The Journal of Neuroscience, August 1, 2002, 22(15):6732–6741

Differential Composition of 5-Hydroxytryptamine$_3$ Receptors Synthesized in the Rat CNS and Peripheral Nervous System

Marisela Morales[1] and Shwun-De Wang[2]

[1]National Institute on Drug Abuse, Cellular Neurophysiology, Baltimore, Maryland 21224, and [2]Department of Biology and Anatomy, National Defense Medical Center, Taipei, 114 Taiwan

The type 3 serotonin (5-HT$_3$) receptor is the only ligand-gated ion channel receptor for serotonin in vertebrates. Two 5-HT$_3$ receptor subunits have been cloned, subunit A (5-HT$_{3A}$) and subunit B (5-HT$_{3B}$). We used *in situ* hybridization histochemistry and reverse transcriptase-PCR amplification to demonstrate that 5-HT$_{3A}$ subunit transcripts are expressed in central and peripheral neurons. In contrast, 5-HT$_{3B}$ subunit transcripts are restricted to peripheral neurons. Thus, the prevalent form of 5-HT$_3$ receptor synthesized within the CNS lacks the 5-HT$_{3B}$ subunit. Because coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits produces heteromeric 5-HT$_{3A/3B}$ receptors with properties that differ from those of 5-HT$_{3A}$ homomeric receptors, we investigated possible coexpression of both subunits at the cellular level. We found that near to 90% of all 5-HT$_{3B}$ expressing neurons coexpress the 5-HT$_{3A}$ subunit in superior cervical and nodose ganglia (NG). In addition, there is a cellular population that expresses only the 5-HT$_{3A}$ subunit. Therefore, peripheral neurons have the capacity to synthesize two different 5-HT$_3$ receptors, 5-HT$_{3A}$+/$_{3B}$− and 5-HT$_{3A}$+/$_{3B}$+ receptors. We also determined that neurons of NG projecting to the nucleus tractus solitarium and those of dorsal root ganglia projecting to superficial layers of the spinal cord express 5-HT$_{3A}$ or 5-HT$_{3A/3B}$ subunits. Thus, presynaptic 5-HT$_3$ receptors containing the 5-HT$_{3B}$ subunit might be present in these target brain areas. The compartmentalized structural composition of the 5-HT$_3$ receptor may be the basis of functional diversity within this receptor. This raises the possibility that 5-HT$_3$ receptors participating in sympathetic, parasympathetic and sensory functions may be functionally different from those involved in cognition and emotional behavior.

Key words: 5-HT$_{3A}$ subunit; 5-HT$_{3B}$ subunit; myenteric plexus; nodose ganglia; superior cervical ganglia; serotonin receptors

The 5-HT$_3$ receptor is the only ligand-gated ion channel receptor for serotonin in vertebrates (Derkach et al., 1989). This receptor modulates visceral afferent information and visceral reflexes, participates in nociception and cognition (for review, see Fozard, 1992), and has been suggested to play a role in the biology of drugs of abuse (for review, see Grant, 1995; Lovinger, 1999).

Binding studies have shown 5-HT$_3$ receptor binding sites in the CNS of rodents and primates (Kilpatrick et al., 1987; Waeber et al., 1988, 1989, 1990; Barnes et al., 1989; Pratt et al., 1990; Gehlert et al., 1991; Jones et al., 1992; Laporte et al., 1992). In recent years, cellular analysis of the pattern of distribution of the functional 5-HT$_3$ receptor subunit A (5-HT$_{3A}$) demonstrated 5-HT$_{3A}$ mRNA (Tecott et al., 1993; Morales et al., 1996b; Morales and Bloom, 1997) and protein (Morales et al., 1996a, 1998) in several brain areas shown previously to contain 5-HT$_3$ receptor binding sites. Neurons of peripheral ganglia are also known to contain 5-HT$_3$ receptor binding sites (Hoyer et al., 1989; Kilpatrick et al., 1989) and 5-HT$_{3A}$ subunit transcripts (Tecott et al., 1993; Rosenberg et al., 1997).

In addition to the 5-HT$_{3A}$ subunit, which has been cloned from tissues of several animal species including humans (Maricq et al., 1991; Hope et al., 1993; Belelli et al., 1995; Miyake et al., 1995), a new class of 5-HT$_3$ receptor subunit (5-HT$_{3B}$) has also been cloned recently (Davies et al., 1999; Dubin et al., 1999; Hanna et al., 2000). In contrast to results with the 5-HT$_{3A}$ subunit, expression of recombinant 5-HT$_{3B}$ subunit alone does not produce a functional 5-HT$_3$ receptor. However, in heteromeric receptor complexes, the 5-HT$_{3B}$ subunit confers unique pharmacological and biophysical properties. Coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in *Xenopus* oocytes and mammalian cell lines yields receptors with a large single-channel conductance, low permeability to calcium ions, and a linear current–voltage relationship (Davies et al., 1999; Dubin et al., 1999).

Electrophysiological studies have indicated heterogeneous properties for native 5-HT$_3$ receptors (Derkach et al., 1989; Peters et al., 1992; Yang et al., 1992; Hussy et al., 1994; Brown et al., 1998). Although the basis of this heterogeneity is unknown, it has been speculated that receptor post-transcriptional modification such as phosphorylation or subunit composition might account for this diversity. Thus, knowledge of expression patterns for 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit genes is fundamental for understanding the possible structural composition of the 5-HT$_3$ receptors present in different cells of the nervous system. To address this question, we first investigated whether the mRNA for the 5-HT$_{3B}$ subunit was present in the brain, spinal cord, and peripheral ganglia. Moreover, because coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in recombinant preparations produces heteromeric 5-HT $_{3A/3B}$ receptors with properties that differ from those of 5-HT$_{3A}$ homomeric receptors, we sought to determine possible coexpression of both subunits at the cellular level. Finally, a combination of *in situ* hybridization and retrograde tracing was

Received Dec. 11, 2001; revised April 22, 2002; accepted May 1, 2002.

This work was supported by the Ministry of Defense, Taiwan, Grant DOD-90-02, and the Intramural Research Program of the National Institute on Drug Abuse. We thank Dr. Ewen F. Kirkness for the 5-HT$_{3B}$ cDNA clones and Dr. Barry J. Hoffer for helpful comments. We also express our appreciation for the technical assistance of Karen McCullough and Nicholas McCollum.

Correspondence should be addressed to Dr. Marisela Morales, National Institute on Drug Abuse, Cellular Neurophysiology, 5500 Nathan Shock Drive, Baltimore, MD 21224. E-mail: mmorales@intra.nida.nih.gov.

