Two Parallel Signaling Pathways Couple 5HT$_{1A}$ Receptors to N- and L-Type Calcium Channels in C-Like Rat Dorsal Root Ganglion Cells

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Cardenas, Carla G., Lucinda P. Del Mar, and Reese S. Scroggs. Two parallel signaling pathways couple 5HT$_{1A}$ receptors to N- and L-type calcium channels in C-like rat dorsal root ganglion cells. J. Neurophysiol. 77: 3284–3296, 1997. The coupling of serotonin receptors to Ca$^{2+}$ channels was studied in a subpopulation of acutely isolated rat dorsal root ganglion (DRG) cell bodies (type 1 DRG cells), which have membrane properties similar to C-type nociceptive sensory neurons. In these cells, serotonin (5HT) inhibited high-threshold Ca$^{2+}$ channel current and decreased action potential duration. The inhibitory effects of 5HT and the 5HT$_{1A}$ agonist 8-OH-DPAT were shown to be antagonized by the 5HT$_{1A}$ antagonists sipiperone and pindolol, respectively, indicating involvement of a 5HT$_{1A}$ receptor. Several observations suggest that 5HT$_{1A}$ receptors couple to N- and L-type Ca$^{2+}$ channels by two different signaling pathways in type 1 DRG cells. The inhibition of Ca$^{2+}$ channel currents produced by 10 µM 5HT occurred in two phases, an initial slowing of current activation rate (kinetic slowing), which was complete within 10 s, and a simultaneous reduction in steady state current amplitude (steady state inhibition), which peaked in ~1 min. The kinetic slowing, but not steady state inhibition, was reversed by a positive prepulse to +70 mV (prepulse). Blockade of N-type Ca$^{2+}$ channels selectively reduced the kinetic slowing and its reversal by prepulses. Chelation of intracellular Ca$^{2+}$ or blockade of L-type Ca$^{2+}$ channels selectively reduced the steady state inhibition. Recordings using the cell-attached patch configuration suggest that steady state inhibition required a component that was diffusible in the cytosol, while kinetic slowing occurred via a membrane delimited pathway. The application of 5HT to the cell body outside the patch pipette reduced macroscopic Ca$^{2+}$ channel currents in 33% of small-diameter DRG cells tested, indicating the participation of a cytosolic diffusible component. Application of 5HT (a membrane impermeant compound) outside the patch pipette produced steady state inhibition only, whereas similar application of membrane permeant 5HT$_{1A}$ agonists, 8-OH-DPAT or 5-methoxy-N,N-dimethyl-tryptamine, produced kinetic slowing and steady state inhibition. Together these data suggest that 5HT$_{1A}$ receptors couple negatively to Ca$^{2+}$ channels via two pathways: a membrane-delimited pathway that couples to N-channels and actsuates voltage-sensitive kinetic slowing and a pathway dependent on a cytosolic diffusible component and free intracellular Ca$^{2+}$, which couples to L channels and actuates steady state inhibition.

INTRODUCTION

Several different biochemical pathways have been implicated in the coupling of neurotransmitter receptors to Ca$^{2+}$ channels in neurons. Initially “membrane-delimited” pathways were observed in several neuronal types, where the signal traverses a very short distance through the membrane from the receptor to a closely associated ion channel (Bean 1989; Brown 1993). Membrane-delimited pathways have been implicated directly in the actions of several neurotransmitters including, serotonin, neuropeptide Y, γ-aminobutyric acid, and norepinephrine (Bernheim et al. 1991; Foehring 1996; Forscher et al. 1986; Green and Cottrell 1988; Hirning et al. 1990; Penington et al. 1991). Subsequently, evidence was obtained in several neuronal types suggesting the coupling of various neurotransmitter receptors to Ca$^{2+}$ channels by cytosolic diffusible pathways. Cytosolic diffusible pathways have been implicated directly in the actions of several neurotransmitters including acetylcholine, angiotensin, dopamine, norepinephrine, and bradykinin (Bernheim et al. 1991; Diverse-Pierluissi et al. 1995; Hille 1994; Shapiro et al. 1994; Surmeier et al. 1995). There appears to be several cytosolic diffusible pathways, including a pathway involving protein kinase A and phosphatase 2A (Surmeier et al. 1995), another pathway involving protein kinase C (Boland et al. 1991; Diverse-Pierluissi and Dunlap 1993), and another pathway that appears not to correspond to any known signaling pathway, but has a dependence on the presence of intracellular Ca$^{2+}$ (Beech et al. 1991; Bernheim et al. 1991; Hille 1992; Howe and Surmeier 1995; Shapiro et al. 1994).

In previous reports, we described the inhibition of high-threshold Ca$^{2+}$ channel currents by serotonin (5HT) in a group of acutely isolated small-diameter rat dorsal root ganglion (DRG) neurons, which had membrane properties consistent with those of C-type nociceptors (referred to as type 1 DRG cells) (Cardenas et al. 1995; Del Mar et al. 1994). Type 1 DRG cells exhibit large high-threshold Ca$^{2+}$ currents of which, on average, 28% is conducted through N-type Ca$^{2+}$ channels, 46% through L-type Ca$^{2+}$ channels, and 26% through other high-threshold Ca$^{2+}$ channels (that are resistant to selective L- and N-channel blockers (Cardenas et al. 1995). Below evidence is presented that suggests that in these type 1 DRG cells, 5HT$_{1A}$ receptors couple to high-threshold Ca$^{2+}$ channels via two parallel pathways, a membrane-delimited pathway as previously reported in dorsal raphe neurons and cortical neurons (Foehring 1996; Penington et al. 1991), and a cytosolic diffusible pathway, which has not been reported elsewhere. Inhibition via the membrane delimitied pathway is characterized by a voltage-sensitive slowing of current activation rate and coupling to N-type Ca$^{2+}$ channels. Inhibition via the cytosolic diffusible pathway is characterized by a voltage-insensitive decrease in steady state current amplitude, dependence on the presence of intracellular Ca$^{2+}$, and coupling to L-type Ca$^{2+}$ channels. Because the two pathways differ in several respects (voltage sensitivity, Ca$^{2+}$ channel coupling, intracellular signal-
ing molecules), modulation via either pathway may be inac-
tivated selectively or enhanced by various physiological in-
put. Such physiological input could include rapid firing rates,
depolarization, and hyperpolarization by impinging affer-
ents, cross-talk from other activated second messenger path-
ways, or prolonged exposure to agonist. Also due to the
differences in the two pathways, selective reduction or en-
hancement of either pathway could modify changes in Ca\textsuperscript{2+}
entry patterns produced by activation of 5HT\textsubscript{1A} receptors.

