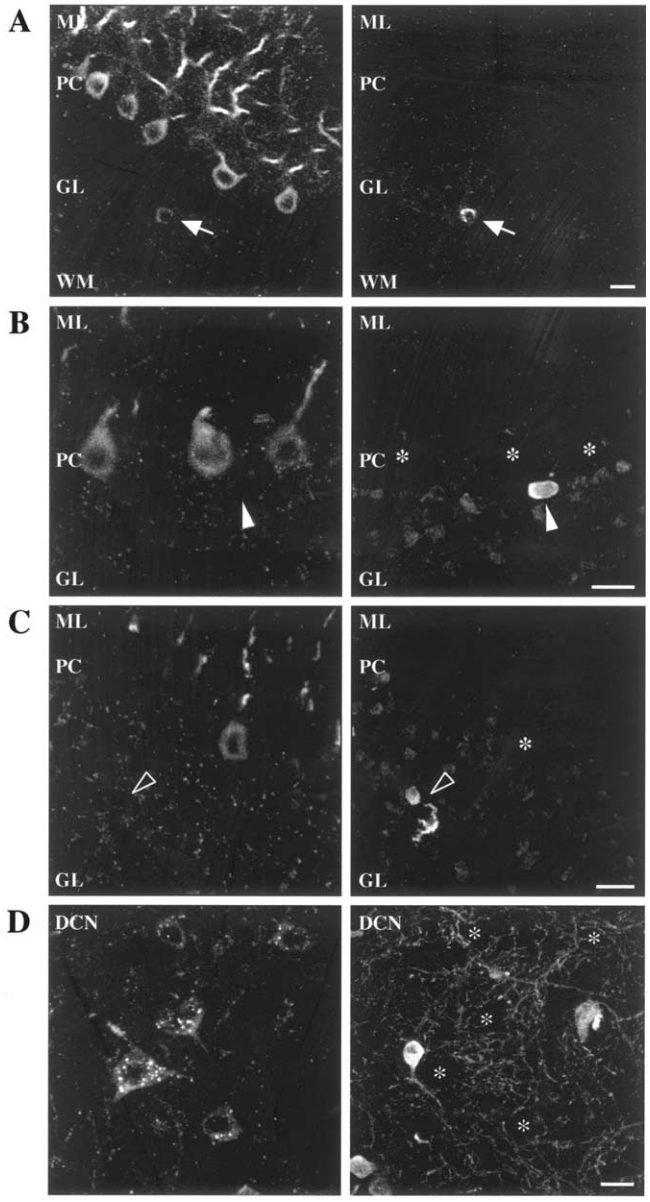

Fig. 4. Localization of 5-HT2A IR in rat cerebellum. (**A**) Double immunohistochemistry for 5-HT2A (left, visualized with FITC) and somatostatin (right, visualized with Cy3) demonstrates 5-HT2A IR in Purkinje cells and in somatostatin-positive Golgi cells (arrow). (**B**–**C**) Double immunostaining for 5-HT2A (left, visualized with FITC) and calretinin (right, visualized with Cy3) reveals no double labeling in the cerebellar cortex. Calretinin-positive Lugaro cells (B, arrowhead) and UBCs (C, open arrowhead) do not show 5-HT2A IR. (**D**) Double staining for 5-HT2A (left, visualized with FITC) and calretinin (right, visualized with Cy3) shows 5-HT2A IR in neuronal cells of the deep cerebellar nuclei. Asterisks indicate the location of 5-HT2A-positive Purkinje cells (**B**–**C**) and neuronal cells in the deep nuclei (**D**), respectively. Abbreviations: DCN, deep cerebellar nuclei; GL, granular layer; ML, molecular layer; PC, Purkinje cell layer; WM, white matter. Scale bars = 20 μm.

represented somatostatin-positive Golgi cells (Fig. 4A). Calretinin-positive Lugaro cells (Fig. 4B, arrowhead) and UBCs (Fig. 4C, open arrowhead), on the other hand, appeared to be 5-HT2A-negative. Calretinin IR was also used to delineate deep cerebellar nuclei. Many neuronal cells in the deep nuclei showed 5-HT2A immunolabeling (Fig. 4D). Occasionally, some calretinin/5-HT2A double-labeled cells were seen.