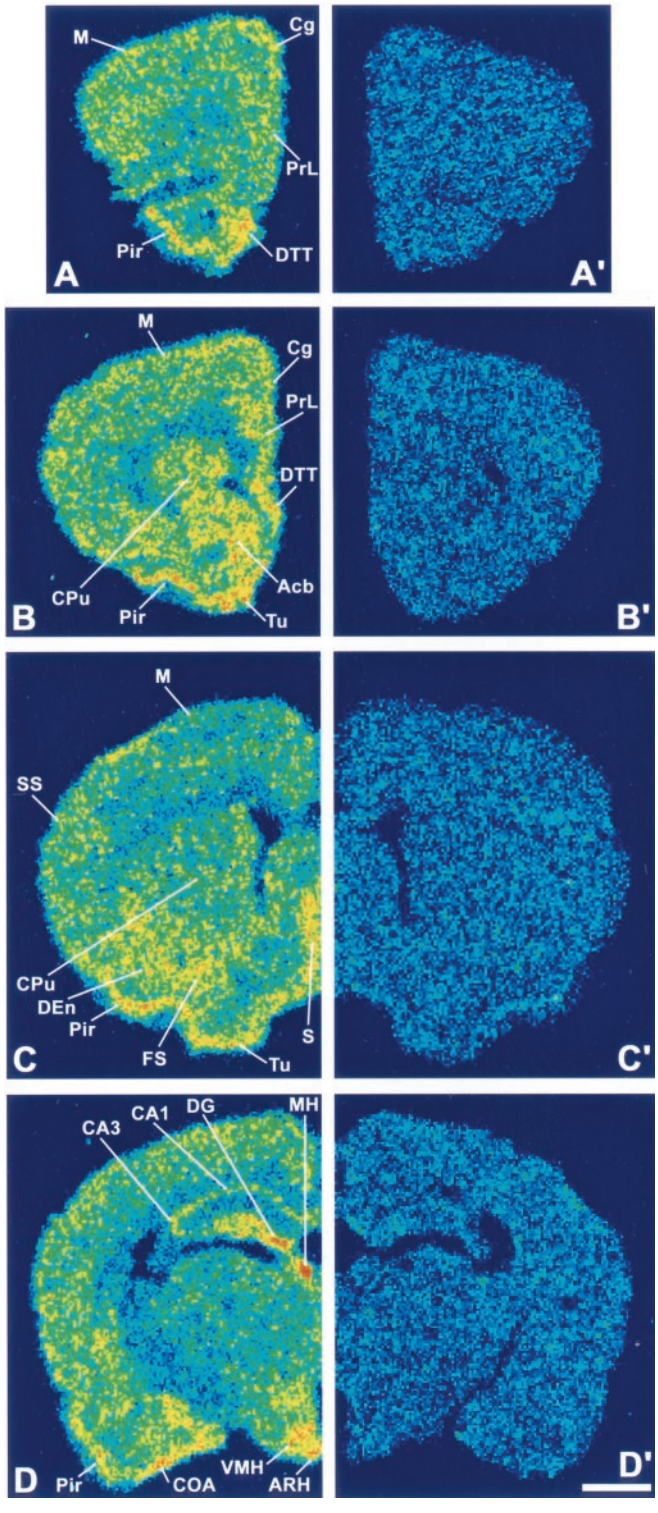

Figure 1. Phosphoimages comparing regional expression of mRNA encoding 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in the rat brain. *A–D*, Widespread expression of 5-HT$_{3A}$ subunit throughout the rat brain. *A′–D′*, Lack of detection of 5-HT$_{3B}$ subunit transcripts in adjacent brain sections. *Acb*, Nucleus accumbens; *ARH*, arcuate nucleus of the hypothalamus; *CA1*, field CA1 of the hippocampus; *CA3*, field CA3 of the hippocampus; *Cg*, cingulate cortex; *COA*, cortical nucleus of the amygdala; *CPu*, caudate putamen; *DEn*, dorsal endopiriform nucleus; *DG*, dentate gyrus; *DTT*, dorsal tecnia tecta; *FS*, fundus of the striatum; *M*, motor cortex; *MH*, medial habenular nucleus; *Pir*, piriform nucleus; *PrL*, prelimbic cortex; *S*, septum; *SS*, somatosensory cortex; *Tu*, olfactory tubercle; *VMH*, ventromedial hypothalamic nucleus. Scale bar, 1.4 mm.

used to investigate whether peripheral neurons that express transcripts for the 5-HT$_{3B}$ subunit, in addition to those of the 5-HT$_{3A}$ subunit, project to specific areas of the CNS.

MATERIALS AND METHODS

Tissue preparation. Adult Sprague Dawley male rats (200–250 gm body weight) were anesthetized with chloral hydrate (35 mg/100 gm) and perfused transcardially with a solution of 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3. Brains, spinal cord, and peripheral ganglia were postfixed in fresh fixative for 15 hr at 4°C, rinsed with PB, and sequentially transferred to 12, 14, and 18% sucrose solutions. Material was frozen on dry ice and then sectioned with a cryostat. All animal procedures used were approved by the National Institute on Drug Abuse Animal Care and Use Committee.

In situ *hybridization. In situ* hybridization was performed as described previously (Morales and Bloom, 1997). Free-floating (25 μm) cryosections of brain tissue and spinal cord and 8–15 μm sections of peripheral ganglia on glass slides were incubated for 10 min in PB containing 0.5% Triton X-100, rinsed two times for 5 min with PB, treated with 0.2N HCl for 10 min, rinsed two times for 5 min with PB, and then acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 10 min. Sections were rinsed two times for 5 min with PB, postfixed with 4% paraformaldehyde for 10 min, rinsed with PB, dehydrated, and hybridized at 55°C for 16 hr in hybridization buffer (50% formamide, 10% dextran sulfate, 5× Denhardt's solution, 0.62 M NaCl; 50 mM DTT, 10 mM EDTA, 20 mM PIPES, pH 6.8, 0.2% SDS, 250 μg/ml single-stranded DNA, and 250 μg/ml tRNA) containing [^{35}S]- and [^{33}P]-labeled single-stranded RNA probes at 10^7 cpm/ml. Sense and antisense riboprobes were prepared for rat 5-HT$_{3A}$ subunit [nucleotides 1500–2230 of the rat 5-HT$_{3A}$ subunit and rat 5-HT$_{3B}$ subunit (nucleotides 335–1346, accession number AF155044; nucleotides 1–1948 and 739–1948, accession number AF303447)] and mouse 5-HT$_{3B}$ subunit (nucleotides 83–1373, accession number AF155045). Sections were treated with 4 μg/ml RNase A at 37°C for 1 hr, washed with 1× SSC and 50% formamide at 55°C for 1 hr, and washed with 0.1× SSC at 68°C for 1 hr. Sections were rinsed with PB. Free-floating sections were mounted on coated slides, air dried, dipped in nuclear track emulsion, and exposed for several weeks at 4°C before development. Sections were counterstained with toluidine blue and analyzed in a Nikon (Tokyo, Japan) Microphot-FX microscope. Material was analyzed and photographed using bright-field or dark-field microscopy.

As control for *in situ* hybridization specificity, sequential sections were incubated with sense or antisense radioactive riboprobes. In another type of control, sequential sections were incubated with antisense radioactive riboprobes in the absence or presence of a 100-fold excess of nonradioactive antisense riboprobe. Silver grains were scattered when hybridization was performed with radioactive sense riboprobes or with an excess of nonradioactive antisense riboprobes. This level of signal was very low and was considered unspecific background.

Reverse transcriptase-PCR. Adult Sprague Dawley male rats (200–250 gm body weight) were anesthetized with chloral hydrate (35 mg/100 gm). Brain and ganglia [nodose ganglia (NG), trigeminal ganglia (TG), superior cervical ganglia (SCG), and dorsal root ganglia (DRG)] were immediately removed and transferred to buffer RNAlater (Ambion, Austin, TX). Specific brain areas were dissected out. Entire brains, selected brain areas, and different ganglia were placed in individual Eppendorf tubes containing RNeasy lysis buffer (RNeasy kit; Qiagen, Valencia, CA) and homogenized with a rotor-stator homogenizer. Total RNA samples were isolated using the Qiagen Rneasy mini kit. cDNA synthesis and amplification of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits were performed using the PCR access kit (Promega, Madison, WI). Reverse transcriptase (RT)-PCR amplification was performed three times using duplicates of RNA samples of central and peripheral tissues. The sequences of primers used to amplify the 5-HT$_3$ subunits were: ATCCAGGACATCAACATTTCCCTGTGGCGAACA and GTCTCAGCGAGGCTTATCACCAGCAGAG for the 5-HT$_{3A}$ subunit and GTGGAAGACATAGACCTGGGCTTCCTGAG and ACCCTGCGCTTCTTGGCACCTCATCAGA for the 5-HT$_{3B}$ subunit.

Retrograde tracing. Adult Sprague Dawley male rats (200–250 gm body weight) were anesthetized with ketamine/xylazine (40 mg/kg ketamine and 10 mg/kg xylazine, i.p.) and placed on a stereotaxic frame. A microinjector needle was lowered into the nucleus tractus solitarium (NTS) (Obex was used as reference point; NTS was located at 1 mm lateral to midline and 1 mm depth from brain surface) or into the