**METHODS**

Male rats (75–150 g, Sprague Dawley purchased from Harlan) were rendered unconscious with methoxyflurane, decapitated, and DRG from thoracic and lumbar regions were dissected out. The ganglia were incubated at 36°C for 1 h in Tyrode’s solution (composition below) containing 2 mg/ml collagenase (Sigma, Type I) and 5 mg/ml Dipase II (Boehringer Mannheim). Individual DRG cell bodies were isolated by trituration and adhered to a poly-L-
lysine coated coverslip stuck to the bottom of a 35-mm Petri dish and superfused with Tyrode’s solution containing (in mM) 140 NaCl, 4 KCl, 2 MgCl\textsubscript{2}, 2 CaCl\textsubscript{2}, 10 glucose, 10 N\textsubscript{7}-2-hydroxyethyl-
piperazine-N\textsubscript{7}-2-ethanesulfonic acid (HEPES), adjusted to pH 7.4 with NaOH. Currents were recorded either in the whole cell or cell-attached patch configuration using an Axopatch 200A (Axon Instruments). Voltage and current steps, holding potential, and data acquisition and analysis were controlled by an on-line IBM PC/AT clone computer programmed with Axobasic 1.0 (Axon Instruments).

Electrodes were fabricated from soda lime capillary glass (Sc-
ientific Products, B4416-1) using a Narishige 2-stage vertical puller, coated with silicone elastomer (Sylgard) to ~200 μm from the tip, and fire polished to a final resistance of 0.8–2.0 MΩ, using a Narishige microforge. For voltage-clamp experiments, series res-
tance was estimated from capacity transients, as described pre-
viously (Scroggs and Fox 1992a). No data were included where series resistance resulted in greater than a 10-mV error in voltage commands.

Solutions were changed using the “sewer pipe” system, which consisted of a glass capillary tube mounted on a micromanipulator. The end of the glass capillary tube was placed near the cell under study, and the flow from it completely isolated the cell from the background flow of Tyrode’s solution, which continuously flowed over the cells via another port. Different solutions were directed out of the capillary tube by means of a small manifold to which various 10-ml aliquots of drug or control solutions were connected. All experiments were done at room temperature (23°C).

Capsaicin (Sigma) was made daily as a 10 mM stock solution in 100% ethanol and diluted to 1 μM in Tyrodes’ for experiments. Previous control experiments determined that the ethanol vehicle had no effect by itself (Del Mar et al. 1994). Omega-conotoxin GVIA (BacChem) was dissolved into 0.01 N acetic acid, aliquoted, lyophilized, and stored at −4°C for future use. Immediately before use the ω-conotoxin GVIA was dissolved in the external solution used for Ca\textsuperscript{2+} channel isolation. Stock solutions (10 mM) of n-midopine and 2-Bay K 8644 (Research Biochemicals) were made in 100% ethanol and stored in the freezer. The dithydropyridines were diluted in external solution for superfusion of cells. Restricted light conditions were present during experiments involving dith-
yropyridines. (+)8-OH-DPAT, serotonin creatinine sulfate, 5-me-
theoxy-N,N-dimethyl tryptamine, 5-carboxamidotryptamine, pin-
dolol, and spiperone (Research Biochemicals) first were dissolved as 1- or 10-mM stock solutions (made fresh daily) and then diluted to appropriate concentrations in the external solution. Pin-
dolol was first dissolved in slightly acidified water to obtain a 1-
mM concentration. Spiperone was dissolved in dimethyl sulfoxide to obtain a 10-mM stock solution.

Experiments using the whole cell patch-clamp configuration were restricted to type I DRG cells identified by their small diam-
eter, sensitivity to capsaicin, and lack of I\textsubscript{n} and I\textsubscript{h}, as described previously (Cardenas et al. 1995). For measuring the above men-
tioned K\textsuperscript{+}-dependent phenomena, cells were superfused with Ty-
rodes externally, and the patch electrodes were filled with (in mM) 120 KCl, 5 2Na-ATP, 0.4 2Na-GTP, 5 ethylene glycol-bis(β-
aminoethylether)-N,N',N'-tetraacetic acid (EGTA), 2.25 CaCl\textsubscript{2}, 20 HEPES, adjusted to pH 7.4 with KOH. Total KCl after pH adjustment was 154 mM. Free [Ca\textsuperscript{2+}], was calculated at 140 nM. Calcium channel currents (carried by Ba\textsuperscript{2+}) were isolated by changing the Tyrodes’ solution superfusing the outside of the DRG cell under study to one containing (in mM) 160 tetraethylammo-
nium (TEA)-Cl, 2 BaCl\textsubscript{2}, 10 HEPES, pH 7.4 with TEA-OH. Eh-
dence that this combination of internal and external solutions ade-
quately isolates Ca\textsuperscript{2+} currents has been presented previously (Del Mar et al. 1994). Furthermore, it has been demonstrated previously that the reduction of Ca\textsuperscript{2+} current amplitude by 5HT under the above conditions is due to a direct action on Ca\textsuperscript{2+} channels rather than activation of an outward K\textsuperscript{+} current (Del Mar et al. 1994). Unless otherwise stated, Ca\textsuperscript{2+} channel currents were evoked using 40-ms test depolarizations from a holding potential of −60 mV.