4. Discussion

The present study describes the distribution of 5-HT3, 5-HT5A and 5-HT7 serotonin receptor-like immunoreactivity in the rat cerebellum based on fluorescence immunohistochemistry. To date, as summarized in the introduction, no detailed study of the cerebellar localization of these receptor subtypes has been reported. In addition, knowledge of the functional role of serotonin on cerebellar function is still limited. The immunohistochemical localization of 5-HT receptor subtypes was facilitated by incorporating cell-specific markers. The calcium-binding protein parvalbumin was used to identify Purkinje cells and stellate/basket cells (Schneeberger et al., 1985). Golgi cells were identified by the expression of mGluR2 (Neki et al., 1996; Geurts et al., 2001) and somatostatin (Geurts et al., 2001), while another calcium-binding protein, calretinin, was used to label Lugaro cells and UBCs (Geurts et al., 2001). As previously reported (Geurts et al., 2001), UBCs also show some very faint immunostaining for mGluR2. Finally, the unidentified antigen Rat-303 (Hockfield, 1987), a marker previously used in our laboratory to identify Lugaro cells and Golgi cells in the rat (Geurts et al., 2001), appeared useful in labeling neuronal cells in the deep nuclei. In this way, we could clearly differentiate distinct populations of cerebellar neurons. As summarized in Fig. 5, this approach allowed a detailed immunolocalization of 5-HT3, 5-HT5A and 5-HT7 receptors, specifically in the cerebellar cortex which is composed of diverse neuronal cell types. In addition, it allowed identification of the recently described population of large 5-HT2A-positive cells located in the granular layer (Maeshima et al., 1998; Cornea-Hebert et al., 1999).

In agreement with results obtained in cerebellar synaptosomes (Nayak et al., 1999), 5-HT3 receptor immunoreactivity is found in rat cerebellar cryosections. The lack of 5-HT3 receptors reported in radioligand-binding studies (Kilpatrick et al., 1987) and mRNA detection studies (Maricq et al., 1991) may therefore be due to the experimental set-up or to the low extent of serotonergic innervation in the cerebellum when compared to other brain areas (Sur et al., 1996). The restricted immunolocalization of 5-HT3 receptors in varicose axon-like fibers distributed throughout the cerebellum fits well to previous reports demonstrating a presynaptic localization of this 5-HT receptor subtype in the central nervous system (Kidd et al., 1993; Nayak