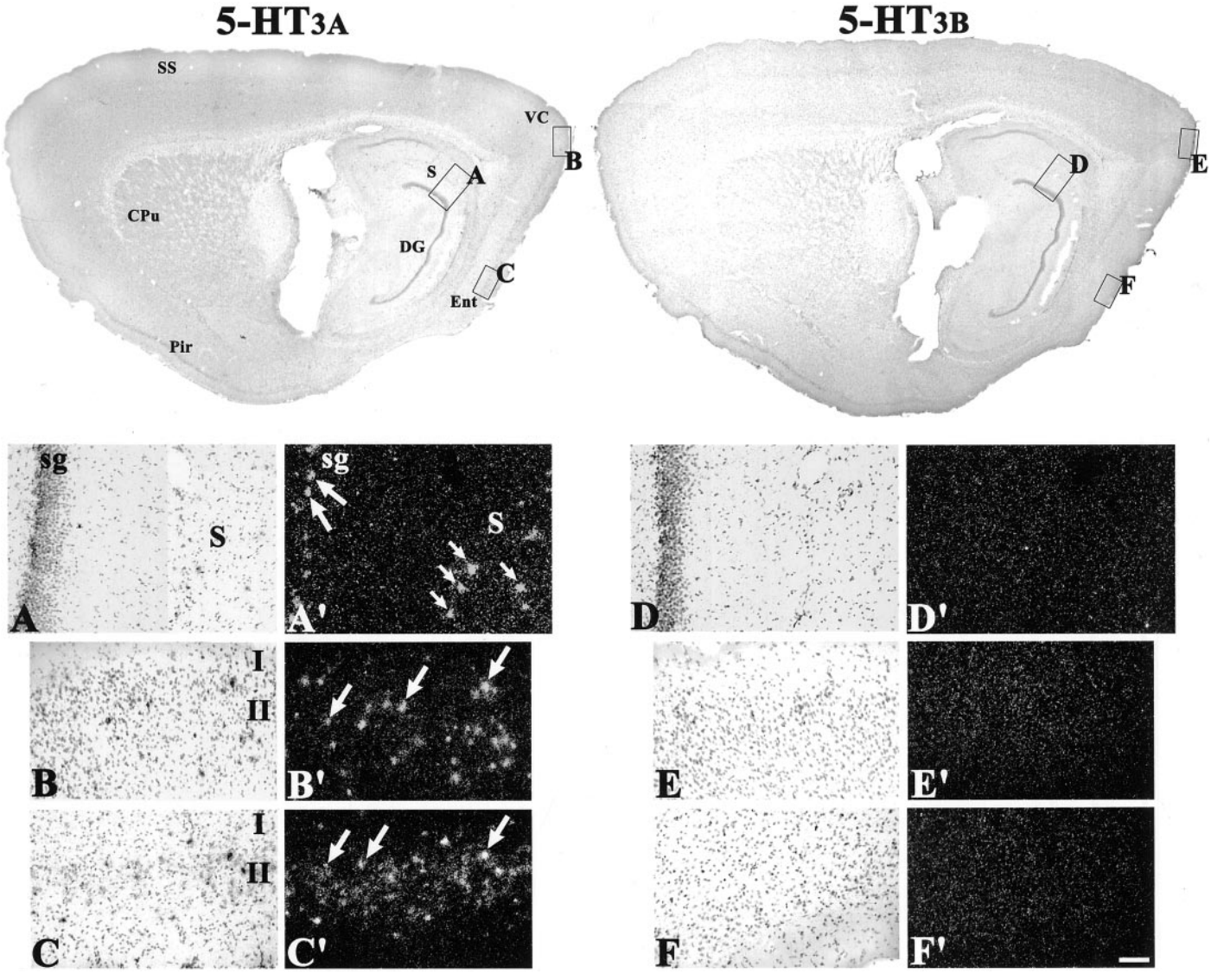

Figure 2. Autoradiograms comparing cellular expression of mRNA encoding 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in sagittal sections of the rat brain. Bright-field (*A–F*) and dark-field (*A′–F′*) microscopy are shown. *A, A′*, High expression of 5-HT$_{3A}$ mRNA was detected in neurons of the subiculum (*S*; *small arrows*) and basket cells (*large arrows*) in the subgranular layer of the dentate gyrus (*large arrows*). *B, B′, C, C′*, Prominent expression of 5-HT$_{3A}$ mRNA was found in cortical neurons (*arrows*) distributed primarily in layer II. *B, B′*, Visual cortex. *C, C′*, Entorhinal cortex. Lack of expression of 5-HT$_{3B}$ mRNA in the hippocampal formation (*D, D′*), visual cortex (*E, E′*), and entorhinal cortex (*F, F′*) is shown. *CPu*, Caudate putamen; *DG*, dentate gyrus; *Ent*, entorhinal cortex; *Pir*, piriform cortex; *S*, subiculum; *SS*, somatosensory cortex; *VC*, visual cortex. Scale bar: bright-field sagittal sections, 750 μm; *A–F, A′–F′*, 75 μm.

superficial layer of the dorsal horn of the spinal cord. The tracer fluorogold (0.2–0.3 μl of 4% fluorogold in saline solution) was unilaterally injected into the NTS or into the superficial layer of dorsal horn. The injector needle was then removed. After suturing the wound and recovery from anesthesia, the animals were placed in their home cages for 6 d. Animals were subsequently anesthetized with chloral hydrate (35 mg/100 gm) and perfused transcardially as indicated under Tissue preparation. Ganglia were removed (NG from rats injected into the NTS and DRG from rats injected into the spinal cord), and 10-μm-thick cryosections were mounted on glass slides.

Data analysis. Toluidine blue-counterstained sections (10–15 μm thickness) were used to calculate the percentage of labeled neurons. Each alternate section was hybridized with antisense riboprobes for detection of either 5-HT$_{3B}$ or 5-HT$_{3A}$ subunit transcripts. Data were collected using a Nikon Eclipse E800 microscope with a ×20 objective lens, equipped with an Optronics International (Chelmsford, MA) video camera, and connected to a C-Imaging (Compix, Inc., Cranberry Township, PA) workstation. Image processing and analysis were done using C-Imaging Systems software (Compix, Inc.). The percentage of labeled neurons was calculated using two alternate sections from five corresponding ganglia of different rats; all neurons in a given field were outlined. Cell diameter was determined from labeled cells that contained a visible nucleus.

Five sets of two alternate sections (8 μm thickness) from five NG and three sets of three SCG were used to calculate the degree of coexpression of mRNA for 5-HT$_{3B}$ and 5-HT$_{3A}$ subunits at the cellular level. Each alternate section was hybridized with antisense riboprobes for detection of either 5-HT$_{3B}$ or 5-HT$_{3A}$ subunit transcripts. Before data analysis, slides were examined under bright-field and epiluminescence microscopy to assess the quality of the tissue and that of the autoradiographic signal. Selected slides were analyzed as described above to determine labeled neurons. This information was used to identify the same cells in micrographs at a final magnification of 40×. Transparency films were overlaid on micrographs, corresponding to either 5-HT$_{3A}$ or 5-HT$_{3B}$ subunit, to outline labeled neurons. Transparency films with outlines of the 5-HT$_{3A}$ subunit-labeled neurons were overlaid on micrographs corresponding to

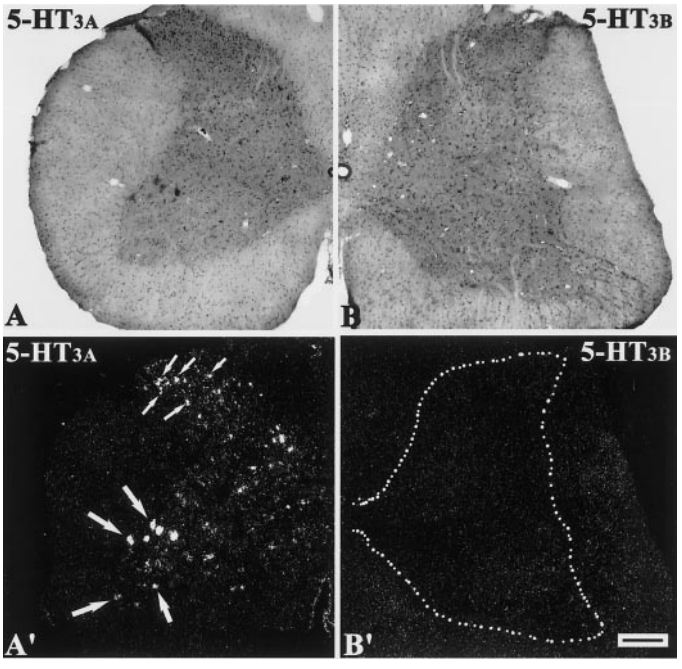

Figure 3. Autoradiograms comparing cellular expression of mRNA encoding 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in the spinal cord. Bright-field (*A, B*) and dark-field (*A′, B′*) microscopy is shown. *A′*, Expression of 5-HT$_{3A}$ subunits in neurons distributed in the dorsal (*small arrows*) and ventral (*large arrows*) horn of the spinal cord. *B′*, Lack of detection of 5-HT$_{3B}$ subunit transcripts in an adjacent section. Scale bar, 290 μm.

5-HT$_{3B}$ subunit-labeled sections to match outlines of 5-HT$_{3A}$ subunit-labeled neurons with corresponding 5-HT$_{3B}$ subunit-labeled neurons. A similar procedure was followed to match outlines of 5-HT$_{3B}$ subunit-labeled neurons with 5-HT$_{3A}$ subunit-labeled neurons.

To calculate the distribution of 5-HT$_{3B}$ or 5-HT$_{3A}$ subunit transcripts within the total population of neurons labeled with fluorogold, before dipping slides in nuclear track emulsion, images of slides of DRG and NG were collected with a video camera under ultraviolet light. After exposure of slides for several weeks, images of DRG and NG were collected under dark-field microscopy. Both images were overlaid using Adobe Photoshop software (Adobe Systems, San Jose, CA), and the total population of fluorogold-labeled neurons with or without transcripts was obtained.

RESULTS

Expression of 5-HT$_{3A}$ but not 5-HT$_{3B}$ subunit in neurons of the CNS

Several riboprobes complementary to the rat 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits (see Materials and Methods) were used to determine the pattern of expression of both subunits within the CNS. To increase sensitivity for mRNA detection, *in situ* hybridization histochemistry was performed in free-floating tissue sections using [^{35}S]- and [^{33}P]-labeled riboprobes. Regardless of hybridization conditions, 5-HT$_{3A}$ subunit but not 5-HT$_{3B}$ subunit was detected in neurons of several brain areas (Figs. 1, 2) and spinal cord (Fig. 3). At low magnification, silver grains slightly above background levels were seen in the pyramidal layer of hippocampus and olfactory tubercle in samples hybridized with riboprobes specific for detection of 5-HT$_{3B}$ subunit. However, at higher magnification silver grains were not clearly associated with cell bodies.