For cell-attached patch experiments the recording electrode was filled with a solution containing (in mM) 90 BaCl\textsubscript{2}, 20 TEA, 10 CsCl, 20 HEPES, pH 7.4 adjusted with TEA-OH. The membrane potential of the cell under study was depolarized to near 0 mV by superfusing the cell with a solution containing (in mM) 140 K\textsuperscript{+} aspartate, 1 MgCl\textsubscript{2}, 10 EGTA, 10 HEPES, adjusted to pH 7.4 with KOH. Data acquisition was achieved using the axobasic 1.0 single channel program “pinched.” A transmembrane potential of −60 mV was achieved by clamping the pipette tip to +60 mV. To trigger peak channel opening, the pipette tip potential was changed to around −20 mV. Macroscopic Ca\textsuperscript{2+} channel currents could be observed routinely by using a patch pipette with a relatively large tip, which included numerous Ca\textsuperscript{2+} channels. Peak currents re-
corded using this technique averaged 45 ± 6.9 pA (mean ± SE; n = 39). Some patches had no detectable current and were not included in the data analysis. In cell-attached patch recordings, DRG cells were not characterized regarding capsaicin sensitivity, I\textsubscript{n}, or I\textsubscript{h}, but recordings were restricted to small diameter cells to increase the likelihood of patching type 1 DRG cells.

In experiments regarding Ca\textsuperscript{2+} channel current amplitude, mea-
surements were made from plots of current versus time. It was necessary to take into consideration the rate Ca\textsuperscript{2+} channel current rundown, which varied from cell to cell and decreased over time. To adjust for rundown in experiments where Ca\textsuperscript{2+} channel currents were blocked with 5HT agonists or Ca\textsuperscript{2+} channel antagonists, a line was drawn through the control data points and extrapolated out to a position over the peak effect of the drug. This position was considered to be the control Ca\textsuperscript{2+} channel current amplitude and was used to calculate the percent change in Ca\textsuperscript{2+} channel current amplitude produced by the drug.

In most cells, a significant portion of the decrease in peak Ca\textsuperscript{2+} channel current produced by 5HT agonists was due to a reduction in current activation rate (kinetic slowing). The percentage inhibi-
tion of peak Ca\textsuperscript{2+} current occurring via kinetic slowing was esti-
mated using the formula: \( \frac{(\text{pA reduction of peak current} \ - \ \text{pA reduction of steady state current})}{\text{pA peak current}} \times 100 \), where “peak current” refers to the time point after the beginning of the test depolarization where peak current amplitude was observed under control conditions, and “steady state current” was the cur-
rent amplitude observed at the end of the test depolarization. This formula likely results in an underestimation of the inhibition occur-
vying via kinetic slowing because in many cells control current, and the current in the presence of 5HT had not quite reached steady
state by the end of the 40-ms sweep. However, due to the slow rate of change in current amplitude around this time point, the error is not large.

Concentration-response curves were generated for 5HT in the absence and presence of 1 μM spiperone and for 8-OH-DPAT. Several concentrations of agonist were tested on individual type 1 DRG cells (1 agonist per cell) starting with the lowest concentration and proceeding to higher concentrations in ascending order. For estimation of EC50, the inhibition of Ca2+ channel current produced by each concentration of agonist in a given cell was normalized as percent of maximal inhibition observed for each agonist in the same cell. Maximal inhibition for each agonist in individual cells was determined either from a plateau in the concentration response curve or, in some cases regarding 5HT concentration response curves, that observed with 5 or 10 μM 5HT, which were supramaximal concentrations in seven cells where 5HT was tested at higher concentrations. For estimation of the EC50 for 5HT and 8-OH-DPAT, the data points were fitted with the binding isotherm \( R = R_{max} / ((1 + EC_{50})/5HT)^n \) using the nonlinear curve fitting program in Systat (SPSS). \( n \) (the number of molecules that have to bind to the receptor to produce activation) was assumed to be 1. For cells where 5HT or 8-OH-DPAT were tested in the presence of an antagonist, the cell first was superfused with the antagonist for ~1 min before testing of agonist. Spiperone and pindolol were without effect by themselves.

**RESULTS**

Whole cell patch-clamp experiments were restricted to type 1 DRG cells, which are characterized by small size, capsaicin sensitivity, lack of I\(_{Na}\), long duration action potentials, and thus resemble C-type nociceptors (Cardenas et al. 1995; Del Mar et al. 1994). We previously reported that 5HT consistently produces a pronounced inhibition of high-threshold Ca\(^{2+}\) channel currents in these type 1 DRG cells (Cardenas et al. 1995). Recent studies on action potential duration (see METHODS) in type 1 DRG cells suggests that the inhibition of Ca\(^{2+}\) channel current by 5HT produces a simultaneous reduction in action potential duration. Figure 1, A and B, illustrates the inhibition of Ca\(^{2+}\) channel current by 5HT and the marked reduction in action potential duration produced by superfusion with 10 μM 5HT in a type 1 DRG cell. In six type 1 cells, which had an average control action potential duration of 8.2 ± 0.56 ms, the reduction of action potential duration at 1/2 peak amplitude by 10 μM 5HT averaged 33 ± 4.0%. In these same six cells, 10 μM 5HT inhibited peak high-threshold Ca\(^{2+}\) channel current by 40 ± 4.0%.