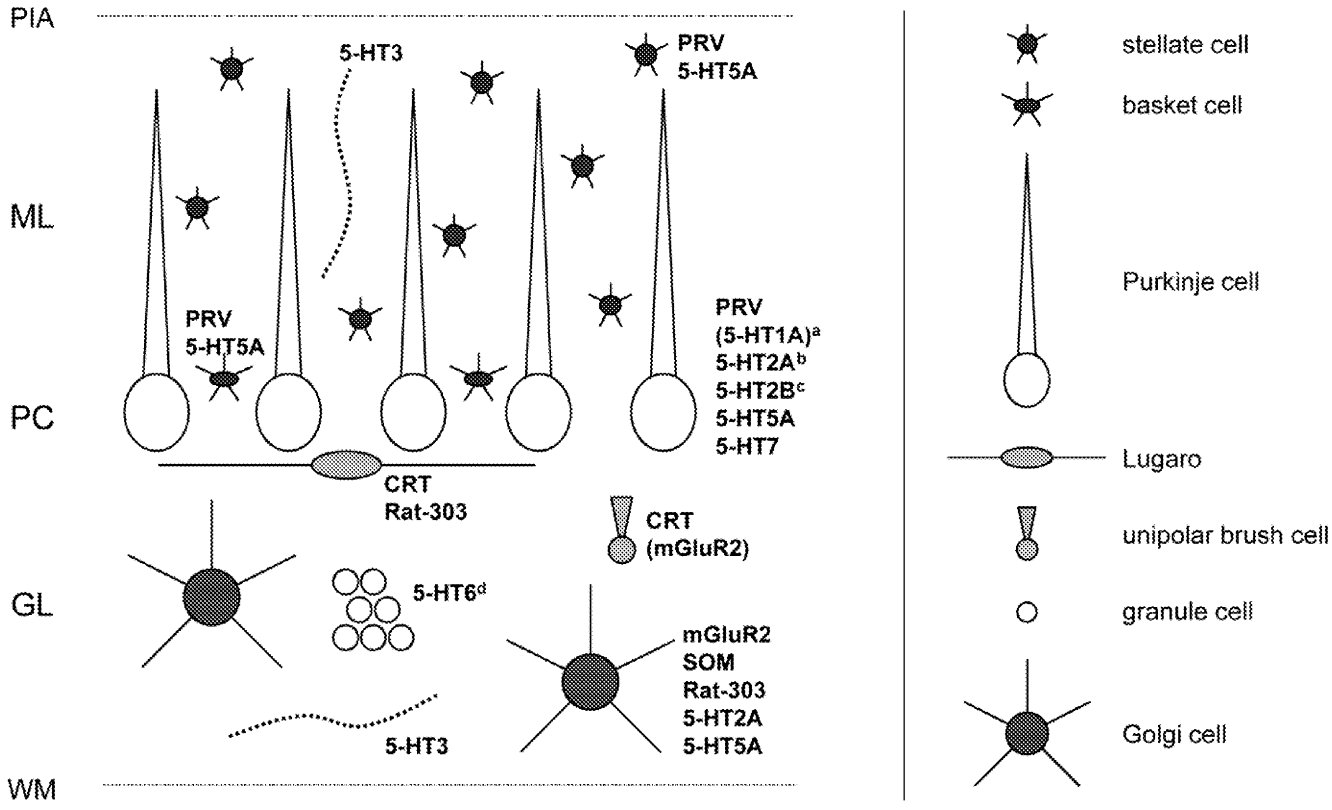

Fig. 5. Schematic drawing illustrating the current knowledge of 5-HT receptor immunolocalization in neurochemically identified cell types of the rat cerebellar cortex. Parvalbumin-positive stellate/basket cells exhibit 5-HT5A IR. Purkinje cells, which also show parvalbumin IR, express 5-HT2A, 5-HT2B, 5-HT5A and 5-HT7 IR. In addition, Purkinje cells transiently express 5-HT1A IR in the immature cerebellum. No 5-HT receptor IR is reported in calretinin-positive Lugaro cells or unipolar brush cells, the latter cell type also being faintly immunoreactive for mGluR2. Golgi cells, which display mGluR2 and somatostatin IR, express 5-HT2A and 5-HT5A IR. In addition, Golgi cells and Lugaro cells exhibit Rat-303 IR. 5-HT6 IR is localized most likely in granule cells. Finally, 5-HT3 IR is present in fine varicose axon-like fibers running in both the molecular layer and granular layer. Abbreviations: ML, molecular layer; PC, Purkinje cell layer; GL, granular layer; WM, white matter; PRV, parvalbumin; CRT, calretinin; SOM, somatostatin. [a] Matthiessen et al., 1993 and Kia et al., 1996; [b] Cornea-Hebert et al., 1999 and Maeshima et al., 1998; [c] Duxon et al., 1997; [d] Gerard et al., 1997.