Expression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit in neurons of the PNS

Because previous studies have shown that the 5-HT$_3$ receptor is abundant in peripheral neurons, we used *in situ* hybridization to determine whether 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits were present in neurons of peripheral ganglia. In contrast to results obtained for neurons of the CNS, both 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits were found in neurons of the NG (Fig. 4), SCG, TG, DRG, and myenteric plexus in the gastrointestinal tract. All tested antisense 5-HT$_{3B}$ subunit riboprobes revealed strong expression of 5-HT$_{3B}$

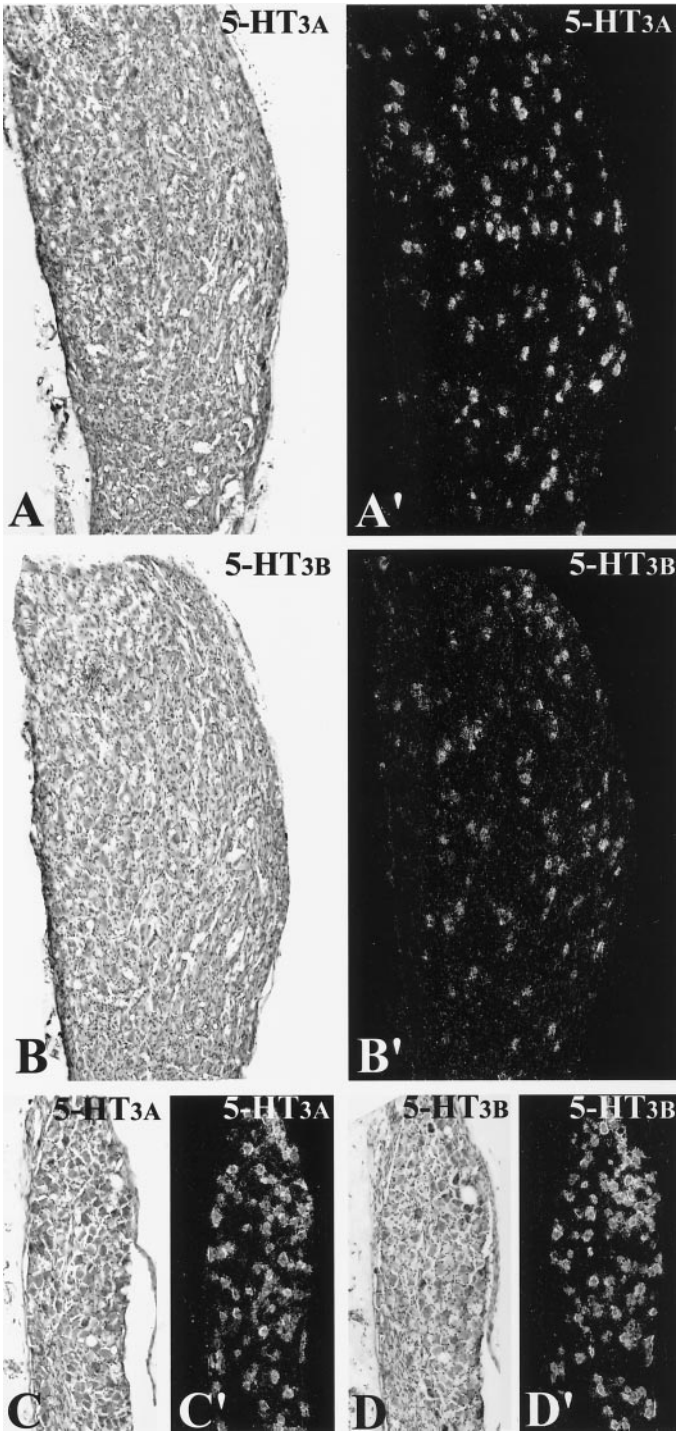

Figure 4. Expression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in peripheral ganglia. Bright-field (*A–D*) and dark-field (*A′–D′*) microscopy are shown. *A, A′, B, B′*, SCG. *C, C′, D, D′*, NG. Expression of 5-HT$_{3A}$ subunit was detected in neurons of the SCG (*A′*) and NG (*C′*). Neuronal expression of 5-HT$_{3B}$ subunit was found in the SCG (*B′*) and NG (*D′*). Scale bar, 175 μm.

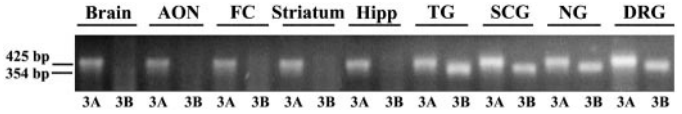

Figure 5. Amplification of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit transcripts from RNA of the CNS and PNS. RT-PCR amplification of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit transcripts using RNA isolated from the entire brain, anterior olfactory nucleus (*AON*), frontal cortex (*FC*), striatum, hippocampus (*Hipp*), TG, SCG, NG, and DRG is shown. PCR products of the predicted size (425 bp) for 5-HT$_{3A}$ transcripts (*3A*) were obtained from RNA of central and peripheral origin. However, PCR products of predicted size (354 bp) for 5-HT$_{3B}$ transcripts (*3B*) were amplified only from peripheral RNA.

subunit in peripheral neurons. As controls for specificity of the *in situ* hybridization signal, addition of either a 100-fold excess of nonradioactive antisense riboprobes or hybridization with radioactive sense riboprobes were performed. Both conditions produced signal at background levels.

RT-PCR amplification of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit transcripts

RT-PCR assays were performed to amply transcripts for 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits using RNA isolated from the entire brain, anterior olfactory nucleus, frontal cortex, striatum, hippocampus, and peripheral ganglia (TG, SCG, NG, and DRG). Although transcripts for the 5-HT$_{3A}$ subunit were amplified from RNA samples of the entire brain, specific brain areas and peripheral ganglia amplification of transcripts for the 5-HT$_{3B}$ subunit was obtained only from peripheral ganglia RNA (Fig. 5). Thus, RT-PCR results confirm data derived from *in situ* hybridization analysis showing expression of mRNA for 5-HT$_{3A}$ subunit in central and peripheral neurons, whereas the 5-HT$_{3B}$ subunit mRNA was detected only in peripheral neurons.

Coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in peripheral neurons

Cellular analysis of neurons expressing 5-HT$_{3A}$ subunit (Fig. 6) demonstrated that approximately one-half of the total population of neurons expressed the 5-HT$_{3A}$ subunit in TG (45.73 ± 6.56%, mean ± SEM; 1557 neurons) and SCG (45.73 ± 3.72%; 1957 neurons). In contrast, a larger population of 5-HT$_{3A}$-expressing neurons was found in the NG (Fig. 6), where 88.64 ± 1.99% (1435 neurons) of the total population of neurons expressed the 5-HT$_{3A}$ subunit (Fig. 6). A smaller population of peripheral neurons expressed the 5-HT$_{3B}$ subunit; approximately one-third of the total population of neurons expressed the 5-HT$_{3B}$ subunit in the SCG (29.95 ± 3.69%; 1489 neurons) and TG (35.99 ± 3.89%; 2551 neurons). As with the 5-HT$_{3A}$ subunit, the NG had the highest proportion of 5-HT$_{3B}$-expressing neurons (51.11 ± 2.41%; 1804 neurons). Thus, within the three ganglia, the population of neurons expressing the 5-HT$_{3A}$ subunit was larger than the one expressing 5-HT$_{3B}$; the difference between these two populations was 15.78% for TG, 9.74% for SCG, and 37.53% for NG.

Additional cellular analysis of cell diameter and expression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits showed that, in the three ganglia, both subunits were expressed in neurons of the same diameter (Table 1), suggesting that the two subunits are coexpressed in some neurons. Thus, serial sections of NG (Fig. 7) and SCG were used to determine the degree of coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits within single neurons. Analysis of the proportion of neurons coexpressing 5-HT$_{3A}$/5-HT$_{3B}$ subunits in the total population of 5-HT$_{3B}$-expressing neurons showed that the vast majority (93.67–96%) of 5-HT$_{3B}$-labeled neurons also expressed

Table 1. Cell diameter of neurons expressing mRNA for the 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in peripheral ganglia[a]

Ganglia	Subunit	Total number of counted cells	Soma diameter
Trigeminal			
	5-HT$_{3A}$ subunit	916	25.41 ± 5.90 μm
	5-HT$_{3B}$ subunit	477	26.77 ± 6.75 μm
Nodose			
	5-HT$_{3A}$ subunit	460	26.28 ± 5.61 μm
	5-HT$_{3B}$ subunit	477	27.71 ± 5.10 μm
Superior cervical			
	5-HT$_{3A}$ subunit	1193	25.86 ± 5.03 μm
	5-HT$_{3B}$ subunit	720	28.34 ± 5.43 μm

[a]Diameter of 5-HT$_{3A}$- or 5-HT$_{3B}$-labeled cell profiles was measured in neurons with visible nucleus in five to six sections from the corresponding ganglia. Diameters are expressed as mean ± SD.