**RECEPTOR SUBTYPE.** A previous study demonstrated that the inhibition of Ca\(^{2+}\) channel currents in type 1 DRG cells was mimicked by the putative 5HT\(_{1A}\) selective agonist 8-OH-DPAT and that the inhibition produced by 8-OH-DPAT was antagonized by the putative 5HT\(_{1A}\)-selective antagonist NAN-190 (Cardenas et al. 1995; Cornfield et al. 1991; Del Mar et al. 1994; Glennon et al. 1988). These data were consistent with the idea that the inhibition of Ca\(^{2+}\) channel currents in type 1 DRG is mediated by a 5HT\(_{1A}\) receptor. However, recently several novel 5HT receptors (5HT\(_{1D}\), 5HT\(_{1F}\), 5HT\(_{1B}\), and 5HT\(_{7}\)) have been demonstrated that also may be targets for 8-OH-DPAT and/or NAN-190 (Boess and Martin 1994). Thus the receptor subtype involved was further explored with pindolol, which is selective for 5HT\(_{1A}\) and 5HT\(_{1B}\) receptors versus the above mentioned 5HT receptor subtypes, and spiperone, which is selective for the 5HT\(_{1A}\) receptor versus the 5HT\(_{1B}\) receptor (Boess and Martin 1994; Sills et al. 1984).

Figure 2, A–C, illustrates concentration-response data for the inhibition of peak high-threshold Ca\(^{2+}\) channel current by 5HT (taken from a group of 12 type 1 DRG cells) and for 5HT in the presence of 1 μM spiperone (taken from a different group of 6 type 1 DRG cells). The EC\(_{50}\) for 5HT was determined to be 196 nM in the absence of antagonist and was shifted rightward to 2.4 μM in the presence of 1 μM spiperone (Fig. 2, A–C). The antagonism by spiperone appeared to be surmountable by high concentrations of 5HT, consistent with a model of competitive antagonism. In the same type 1 cells where concentration-response was studied, the maximal inhibition of Ca\(^{2+}\) channel currents by 5HT alone (1–10 μM) averaged 37 ± 4.1% (\( n = 12 \)), compared with 49 ± 7.3% (\( n = 6 \)) observed for 5HT (5–50 μM) in the presence of 1 μM spiperone.

The EC\(_{50}\) for 8-OH-DPAT regarding inhibition of Ca\(^{2+}\)
channel current was determined to be 276 nM in a group of seven type 1 DRG cells tested (not illustrated). The inhibitory effect of 1 μM 8-OH-DPAT on Ca\(^{2+}\) channel current in type 1 cells was antagonized potently by 500 nM pindolol (Fig. 2, D–G). In six cells tested, 1 μM 8-OH-DPAT produced an inhibition of peak Ca\(^{2+}\) current averaging 35 ± 3.8% under control conditions compared with an average inhibition of only 9.4 ± 1.9% when added in the presence of 500 nM pindolol. After washout of antagonist for several minutes, the inhibition produced by 8-OH-DPAT recovered to 31 ± 4.6%. The inhibition produced by 8-OH-DPAT after the washout of pindolol was significantly greater than that observed in the presence of pindolol (P < 0.05, paired-difference t-test).

5-carboxamidotryptamine and 5-methoxy-N,N-dimethyl tryptamine, two additional 5HT\(_{1A}\) agonists known to activate Ca\(^{2+}\) channel current in type 1 DRG cells. In group of four cells tested, 5-carboxamidotryptamine (10 μM) inhibited Ca\(^{2+}\) channel current by 77.5 ± 3.1%, whereas 5-methoxy-N,N-dimethyl tryptamine (10 μM) inhibited Ca\(^{2+}\) channel current by 41 ± 5.8%, in another group of three cells tested.

EVIDENCE FOR TWO PARALLEL PATHWAYS. Several observations suggest that two parallel signaling pathways couple 5HT receptors to Ca\(^{2+}\) channels in type 1 DRG cells. One such observation was that the inhibition of Ca\(^{2+}\) channel currents by 5HT appeared to involve two components with different time courses: a slowing of current activation rate with a rapid time course and a decrease in overall current amplitude with a slower time course (Fig. 3, A and B). Similar components of neurotransmitter induced inhibition of Ca\(^{2+}\) currents have been observed previously and frequently are referred to as “kinetic slowing” and “steady state inhibition,” respectively (Luebke and Dunlap 1994).

In 37 type 1 DRG cells challenged once with 10 μM 5HT, peak inhibition of Ca\(^{2+}\) channel current (averaging 53 ± 2.2%) occurred in an average time of 61 ± 2.5 s (Fig. 3A). Kinetic slowing was completed within the first 10 s after exposure to 5HT, whereas Ca\(^{2+}\) channel current amplitude continued to decrease for up to ~1 min via steady state inhibition (Fig. 3B). These two components of inhibition were observed in >90% of type 1 cells, regardless of whether 5HT, 8-OH-DPAT, 5-carboxamidotryptamine, or 5-methoxy-N,N-dimethyl tryptamine was used as the agonist. As calculated using the formula described in METHODS, kinetic slowing reduced peak Ca\(^{2+}\) channel current by 11.9 ± 1.4%, which accounted for an average of 22.5 ± 2.6% of the total inhibition (n = 37). Thus steady state inhibition accounted for ~75% of the total decrease in Ca\(^{2+}\) channel current amplitude.