et al., 1999). As a consequence, 5-HT3 receptors in the cerebellum may be involved in the regulation of neurotransmitter release, as described in the cerebral cortex and nucleus accumbens for cholecystokinin release (Paudice and Raiteri, 1991), in the corpus striatum and olfactory tubercle for dopamine release (Blandina et al., 1989; Zazpe et al., 1994) and in the cerebral cortex for acetylcholine release (Maura et al., 1992). In the cerebellum, 5-HT3 receptors are most likely involved in the release of dopamine, noradrenaline or acetylcholine, which have been reported as beaded fibers projecting aside climbing and mossy fibers (Barmack et al., 1992; Ottersen, 1993). Previous immunohistochemical studies on 5-HT3 receptors revealed different staining patterns in the rat brain, depending on the antibodies used. Morales et al. (1998) described labeling of perikarya and dendrites throughout the rat brain using an antibody raised against a peptide which corresponds to part of the second intracytoplasmatic loop of the receptor (amino acids 444–457). Doucet et al. (2000), on the other hand, reported restricted immunolabeling of terminals and axonal profiles using an antibody raised against another peptide chosen in the second intracytoplasmatic loop (amino acids 375–395). The difference in staining pattern was explained in that these antibodies might not recognize the same form of the receptor. The antibody described by Morales et al. (1998) might label only unassembled subunits of the receptor, whereas the antibody described by Doucet et al. (2000) recognizes the assembled, functional form. Fu et al. (2001), however, demonstrated functional 5-HT3 receptor expression using an antibody raised against amino acids 444–457. In addition, Spier et al. (1999) reported labeling of cell bodies, dendrites and axons in the rat brain based on immunohistochemistry with an antibody raised against a peptide corresponding to amino acids 23–36 of the extracellular domain of the 5-HT3 receptor. Using the latter antibody, Nayak et al. (1999) demonstrated, as described above, the presence of 5-HT3 receptors in rat cerebellar synaptosomes, suggesting the presynaptic nature of 5-HT3 receptors in this part of the brain.

Immunostaining for the 5-HT5A receptor, on the other hand, is found in neuronal cells of the cerebellar cortex and deep nuclei. This finding is compatible with a previous report demonstrating 5-HT5A IR in the rat cerebellum (Oliver et al., 2000). Double labeling experiments unequivocally identify 5-HT5A-positive cortical cells as either Purkinje cells, molecular layer interneurons or Golgi cells. In electrophysiological studies, increased inhibitory postsynaptic currents were observed in Golgi cells after application of serotonin, and Lugaro cells were considered to be the serotonin-sensitive inhibitory interneurons presynaptic to Golgi cells (Dieudonné and Dumoulin, 2000). In that case, however, serotonin should act on other 5-HT receptors present on Lugaro cells than those studied here. On the other hand, the serotonin-evoked inhibition of Golgi cells may be mediated, at least partially, by 5-HT5A receptors present on Golgi cells or molecular layer interneurons. The presence of 5-HT5A receptors on molecular layer interneurons may also influence the synaptic transmission between stellate/basket cells and Purkinje cells. They may be involved, for example, in the long-lasting enhancement of GABA-mediated inhibitory postsynaptic currents observed in Purkinje cells after application of serotonin (Mitoma et al., 1994), which is unlikely to be mediated by 5-HT1A, 5-HT2, 5-HT3 or 5-HT7 receptors (Mitoma and Konishi, 1999). Given the known involvement of 5-HT in motor activity (Jacobs, 1991), 5-HT5A knock-out mice have been generated in which locomotor behavior was investigated (Grailhe et al., 1999). The main difference between 5-HT5A knock-out and wild-type mice was an increase in exploratory activity when exposed to novel environments (Grailhe et al., 1999). Likewise, cerebellar-lesioned rats presenting obvious autistic-like symptoms showed an increase in exploratory activity in response to novelty (Bobée et al., 2000). These results are in line with previous observations suggesting the involvement of both serotonergic abnormalities (Cook, 1990; Rumsey and Ernst, 2000) and cerebellar damage (Cook, 1990; Townsend et al., 1999) in at least some forms of autistic behavior characterized by spatial attention deficits.