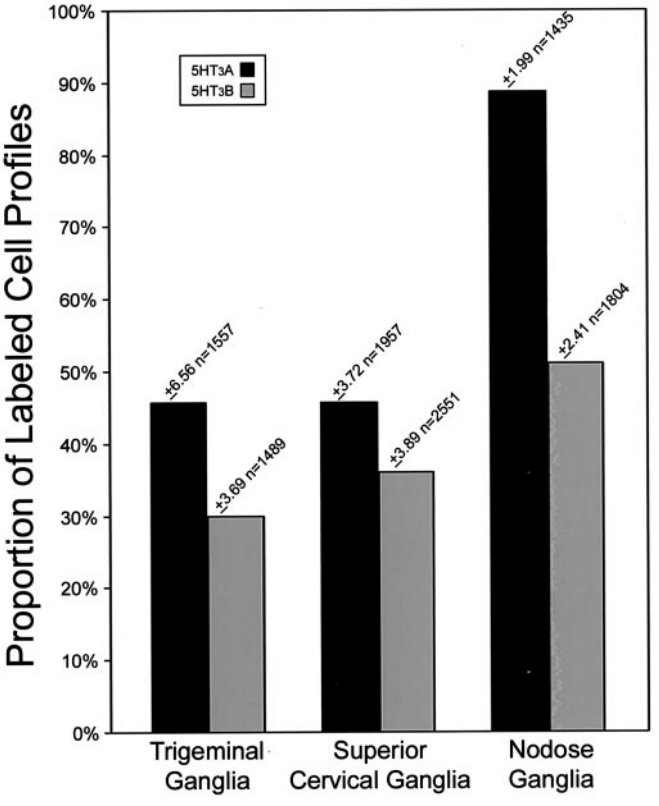

Figure 6. Frequency histogram showing percentage of neurons expressing either 5-HT$_{3A}$ or 5-HT$_{3B}$ subunits in the total neuronal population of TG, SCG, and NG. The data are presented as mean ± SEM; n = number of cells.

the 5-HT$_{3A}$ subunit in the SCG (93.67 ± 1.45%; 143 neurons) and NG (96 ± 0.89%; 330 neurons). Comparison of these results with those detailed above (Fig. 6) indicated that of the total population of neurons containing 5-HT$_{3A}$ subunit, a small proportion (9.74% for SCG and 37.53% for NG) lacks the 5-HT$_{3B}$ subunit.

Peripheral neurons expressing 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits innervate specific areas of the CNS

We have found previously that 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits are expressed in the DRG in a pattern similar to that of the NG (Morales et al., 2001). Because both ganglia project to the PNS

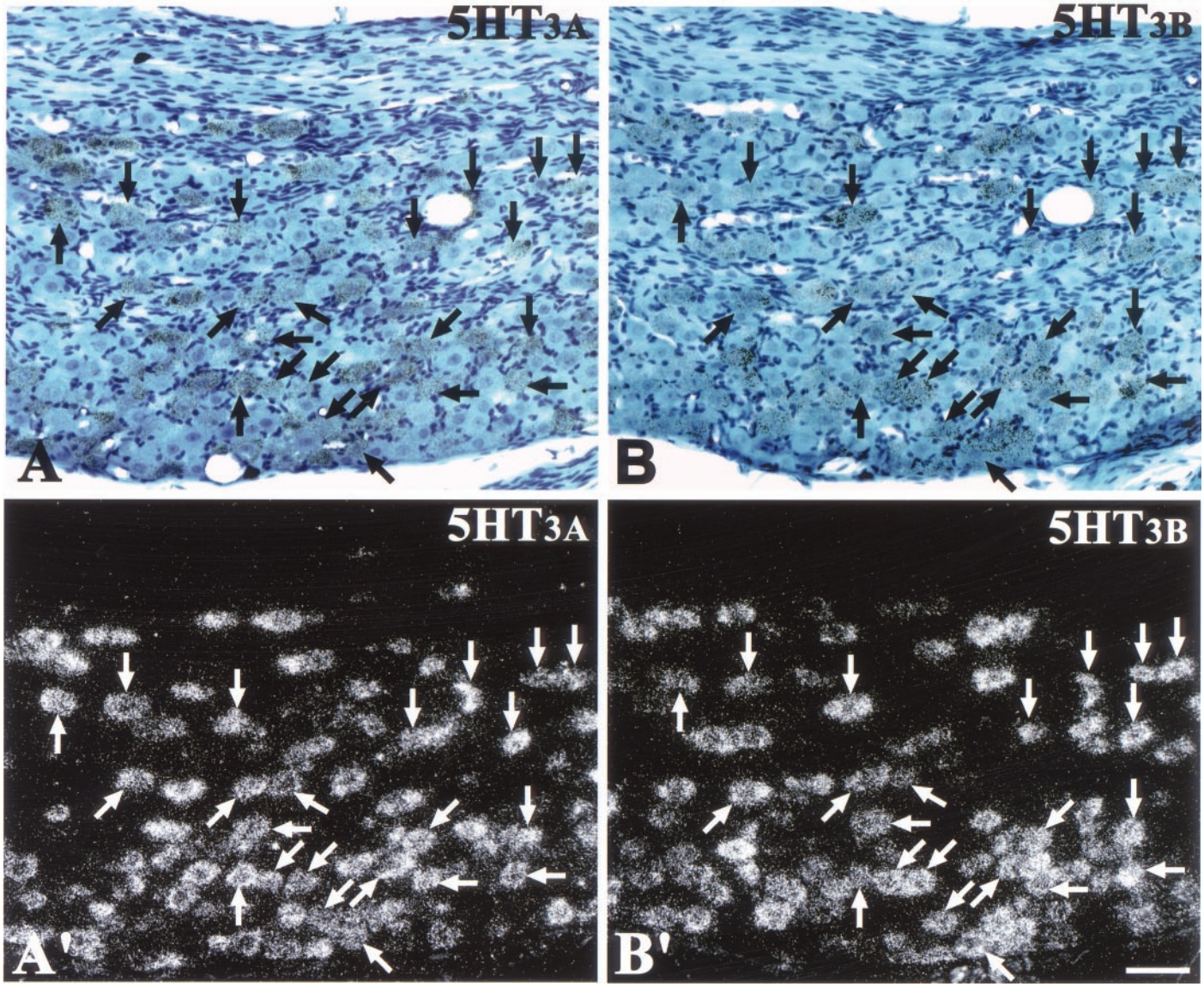

Figure 7. Coexpression of 5-HT_{3A} and 5-HT_{3B} subunits in the NG. Pairs of micrographs of adjacent serial sections show expression of 5-HT_{3A} (*A, A′*) and 5-HT_{3B} (*B, B′*) subunits. Bright-field (*A, B*) and dark-field (*A′, B′*) microscopy is shown. *Arrows* indicate examples of neurons coexpressing 5-HT_{3A} and 5-HT_{3B} subunits. Scale bar, 50 μm.

Table 2. Percentage of neurons expressing mRNA for 5-HT_{3A} or 5-HT_{3B} subunits in the total population of fluorogold-labeled neurons in DRG and NG

Dorsal root ganglia	
Total population of fluorogold-labeled neurons (951 cells = 100%)[a]	
Fluorogold(+)/5-HT_{3A}+	41.91 ± 0.06%
Total population of fluorogold-labeled neurons (949 cells = 100%)[b]	
Fluorogold(+)/5-HT_{3B}+	28.65 ± 0.03%
Nodose ganglia	
Total population of fluorogold-labeled neurons (626 cells = 100%)[c]	
Fluorogold(+)/5-HT_{3A}+	33.88 ± 0.06%
Total population of fluorogold-labeled neurons (636 cells = 100%)[d]	
Fluorogold(+)/5-HT_{3B}+	26.03 ± 0.05%

[a,c]All fluorogold-labeled cell profiles were counted in three to four alternate sections from three DRG (*a*) or three NG (*c*); the proportion of fluorogold (+)/5-HT_{3A}+ cells was expressed as a percentage (mean ± SEM) of the total number of cell profiles containing fluorogold.

[b,d]All fluorogold-labeled cell profiles were counted in three to four alternate sections from three DRG (*b*) or three NG (*d*); the proportion of fluorogold (+)/5-HT_{3B}+ was expressed as a percentage (mean ± SEM) of the total number of cell profiles containing fluorogold.