The two components of inhibition were further differentiated by their sensitivity to reversal by strong depolarizing voltage commands (prepulses) delivered 3 ms before peak Ca\(^{2+}\) channel current was evoked. 5HT-induced kinetic slowing was reversible by strong depolarizing prepulses, whereas steady state inhibition was little affected. Figure 3C illustrates that under control conditions, a prepulse to +70 mV increased peak current amplitude (facilitation) in type 1 DRG cells only slightly by an average of 4.7 ± 0.4% (n = 34). However, when the prepulse was delivered during
peak inhibition produced by 10 μM 5HT in the same cells, facilitation (relative to pre-5HT control current amplitude) was increased to 19 ± 1.1% \((n = 34); \text{Fig. 3D}\). During peak inhibition of \(\text{Ca}^{2+}\) channel currents by 5HT, the prepulse predominately reversed the kinetic slowing (Fig. 3D). These data suggest the participation of parallel pathways: one that mediates voltage-sensitive kinetic slowing and one that mediates steady state inhibition.

The idea of two pathways is supported by the observation that chelation of intracellular \(\text{Ca}^{2+}\) antagonized steady state inhibition but not voltage-sensitive kinetic slowing. Significantly less inhibition of \(\text{Ca}^{2+}\) currents was produced by 5HT when the patch electrodes were filled with a solution containing 10 mM bis-\((\alpha\text{-aminophenoxy})-N,N,N',N'\text{-tetraacetic acid (BAPTA)}\) and no added \(\text{Ca}^{2+}\) \((\text{BAPTA} \text{treatment})\) instead of the usual solution, which buffered intracellular \(\text{Ca}^{2+}\) at 140 mM \((\text{Fig. 4, A, B, and E})\). In BAPTA-treated type 1 DRG cells, 5HT reduced \(\text{Ca}^{2+}\) channel current by 27.2 ± 3.9% \((n = 8)\) compared with a reduction of 54.5 ± 4.0% in controls \((n = 15); \text{Students’} \text{t-test, } P < 0.05)\). This series of experiments included control and BAPTA-treated cells from the same group of rats to reduce the possibility of a sampling error.

A closer inspection of the above data revealed that the chelation of intracellular \(\text{Ca}^{2+}\) selectively affected steady state inhibition versus voltage-sensitive kinetic slowing. 5HT-induced kinetic slowing occurred in BAPTA-treated cells as well as controls, reducing \(\text{Ca}^{2+}\) channel current amplitude by 9 ± 1.1% in control cells \((n = 15)\) compared with 11.2 ± 3.6% in BAPTA-treated cells \((n = 8); \text{Fig. 4, C, D, and F})\. Also, the voltage sensitivity of 5HT inhibition was not affected by \(\text{Ca}^{2+}\) chelation (Fig. 4, C, D, and G). In control cells, a prepulse to +70 mV during peak inhibition by 5HT facilitated \(\text{Ca}^{2+}\) channel current amplitude by 18 ± 1.9% \((n = 12)\) compared with a facilitation of 16.2 ± 1.6% \((n = 8)\) in BAPTA-treated cells \((\text{relative to pre-5HT control \(\text{Ca}^{2+}\) channel current amplitude})\) . Conversely, BAPTA treatment selectively reduced the inhibition occurring after 10 s post 5HT, where steady state inhibition normally predominated (Fig. 4, A, B, and H). On average, 45.3 ± 4.6% \((n = 15)\) of the total 5HT-induced inhibition occurred after 10 s post 5HT in controls versus only 24.3 ± 4.5% \((n = 8)\) in BAPTA-treated cells \((P < 0.5, \text{Students’} \text{t-test})\). Experiments conducted using the cell-attached patch configuration suggest that steady state inhibition occurred via a diffusible cytosolic pathway, whereas kinetic slowing occurred via a membrane delimited pathway. In the cell-attached patch configuration, superfusion of the cell body outside the patch pipette with 5HT \((\text{a lipophobic molecule})\) can only affect the \(\text{Ca}^{2+}\) channels in the patch under the pipette via a cytosolic diffusible pathway (Fig. 5F). Under these conditions, 5HT \((10 \mu M)\) was observed to inhibit \(\text{Ca}^{2+}\) channel current by an average of 36 ± 5.1% in 13 of 39 small diameter cells tested. On average, peak inhibition occurred in 58 ± 4.5 s \((n = 13)\), which is similar to the 61 ± 2.5 s to
FIG. 4. Interaction of chelation of intracellular Ca$^{2+}$ with time course and voltage dependence of 5HT-induced inhibition of Ca$^{2+}$ channel currents. A and B: plots of Ca$^{2+}$ channel current vs. time in 2 different type 1 cells illustrating effects of internal Ca$^{2+}$ chelation on magnitude and time course of 5HT-induced Ca$^{2+}$ channel current inhibition. In A, internal Ca$^{2+}$ was buffered to 140 nM with EGTA as per usual procedure. In B, internal Ca$^{2+}$ was buffered to near 0 using an internal solution containing 10 mM bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA) plus no added CaCl$_2$. C and D: current sweeps taken from cells in A and B, respectively, under control conditions, during peak inhibition by 5HT, and after a pre-pulse delivered during peak inhibition by 5HT. E–H: summary of effects of BAPTA treatment on 5HT-induced inhibition of Ca$^{2+}$ channel currents (E), percent of inhibition via kinetic slowing (F), facilitation of Ca$^{2+}$ current in presence of 5HT, relative to control current amplitude (G), and percent of total inhibition occurring after 10 s post 5HT (H). Asterisk, responses statistically significant from controls, $P \leq 0.05$, Student’s t-test. For A and C and for “control” in E–H, solutions were same as that used in Fig. 1B. For B and D and for “BAPTA” in E–H, solutions were same as that used in Fig. 1B, except that for internal solution, Ca$^{2+}$ was omitted and 5 mM EGTA was replaced with 10 mM BAPTA.

peak inhibition observed in the above mentioned whole cell patch experiments.