The third receptor investigated, 5-HT7, appears to be exclusively present in Purkinje cells within the cerebellum. This finding is consistent with previous in situ hybridization studies (Ruat et al., 1993). Purkinje cells have been described to be inhibited after iontophoretic application of 5-HT1A agonists such as 8-hydroxy-2(di-*n*-propyl-amino)tetralin (8-OH-DPAT) and ipsapirone (Darrow et al., 1990). 5-HT1A receptors, however, are only transiently expressed in the immature cerebellum (Miquel et al., 1994). In addition, 5-HT1A agonists also exhibit high affinity for the 5-HT7 receptor (Ying and Rusak, 1997). Therefore, as previously suggested (Dieudonné, 1998), the inhibitory effect of 5-HT on Purkinje cells might be mediated by 5-HT7 receptors. Ritanserin (a high affinity 5-HT7 antagonist), on the other hand, failed to antagonize the effect of 8-OH-DPAT in the cerebellar cortex, although it antagonized the actions of 8-OH-DPAT in the hippocampus (Ying and Rusak, 1997). Consequently, the functional role of 5-HT7 receptors in the cerebellar cortex is still unclear.

In general, immunohistochemical information about 5-HT receptors in the cerebellum is sparse. Besides the 5-HT3, 5-HT5A and 5-HT7 receptors investigated in this study, mainly 5-HT1 and 5-HT2 receptor subtypes have been immunohistochemically characterized, predominantly in the rat cerebellum. 5-HT1A receptor IR is demonstrated early in life in Purkinje cell somata and dendrites (Matthiessen et al., 1993; Miquel et al., 1994) but gradually decreases to very low levels in adulthood (Miquel et al., 1994; Kia et al., 1996), although some 5-HT1A-mediated activity is reported at this age (Maura and Raiteri, 1996; Trouillas et al., 1997). Abnormal persistence of 5-HT1A receptors in adulthood has been described in schizophrenic patients (Slater et al., 1998). Immunostaining for the 5-HT1B receptor is reported within the deep cerebellar nuclei (Sari et al., 1999), presumably in Purkinje cell axon terminals (Boschert et al., 1994). 5-HT2A receptor IR is described in Purkinje cells, neuronal cells in the deep nuclei and large cells in the granular layer (Maeshima et al., 1998; Cornea-Hebert et al., 1999). It is demonstrated here that the latter population of large 5-HT2A-positive cells represent Golgi cells. In schizophrenia, partial redistribution of 5-HT2A receptors in Purkinje cells has been reported (Eastwood et al., 2001). Immunolabeling for the 5-HT2B receptor is demonstrated in Purkinje cells (Duxon et al., 1997). Data about the 5-HT2C receptor are inconsistent. Although 5-HT2C receptor IR has been reported in the cerebellum (Abramowski et al., 1995), a recent study did not confirm these results (Clemett et al., 2000). Finally, also 5-HT6 receptor IR is demonstrated in both the molecular and granular layers of the rat cerebellum, most likely in granule cells (Gerard et al., 1997). Fig. 5 schematically summarizes the current knowledge of 5-HT receptor immunolocalization in the different neuronal cell types of the rat cerebellar cortex. Whether the expression of 5-HT receptor IR varies among different lobules, as reported for 5-HT IR (Bishop and Ho, 1985) and for 5-HT1A IR in the young rat (Matthiessen et al., 1992), is beyond the scope of this study. However, in order to fully understand serotonergic actions in this part of the brain, the lobular distribution of 5-HT receptor IR needs to be resolved and compared to the distribution of 5-HT IR. In addition, future analyses should provide quantitative data on the number of 5-HT receptor-labeled neuronal cells and fibers in the different lobules, and the neurochemical content of 5-HT3-positive fibers in the rat cerebellum needs to be determined.

In conclusion, the present study clearly demonstrates the presence and localization of 5-HT2A, 5-HT3, 5-HT5A and 5-HT7 receptors in the rat cerebellum using immunohistochemical techniques. In addition to previous radioligand-binding, in situ hybridization and immunohistochemical studies, our data relate these receptor subtypes to specific cerebellar cell types and may consequently contribute to revealing the modulatory actions of serotonin on cerebellar function.

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