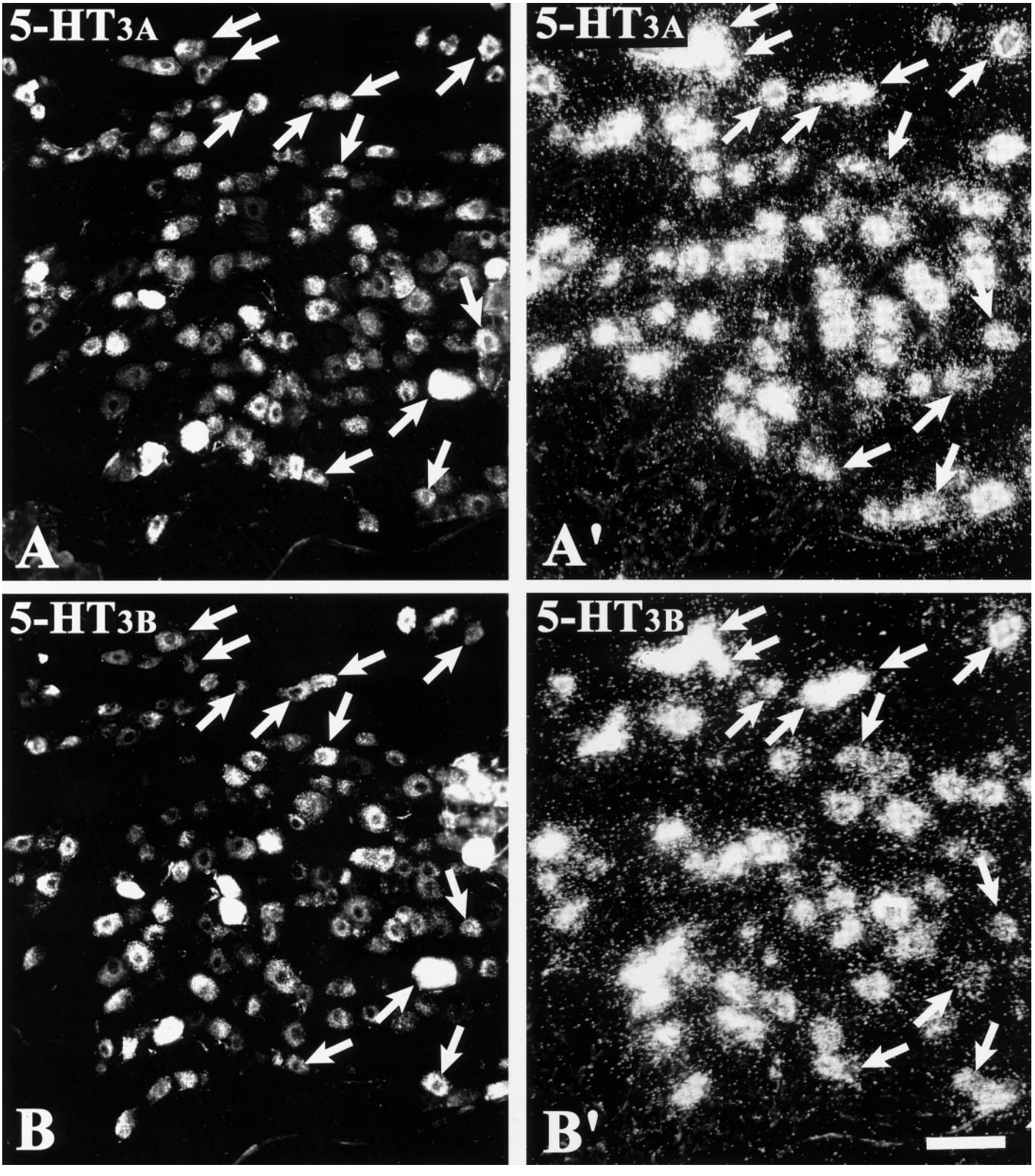

Figure 8. Detection of 5-HT_{3A} and 5-HT_{3B} transcripts in fluorogold retrogradely-labeled neurons in the NG. *A, B*, Fluorogold-labeled neurons. *A′, B′*, Dark-field microscopy indicating detection of transcripts encoding 5-HT_{3A} (*A′*) or 5-HT_{3B} (*B′*) subunits. *Arrows* indicate examples of retrogradely labeled neurons coexpressing 5-HT_{3A} and 5-HT_{3B} transcripts. Scale bar, 60 μm.

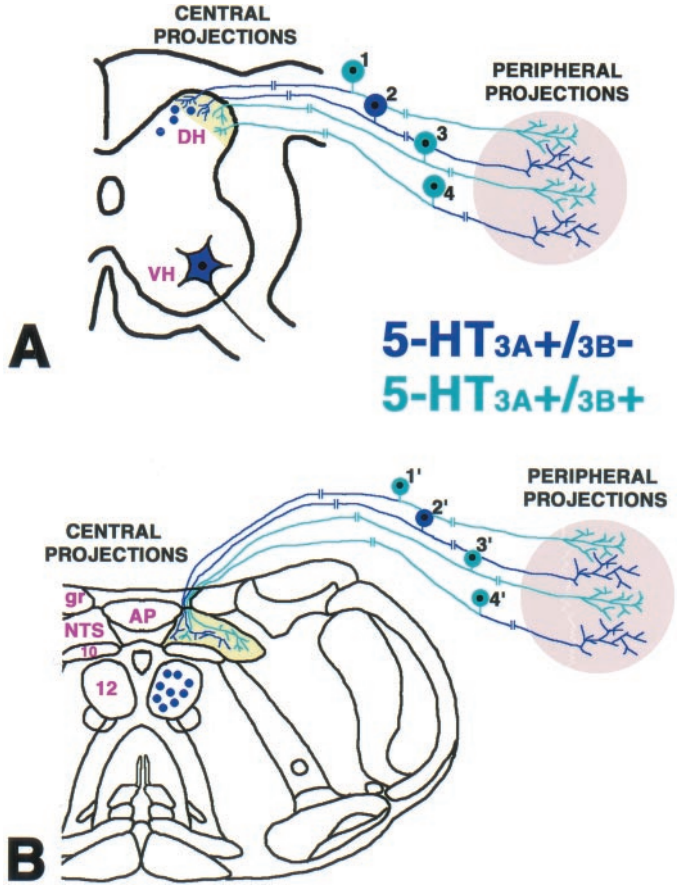

Figure 9. The compartmentalized structural composition of the 5-HT$_3$ receptors may be the basis of pharmacological and electrophysiological diversity within this receptor. The 5-HT$_{3A}$ but not 5-HT$_{3B}$ subunit mRNA (5-HT$_{3A+/3B-}$) is expressed in central neurons [for example, neurons of the dorsal horn (*DH*) and ventral horn (*VH*) of the spinal cord and neurons of the hypoglossal nucleus (*12*)]. However, two major subpopulations of neurons are present in the periphery: one that coexpresses 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits [(5-HT$_{3A+/3B+}$); see neurons 1, 3, and 4 and 1′, 3′, and 4′] and another expressing only 5-HT$_{3A}$ subunit [(5-HT$_{3A+/3B-}$); see neurons 2 and 2′]. Both types of neurons are present in DRG (*A*; 1–4) and NG (*B*; 1′–4′) and might have central and peripheral targets. These results suggest that central nerve endings of peripheral neurons might contain homomeric (5-HT$_{3A}$) and heteromeric (5-HT$_{3A/3B}$) receptors. Thus, despite the lack of detection of 5-HT$_{3B}$ subunit mRNA in the CNS, 5-HT$_{3B}$ subunit protein might be present in central areas innervated by DRG (i.e., superficial layers of the spinal cord) and NG (i.e., the NTS). Central and peripheral compartmentalization of receptors containing or lacking 5-HT$_{3B}$ subunit might occur in neurons endowed with both subunits (see neurons 1, 1′, 4, and 4′). We cannot discount the possibility that additional as yet unidentified subunits (5-HT$_{3x}$) might be present in central and peripheral neurons. *AP*, Area postrema; *gr*, gracile fasciculus; *10*, dorsal motor nucleus of the vagus.

and CNS, a combination of *in situ* hybridization and retrograde labeling was used to determine whether DRG and NG neurons expressing 5-HT$_{3A}$ or 5-HT$_{3B}$ subunits project to defined target areas in the CNS. Within the DRG (Table 2), nearly one-half of all fluorogold-labeled neurons expressed the 5-HT$_{3A}$ subunit (41.91 ± 0.06%), and approximately one-third expressed the 5-HT$_{3B}$ subunit (28.65 ± 0.03%). For the NG (Table 2), one-third of all fluorogold-labeled neurons expressed the 5-HT$_{3A}$ subunit (33.88 ± 0.06%), and one-quarter expressed the 5-HT$_{3B}$ subunit (26.03 ± 0.05%). Expression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits was often seen within the same retrogradely labeled neuron (Fig. 8), indicating coexpression of both subunits in neurons of the NG projecting to the NTS and neurons of the DRG innervating superficial layers of the spinal cord.

DISCUSSION

Differential expression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in the CNS and PNS

In the present study, we used *in situ* hybridization histochemistry and RT-PCR amplification to demonstrate that 5-HT$_{3A}$ subunit transcripts are expressed in central and peripheral neurons in the rat. In contrast, 5-HT$_{3B}$ subunit transcripts are restricted to peripheral neurons. The lack of detectable levels of mRNA encoding the 5-HT$_{3B}$ subunit in neurons of the CNS suggests that 5-HT$_3$ receptors synthesized in the CNS might be 5-HT$_{3A}$ homomeric receptors or heteromeric receptors containing 5-HT$_{3A}$ subunits in combination with subunits different from the 5-HT$_{3B}$ subunit. These putative subunits may participate in the formation of 5-HT$_3$ receptors with high conductance, such as those reported for hippocampal primary neurons in culture (Jones and Surprenant, 1994). The absence of 5-HT$_{3B}$ subunit mRNA in rat central neurons might reflect a species-specific difference in the pattern of expression of this subunit, because 5-HT$_{3B}$ subunit mRNA in human brain tissue has been detected by Northern blot analysis (Davies et al., 1999) and RT-PCR amplification (Dubin et al., 1999).