An example of the 5HT-induced inhibition of Ca$^{2+}$ channel currents under cell-attached patch conditions is illustrated in Fig. 5, A and B. Note that only steady state inhibition was induced and that no kinetic slowing took place (Fig. 5B). This lack of kinetic slowing was observed in each of the 13 cells where 5HT produced inhibition under cell-attached patch conditions and is consistent with the idea that steady state inhibition observed in whole cell patch experiments was mediated by a diffusible cytosolic pathway. In contrast, both kinetic slowing and steady state inhibition were produced on application of 5-methoxy-N,N-dimethyl tryptamine (a membrane permeant 5HT$_{1A}$ agonist) outside the patch pipette to the same cell depicted in Fig. 5, A and B (Fig. 5C). Similarly, kinetic slowing and steady state inhibition were produced by 8-OH-DPAT (also membrane permeant) outside the patch pipette in three DRG cells recorded from in cell-attached patch configuration (Fig. 5E). In two of these cells, 5HT also was tested in the same manner and, as usual, only produced steady state inhibition (Fig. 5D). As reported above, both 5-methoxy-N,N-dimethyl tryptamine and 8-OH-DPAT produced kinetic slowing and steady state inhibition in type 1 DRG cells recorded from using the whole cell patch configuration. Thus the induction of kinetic slowing by 5-methoxy-N,N-dimethyl tryptamine and 8-OH-DPAT in the above experiments could be due to their penetration of the cell membrane, allowing access to 5HT$_{1A}$ receptors under the patch pipette. Activated 5HT$_{1A}$ receptors under the patch pipette then could couple to immediately adjacent Ca$^{2+}$ channels via a membrane-delimited pathway.

Although recordings were limited to small diameter cells in these cell-attached patch experiments, the DRG cells could not be characterized as to action potential duration, expression of $I_{\text{Na}}$, or sensitivity to capsaicin. The presence of 90 mM Ba$^{2+}$ in the pipette tip precluded whole cell recordings. However, the percentage of cells where 5HT inhibited Ca$^{2+}$ channel currents using the cell-attached patch configuration (13 out of 39 = 33%) was similar to the proportion of type 1 DRG cells randomly recorded from in previous whole cell patch experiments (≈ 1/3), when recordings were limited to small-diameter cells (Cardenas et al. 1995).

**CALCIUM CHANNEL COUPLING.** To test for 5HT$_{1A}$ receptor coupling to N- and L-type Ca$^{2+}$ channels, the occlusion of 5HT-induced Ca$^{2+}$ channel current inhibition by 1 $\mu$M $\omega$-conotoxin GVIA (CTX) and 2 $\mu$M nimodipine, respectively, was measured (Bean 1991; Boland et al. 1994; McCarthy...
channel currents persisted in cells simultaneously superfused with 2 μM nimodipine and 1 μM CTX (summarized in Fig. 6G). In four type 1 DRG cells tested, 5HT inhibited Ca\(^{2+}\) channel currents by 56 ± 4.9% under control conditions and by 7.5 ± 0.6% (relative to control Ca\(^{2+}\) current amplitude before addition of antagonists) after superfusion with nimodipine and CTX. Thus it is possible that some non-N/L Ca\(^{2+}\) channel type is coupled to 5HT\(_{1A}\) receptors in these cells. An alternate possibility is that 5HT was modulating L channels that were not blocked by 2 μM nimodipine, due to a reduction in efficacy of the dihydropyridine antagonist at negative membrane potentials (Bean 1983). It is unlikely that the nimodipine/CTX-resistant modulation was achieved by inhibition of T-type Ca\(^{2+}\) channels, which were little affected in type 1 cells where 5HT produced a large inhibition of high-threshold Ca\(^{2+}\) channel current (n = 2; Fig. 8).

Further inspection of the interaction between 5HT inhibition and CTX and nimodipine indicated that 5HT-induced kinetic slowing predominately involved a decrease in N-channel activity, whereas the steady state inhibition included a decrease in L-channel activity. CTX treatment reduced kinetic slowing by 82 ± 3.4% (n = 12) relative to pre-CTX controls (Fig. 6, B, C, and H). Also, relief of 5HT-induced inhibition by a prepulse was abolished completely by CTX treatment (Fig. 6, A–C and I). After CTX treatment, facilitation produced by a prepulse during peak inhibition by 5HT was reduced to an average of −9.7 ± 6.5% (n = 12) of pre-CTX control values (after subtraction of the pre-5HT control facilitation). Consistent with the above mentioned effects, blockade of N channels reduced the 5HT-induced inhibition occurring within the first 10 s after 5HT challenge when kinetic slowing normally occurred. Accordingly, N-
FIG. 6. Effects of selective N- and L-channel antagonists on 5HT-induced inhibition, kinetic slowing, and facilitation of Ca\(^{2+}\) channel current by prepulses. A: plot of Ca\(^{2+}\) channel current amplitude vs. time illustrating inhibition of Ca\(^{2+}\) current by 5HT and effects of prepulses (PP) before and during N-channel blockade with 1 μM ω-conotoxin GVIA (CTX). B and C: representative current sweeps taken from experiment depicted in A under different conditions as marked. D–F: same as A–C except that L-channels were blocked with 2 μM nimodipine. G and H: bar graphs summarizing data obtained by repeating experiments depicted in A–F. G summarizes inhibition produced by 5HT under control conditions (Control) and after blockade of N channels (CTX) or L channels (NIM) or both N and L channels (CTX + NIM). Control responses did not vary significantly between these 3 sets of experiments and are grouped together. H–J summarize effects of CTX or nimodipine treatment on percent inhibition occurring via kinetic slowing (H), facilitation by a prepulse during peak inhibition by 5HT (I), and percent of total inhibition that occurs after 10 s post 5HT (J). * and ** responses statistically significant from pair matched controls, \(P < 0.05\), paired difference test. Solutions were same as those used in Fig. 1B.

Channel blockade increased the proportion of inhibition occurring after 10 s post 5HT, when steady state inhibition typically predominated (Fig. 6, A and J).

An opposite pattern was observed regarding the above phenomena in cells where L channels were blocked with nimodipine. Kinetic slowing was reduced by only 34 ± 5.0%
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FIG. 7. Blockade of Bay K 8644-induced L-type Ca²⁺ channel tail currents by 5HT in a type 1 DRG cell. **A**: plot of current vs. time illustrating effects of 10 μM 5HT on peak Ca²⁺ channel currents and tail currents in a type 1 DRG cell under control conditions and after superfusion of cell with 100 nM Bay K 8644. **Top**: open circle, peak whole cell Ca²⁺ channel current; **bottom**: filled circle, represents tail current measured at 4 ms after cessation of depolarizing test command. **B and C**: current traces from experiment depicted in **A** before and after 5HT under control conditions and before and after 5HT in presence of 5HT. Letters near each current trace indicate where traces were taken from in plot of current vs. time shown in **A**. **D**: current voltage relationship of Ca²⁺ current in presence of 100 nM Bay K 8644. Series resistance was 0.98 MΩ. **E**: current traces from I-V curve depicted in **D**. **F**: effects of 10 μM 5HT on Bay K 8644 prolonged tail currents in a type 1 cell held at −40 mV to reduce current amplitude. Solutions were same as in Fig. 1B.

(n = 10; Fig. 6, E, F, and H). Facilitation resulting from a prepulse during peak inhibition by 5HT was little affected, averaging 82 ± 8.2% (n = 10) relative to pre-nimodipine controls (Fig. 6, D–F and I). Finally, blockade of L channels increased the proportion of inhibition that occurred within the first 10 s after 5HT challenge (when kinetic slowing took place) and reduced the inhibition of Ca²⁺ channel current that occurred after 10 s post 5HT (when steady state inhibition normally predominated; Fig. 6, D and J). In this regard, the L-channel blocker nimodipine had a similar effect as chelation of intracellular Ca²⁺ with BAPTA, described above.

**FIG. 8.** Lack of effect of 5HT on T-type Ca²⁺ currents in a type 1 cell. Cell was held at −90 mV and depolarized to −40 mV to evoke T currents. A subsequent depolarization to −10 mV 3 ms later evoked high-threshold Ca²⁺ channel current. Superimposed current traces are shown under control conditions and after peak effect of 10 μM 5HT on high-threshold Ca²⁺ channel current. Solutions were same as in Fig. 1B.

**DISCUSSION**

Several lines of evidence, presented above, suggest that 5HT₁A receptors are coupled to high-threshold Ca²⁺ channels by two parallel pathways in type 1 DRG cells. Regarding the 5HT receptor subtype, in a previous report we concluded that a 5HT₁A receptor was involved, based on the activity of the putative 5HT₁A selective ligands 8-OH-DPAT and NAN-190 (Del Mar et al. 1994; Glennon et al. 1988). However, it is now apparent that the Del Mar et al. (1994) study was inconclusive, due to the demonstration of novel 5HT receptor subtypes (5HT₁D, 5HT₁E, and 5HT₁F), which potentially are affected by concentrations of 8-OH-DPAT and/or NAN-190 close to those used in the Del Mar et al. (1994) study (Boess and Martin 1994). However, our new data, demonstrating that pindolol and spiperone are potent antagonists of 5HT-receptor-mediated inhibition of Ca²⁺ channel current in type 1 DRG cells, reaffirms the involvement of a 5HT₁A receptor. The nearly complete antagonism by 500 nM pindolol of inhibition of Ca²⁺ channel currents by a nearly maximal concentration of 8-OH-DPAT (1 μM), fits best with its affinity at the 5HT₁A receptor (pKi, 7.5) or 5HT₁B receptor (pKi, 6.3) versus its affinity at 5HT₅, 5HT₇, and 5HT₁D₁ receptors (pKi < 6) (Boess and Martin 1994; Dumis et al. 1988; Harwood et al. 1995; Lovenberg et al. 1993; Wisden et al. 1993). The potent antagonism of 5HT’s action by 1 μM spiperone finds support with spiperone’s affinity for the 5HT₁A receptor (pKi, 7.2) versus the 5HT₁B receptor (pKi, < 5) (Boess and Martin 1994; Sills et al. 1984).

The EC₅₀ regarding 5HT inhibition of Ca²⁺ channel currents in type 1 DRG cells (196 nM) is somewhat higher.
than the EC$_{50}$ observed for 5HT regarding some other reputed 5HT$_{1A}$-mediated responses including inhibition of Ca$^{2+}$ currents in acutely isolated rat cortical neurons (44 nM), increase in a K$^+$ conductance in acutely isolated rat dorsal raphe neurons (30 nM), or inhibition of adenylate cyclase activity in cultured hippocampal neurons (50 nM) (Dumuis et al. 1987; Foehring 1996; Penington et al. 1993). However, the EC$_{50}$ for 5HT has been shown to vary from low nanomolar to low micromolar concentrations in different expression systems transfected with 5HT$_{1A}$ receptors (reviewed in Boess and Martin 1994). This variation appears to be related to 5HT$_{1A}$ receptor density and 5HT$_{1A}$ receptor coupling to different signaling pathways (Boess and Martin 1994).

The differences in the ED$_{50}$ regarding the effects of 5HT in the above physiological systems versus that observed regarding type 1 DRG cells also may reflect variations in 5HT$_{1A}$ receptor density and/or coupling to second messenger systems. The ion channel coupling to 5HT$_{1A}$ receptors in cortical and dorsal raphe neurons appears to exclusively involve membrane-delimited pathways (Foehring 1996; Penington et al. 1993), whereas in type 1 DRG cells, a cytosolic diffusible component is included. In addition, membrane-permeant adenosine 3’、5’-cyclic monophosphate analogs neither mimic nor occlude inhibition of Ca$^{2+}$ channel currents by 5HT in type 1 DRG cells, suggesting that adenylate cyclase is not involved (Cardenas, unpublished observations). It is also possible that 5HT$_{1A}$ receptor density varies between type 1 DRG cells and the above mentioned central neurons.