Comparison of the cellular population containing 5-HT$_{3A}$ and 5-HT$_{3B}$ mRNA subunits demonstrated that the population of peripheral neurons expressing the 5-HT$_{3A}$ subunit is larger than the one expressing 5-HT$_{3B}$ subunit. Moreover, analysis of the proportion of neurons coexpressing both subunits showed that >90% of 5-HT$_{3B}$-labeled neurons coexpressed the 5-HT$_{3A}$ subunit. It is not clear whether the small number of 5-HT$_{3B}$-expressing cells lacking 5-HT$_{3A}$ signal represent neurons with levels of 5-HT$_{3A}$ transcripts below the detectable threshold of *in situ* hybridization or express a 5-HT$_3$ subunit distinct from the 5-HT$_{3A}$. However, coexpression of 5-HT$_{3A/3B}$ subunits in most of the 5-HT$_{3B}$-expressing cells indicates that these peripheral neurons have the potential to synthesize heteromeric 5-HT$_{3A/3B}$ receptors. In addition, there is a neuronal population containing 5-HT$_{3A}$ transcripts that lacks expression of the 5-HT$_{3B}$ subunit. These results provide anatomical evidence suggesting that at least two subpopulations of cells are present in the PNS: one having the potential for synthesizing homomeric 5-HT$_{3A}$ receptors and the other for producing heteromeric 5-HT$_{3A/3B}$ receptors. Consistent with this suggestion, electrophysiological evidence indicates that 5-HT$_3$ receptors of different conductance are present in the SCG (Yang et al., 1992; Hussy et al., 1994). In this regard, coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in *Xenopus* oocytes and mammalian cell lines yields receptors with a large channel conductance and low permeability to calcium ions (Davies et al., 1999; Dubin et al., 1999), whereas the 5-HT$_{3A}$ monomeric receptors display inwardly rectifying currents and low single-channel conductance (Davies et al., 1999; Dubin et al., 1999). These electrophysiological observations are in agreement with our anatomical results and support the notion that homomeric 5-HT$_{3A}$ and heteromeric 5-HT$_{3A/3B}$ receptors constitute functional distinct receptors in the peripheral ganglia.

Peripheral neurons expressing 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits innervate specific areas in the CNS

We have found previously that 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits are expressed in the DRG in a proportion similar to that of the NG (Morales et al., 2001). Because both ganglia project to the CNS in

addition to the PNS, we sought to determine whether DRG neurons innervating superficial layers of the dorsal horn and NG neurons projecting to the NTS express 5-HT$_{3A}$ or 5-HT$_{3B}$ subunits. We found that approximately one-quarter of all retrogradely labeled neurons of the DRG and NG express the 5-HT$_{3B}$ subunit and that more than one-third express the 5-HT$_{3A}$ subunit. Because one-half (DRG) to one-third (NG) of all 5-HT$_{3A}$ subunit-containing neurons expressed the 5-HT$_{3B}$ subunit and >90% of all 5-HT$_{3B}$ subunit-labeled neurons have the 5-HT$_{3A}$ subunit, these results suggest that DRG and NG central nerve endings might contain homomeric (5-HT$_{3A}$) and heteromeric (5-HT$_{3A/3B}$) receptors (Fig. 9). Thus, despite the lack of detection of 5-HT$_{3B}$ subunit mRNA in the CNS (Fig. 9), 5-HT$_{3B}$ subunit protein might be present in areas innervated by NG (i.e., the NTS) (Fig. 9) and DRG (i.e., superficial layers of the dorsal horn) (Fig. 9). Although these results support the suggestion that terminals from peripheral neurons with different structures will innervate the CNS, the compartmentalization of these different receptors at the molecular and structural level remains to be determined.

Functional implications of the presence of homomeric and heteromeric 5-HT$_3$ receptors in the nervous system

Studies with recombinant preparations indicate that 5-HT$_3$ receptors with distinct structural composition result in receptors with different pharmacological and biophysical characteristics. Thus, knowledge of expression patterns for endogenous 5-HT$_{3A}$ and 5-HT$_{3B}$ subunit transcripts and proteins will be helpful in understanding the possible structural composition of 5-HT$_3$ receptors present in different cells of the nervous system. In this regard, the lack of detection of 5-HT$_{3B}$ mRNA in the rat central neurons indicates that the 5-HT$_{3B}$ subunit is not a prominent component of the 5-HT$_3$ receptor synthesized in the rat CNS. However, it does not discard the possibility that heteromeric 5-HT$_{3A/3B}$ receptors of peripheral origin, such as those from NG and DRG, might be present in nerve endings innervating the NTS and dorsal horn (Fig. 9). The compartmentalized structural composition of the 5-HT$_3$ receptors may be the basis of pharmacological and electrophysiological diversity within native 5-HT$_3$ receptors. The distinct distribution of 5-HT$_{3A}$ and5-HT$_{3B}$ subunits indicates that 5-HT$_3$ receptors originating in central or peripheral neurons with specific pharmacological and electrophysiological properties are likely to participate in different neuronal pathways and animal behaviors.

The 5-HT$_3$ receptor modulates visceral afferent information and visceral reflexes, participates in nociception and cognition (for review, see Fozard, 1992), and has been suggested to play a role in the biology of drugs of abuse (for review, see Grant, 1995; Lovinger, 1999). It is well established that vagal afferent fibers are the main source of 5-HT$_3$ receptors in the NTS, because ablation of the NG in the rat and ferret results in a dramatic reduction of 5-HT$_3$ receptor binding sites in the NTS (Pratt and Bowery, 1989; Leslie et al., 1990). The 5-HT$_3$ receptors in the NTS and gastrointestinal tract have been suggested as the site of action of 5-HT$_3$ receptor antagonists used in the treatment of emesis associated with cancer chemotherapy (Costall et al., 1986; Miner and Sanger, 1986; Andrews et al., 1988; Leslie et al., 1990; Naylor and Rudd, 1996). Although most of the interest on vagal 5-HT$_3$ receptors has been focused on their role in emesis, a more widespread role in the modulation of visceral afferent information and visceral reflexes might be expected, because functional studies in the rat have shown that 5-HT$_3$ receptors located on sensory efferent fibers within the NTS regulate blood pressure (Merahi et al., 1992; Veelken et al., 1993) and heart rate (Veelken et al., 1993). Within the superficial layers of the dorsal horn, 5-HT$_3$ receptor-binding sites are prominent (Glaum and Anderson, 1988; Gehlert et al., 1991; Laporte et al., 1992) and represent, in part, primary sensory terminals that contain 5-HT$_3$ receptors (Hamon et al., 1989; Kidd et al., 1993). In addition to 5-HT$_3$ receptors distributed on primary afferents, intrinsic interneurons may contribute to the pool of 5-HT$_3$ receptors present in the dorsal horn (Alhaider et al., 1991), because these interneurons were found to express the 5-HT$_{3A}$ (Morales et al., 1998) but not 5-HT$_{3B}$ subunit (present study). Interestingly, these observations imply that 5-HT$_3$ receptors in the dorsal horn may represent receptors not only of different origins but also of different composition. It has long been recognized that 5-HT$_3$ receptors present on sensory nerve terminals are involved in serotonin-induced pain (Richardson et al., 1985; Giordano and Rogers, 1989; Glaum et al., 1990). In this regard, detection of the 5-HT$_{3A}$ subunit in DRG neurons of different sizes and coexpression of 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits in medium and large neurons (Morales et al., 2001) suggest that 5-HT$_3$ receptors of different structures may convey nociceptive or proprioceptive information.

The specific contribution of homomeric (5-HT$_{3A}$) versus heteromeric (5-HT$_{3A/3B}$) receptors in functions mediated by the PNS and CNS is difficult to evaluate, because neither 5-HT$_{3B}$ knock-out mice nor specific antagonists capable of differentiating these receptors are as yet available. However, the participation of the 5-HT$_{3B}$ subunit in sympathetic, parasympathetic, and sensory functions is underscored by the high levels of expression of this subunit in SCG, NG, TG, and DRG. We suggest that contrary to 5-HT$_3$ receptors originating in the PNS, 5-HT$_3$ receptors synthesized in the CNS participating in cognition and emotional behavior will have unique structural and functional properties. Information on the distribution, electrophysiological and pharmacological characteristics, and possible participation of the different 5-HT$_3$ receptors in peripheral and central circuits will be useful for the development of specific antiemetic and analgesic drugs. This characterization will also be important to further evaluate the controversial participation of the 5-HT$_3$ receptor in the neuronal effects of drugs of abuse.

REFERENCES

Alhaider AA, Lei SZ, Wilcox GL (1991) 5-HT3 receptor-mediated antinociception: possible release of GABA. J Neurosci 11:1881–1888.

Andrews PL, Rapeport WG, Sanger GJ (1988) Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol Sci 9:334–341.

Barnes JM, Barnes NM, Costall B, Ironside JW, Naylor RJ (1989) Identification and characterization of 5-hydroxytryptamine$_3$ recognition sites in human brain tissue. J Neurochem 53:1787–1793.

Belelli D, Balcarek JM, Hope AG, Peters JA, Lambert JJ, Blackburn TP (1995) Cloning and functional expression of a human 5-hydroxytryptamine type 3As receptor subunit. Mol Pharmacol 48:1054–1062.

Brown AM, Hope AG, Lambert JJ, Peters JA (1998) Ion permeation and conduction in a human recombinant 5-HT3 receptor subunit (h5-HT3A). J Physiol (Lond) 507:653–665.

Costall B, Domeney AM, Naylor RJ, Tattersall FD (1986) 5-Hydroxytryptamine M-receptor antagonism to prevent cisplatin-induced emesis. Neuropharmacology 8:959–961.

Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG, Kirkness EF (1999) The 5-HT3B subunit is a major determinant of serotonin-receptor function. Nature 397:359–363.

Derkach V, Surprenant A, North RA (1989) 5-HT$_3$ receptors are membrane ion channels. Nature 339:706–709.

Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, Wilson SJ, Galindo JE, Glass CA, Luo L, Jackson MR, Lovenberg TW, Erlander MG (1999) The pharmacological and functional characteristics of the

serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 274:30799–30810.
Fozard JR (1992) Pharmacological relevance of 5-HT3 receptors. In: Serotonin receptor subtypes: pharmacological significance and clinical implications. International Academy of Biomedicine and Drug Research (Langer SZ, Brunello N, Racagni G, Mendlewicz J, eds), pp 44–55. Basel: Karger.
Gehlert DR, Gackenheimer SL, Wong DT, Robertson DW (1991) Localization of 5-HT$_3$ receptors in the rat brain using [^{3}H]LY278584. Brain Res 553:149–154.
Giordano J, Rogers LV (1989) Peripherally administered serotonin 5-HT3 receptor antagonists reduce inflammatory pain in rats. Eur J Pharmacol 170:83–86.
Glaum SR, Anderson EG (1988) Identification of 5-HT3 binding sites in rat spinal cord synaptosomal membranes. Eur J Pharmacol 156:287–290.
Glaum SR, Proudfit HK, Anderson EG (1990) 5-HT3 receptors modulate spinal nociceptive reflexes. Brain Res 510:12–16.
Grant KA (1995) The role of 5-HT3 receptors in drug dependence. Drug Alcohol Depend 38:155–171.
Hamon M, Gallissot MC, Menard F, Gozlan H, Bourgoin S, Verge D (1989) 5-HT3 receptor binding sites are on capsaicin-sensitive fibres in the rat spinal cord. Eur J Pharmacol 164:315–322.
Hanna MC, Davies PA, Hales TG, Kirkness EF (2000) Evidence for expression of heteromeric serotonin 5-HT(3) receptors in rodents. J Neurochem 75:240–247.
Hope AG, Downie DL, Sutherland L, Lambert JJ, Peters JA, Burchell B (1993) Cloning and functional expression of an apparent splice variant of the murine 5-HT3 receptor-A subunit. Eur J Pharmacol 245:187–192.
Hoyer D, Waeber C, Karpf A, Neijt H, Palacios JM (1989) [^{3}H]ICS 205–930 labels 5-HT3 recognition sites in membranes of cat and rabbit vagus nerve and superior cervical ganglion. Naunyn Schmiedebergs Arch Pharmacol 340:396–402.
Hussy N, Lukas W, Jones KA (1994) Functional properties of a cloned 5-hydroxytryptamine ionotropic receptor subunit: comparison with native mouse receptors. J Physiol (Lond) 481:311–323.
Jones DN, Barnes NM, Costall B, Domeney AM, Kilpatrick GJ, Naylor RJ, Tyers MB (1992) The distribution of 5-HT$_3$ recognition sites in the marmoset brain. Eur J Pharmacol 215:63–67.
Jones KA, Surprenant A (1994) Single channel properties of the 5-HT$_3$ subtype of serotonin receptor in primary cultures of rodent hippocampus. Neurosci Lett 174:133–136.
Kidd EJ, Laporte AM, Langlois X, Fattaccini CM, Doyen C, Lombard MC, Gozlan H, Hamon M (1993) 5-HT3 receptors in the rat central nervous system are mainly located on nerve fibres and terminals. Brain Res 612:289–298.
Kilpatrick GJ, Jones BJ, Tyers MB (1987) Identification and distribution of 5-HT$_3$ receptors in rat brain using radioligand binding. Nature 330:746–748.
Kilpatrick GJ, Jones BJ, Tyers MB (1989) Binding of the 5-HT$_3$ ligand, [^{3}H]GR65630, to rat area postrema, vagus nerve and the brains of several species. Eur J Pharmacol 159:157–164.
Laporte AM, Koscielniak T, Ponchant M, Verge D, Hamon M, Gozlan H (1992) Quantitative autoradiographic mapping of 5-HT3 receptors in the rat CNS using [^{125}I]iodo-zacopride and [^{3}H]zacopride as radioligands. Synapse 10:271–281.
Leslie RA, Reynolds DJM, Andrews PLR, Grahame-Smith DG, Davis CJ, Harvey JM (1990) Evidence for presynaptic 5-hydroxytryptamine and recognition sites on vagal afferent terminals in the brainstem of the ferret. Neuroscience 38:667–673.
Lovinger DM (1999) 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. Neurochem Int 35:125–130.
Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991) Primary structure and functional expression of the 5HT$_3$ receptor, a serotonin-gated ion channel. Science 254:432–437.
Merahi N, Orer HS, Laporte AM, Gozlan H, Hamon M, Laguzzi R (1992) Baroreceptor reflex inhibition induced by the stimulation of serotonin3 receptors in the nucleus tractus solitarius of the rat. Neuroscience 46:91–100.
Miner WD, Sanger GJ (1986) Inhibition of cisplatin-induced vomiting by selective 5-hydroxytryptamine M-receptor antagonism. Br J Pharmacol 88:497–499.
Miyake A, Mochizuki S, Takemoto Y, Akuzawa S (1995) Molecular cloning of human 5-hydroxytryptamine3 receptor: heterogeneity in distribution and function among species. Mol Pharmacol 48:407–416.
Morales M, Bloom FE (1997) The 5-HT3 receptor is present in different subpopulations of GABAergic neurons in the rat telencephalon. J Neurosci 17:3157–3167.
Morales M, Battenberg E, DeLecea L, Sanna PP, Bloom FE (1996a) Cellular and subcellular immunolocalization of the type 3 serotonin receptor in the rat central nervous system. Brain Res Mol Brain Res 36:251–260.
Morales M, Battenberg E, DeLecea L, Bloom FE (1996b) The 5-HT$_3$ receptor is expressed in cortical and hippocampal GABAergic neurons. Brain Res 731:199–202.
Morales M, Battenberg E, Bloom FE (1998) Distribution of neurons expressing immunoreactivity for the 5HT3 receptor subtype in the rat brain and spinal cord. J Comp Neurol 1998:385–401.
Morales M, McCollum N, Kirkness EF (2001) 5-HT$_3$-receptor subunits A and B are coexpressed in neurons of the dorsal root ganglion. J Comp Neurol 438:163–172.
Naylor RJ, Rudd J (1996) Mechanisms of chemotherapy/radiotherapy-induced emesis in animal models. Oncology 53:8–17.
Peters JA, Malone HM, Lambert JJ (1992) Recent advances in the electrophysiological characterization of 5-HT3 receptors. Trends Pharmacol Sci 13:391–397.
Pratt GD, Bowery NG (1989) The 5-HT$_3$ receptor ligand, [^{3}H]BRL 43694, binds to presynaptic sites in the nucleus tractus solitarius of the rat. Neuropharmacology 28:1367–1376.
Pratt GD, Bowery NG, Kilpatrick GJ, Leslie RA, Barnes NM, Naylor R, Jones BJ, Nelson DR, Palacios J, Slater P, Reynolds DJM (1990) Consensus meeting agrees with distribution of 5-HT$_3$ receptors in mammalian hindbrain. Trends Pharmacol Sci 11:135–137.
Richardson BP, Engel G, Donatsch P, Stadler PA (1985) Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316:126–131.
Rosenberg M, Pie B, Cooper E (1997) Developing neonatal rat sympathetic and sensory neurons differ in their regulation of 5-HT3 receptor expression. J Neurosci 17:6629–6638.
Tecott LH, Maricq AV, Julius D (1993) Nervous system distribution of the serotonin 5-HT$_3$ receptor mRNA. Proc Natl Acad Sci USA 90:1430–1434.
Veelken R, Hilgers KF, Leonard M, Scrogin K, Ruhe J, Mann JF, Luft FC (1993) A highly selective cardiorenal serotonergic 5-HT3-mediated reflex in rats. Am J Physiol 264:H1871–H1877.
Waeber C, Dixo K, Hoyer D, Palacios JM (1988) Localisation by autoradiography of neuronal 5-HT3 receptors in the mouse CNS. Eur J Pharmacol 151:351–352.
Waeber C, Hoyer D, Palacios JM (1989) 5-hydroxytryptamine$_3$ receptors in the human brain: autoradiographic visualization using [^{3}H]ICS 205–930. Neuroscience 31:393–400.
Waeber C, Pinkus LM, Palacios JM (1990) The (S)-isomer of [^{3}H]zacopride labels 5-HT$_3$ receptors with high affinity in rat brain. Eur J Pharmacol 181:283–287.
Yang J, Mathie A, Hille B (1992) 5-HT3 receptor channels in dissociated rat superior cervical-ganglion neurons. J Physiol (Lond) 448:237–256.