Another possible source of variation in the affinity of the 5HT$_{1A}$ receptor for 5HT and other ligands is alteration of the receptor by the enzyme treatment used in the cell dissociation procedure, which differed between the studies on acutely isolated neurons mentioned above and this study (Foehring 1996; Penington et al. 1993). However the pharmacological profile of the putative 5HT$_{1A}$ receptor in type 1 cells suggests that it is not severely altered. The ED$_{50}$s for (+)-8-OH-DPAT and 5HT and are not greatly different regarding Ca$^{2+}$ channel inhibition in type 1 DRG cells (276 and 196 nM, respectively), similar to the situation at 5HT$_{1A}$ receptors in rat cortex neurons (15 and 44 nM, respectively), 5HT$_{1A}$ receptors transfected into hamster CHO cells (73 and 146 nM, respectively), and 5HT$_{1A}$ receptors expressed by cultured rat hippocampal neurons (8 and 50 nM, respectively) (Dumuis et al. 1987; Foehring 1996; Raymond et al. 1992). Furthermore, the putative 5HT$_{1A}$ receptor in type 1 DRG cells is sensitive to antagonism by typical 5HT$_{1A}$-selective antagonists such as spiperone and NAN-190 (Del Mar et al. 1994; this study). Also the receptor is strongly activated by 5-carboxamidotryptamine and 5-methoxy-N,N-dimethyl tryptamine, which have been shown to activate 5HT$_{1A}$ receptors in other systems (Boess and Martin 1994).

The experiments demonstrating that the inhibition of Ca$^{2+}$ channel currents is occluded partially by blockade of Ca$^{2+}$ current with CTX and/or nifedipine indicate that N and L channels are negatively coupled to 5HT receptors in type 1 DRG cells. In addition, the reduction of Bay K 8644 induced tail currents by 5HT is further evidence that L-channel currents are involved. The pharmacological data indicating involvement of N and L channels is consistent with the observation that frequently 5HT$_{1A}$ agonists inhibit 60–75% of peak high-threshold Ca$^{2+}$ current in type 1 cells. This degree of inhibition is more than can be explained by the blockade of N or L channels alone, because high-threshold Ca$^{2+}$ current in type 1 cells is on average 28% N type and 46% L type and 26% CTX/nifedipine resistant (Cardenas et al. 1995).

Some modulation of Ca$^{2+}$ currents by 5HT was observed even after both N and L channels were blocked with supposedly saturating concentrations of CTX (1 μM) or nifedipine (2 μM) (Bean 1991; Boland et al. 1994; McCarthy and TanPiengco 1992), indicating the possibility that some other high-threshold Ca$^{2+}$ channel type also is coupled to 5HT receptors in type 1 cells. However, because we have not directly determined that 2 μM nifedipine is a saturating concentration regarding L channels at the membrane potential of −60 mV used in the experiment, it is possible that some or all of the residual modulation of Ca$^{2+}$ channel current in the presence of 2 μM nifedipine and 1 μM CTX may be due to modulation of unblocked L channels.

Several observations made in the present study are consistent with the idea that the 5HT$_{1A}$ receptors couple negatively to N- and L-type Ca$^{2+}$ channels via two different signaling pathways. The inhibition of Ca$^{2+}$ channel currents by 5HT consisted of two components with different time courses: a rapidly occurring kinetic slowing and a slower steady state inhibition. Also, the steady state inhibition was antagonized by chelation of intracellular Ca$^{2+}$, whereas the kinetic slowing was not, suggesting that the two components involve different signaling molecules.

The data obtained from cell-attached patch recordings provides evidence that the kinetic slowing occurred via a membrane delimited pathway, whereas steady state inhibition required a cytosolic diffusible component. When recording in the cell-attached patch configuration, 5HT applied outside the patch produced only steady state inhibition. 5HT is not membrane permeant and thus would not be expected to gain access to the receptors under the patch pipette to activate receptors tightly coupled to channels in a membrane-delimited pathway. Thus only a diffusible cytosolic pathway was available to actuate the 5HT-induced steady state inhibition of Ca$^{2+}$ channels under the patch pipette. On the other hand, membrane-permeant 5HT$_{1A}$ agonists (8-OH-DPAT and 5-methoxy-N,N-dimethyl tryptamine) produced both steady state inhibition and kinetic slowing. These agonists could be expected to gain access to the receptors under the patch pipette as well as those outside the patch pipette and thus activate both membrane-delimited pathways and cytosolic diffusible pathways.

The interaction of Ca$^{2+}$ channel blockers with 5HT-induced inhibition suggests that the kinetic slowing involved modulation of N-type Ca$^{2+}$ channels, whereas steady state inhibition involved modulation of L channels. Blockade of N-type Ca$^{2+}$ channels with CTX occluded kinetic slowing induced by 5HT. Accordingly, blockade of N channels selectively reduced the proportion of inhibition that occurred in the first 10 s after addition of 5HT, which is the time period when kinetic slowing took place under control conditions. On the other hand, blockade of L-type channels with nifedipine did not greatly affect 5HT-induced kinetic slowing. However, L-channel blockade significantly reduced the pro-
portion of inhibition that occurred after the first 10 s of exposure to 5HT, a time when steady state inhibition predominated under control conditions.

The above data do not answer the question as to whether N channels participate in steady state inhibition. As mentioned above, a small amount of steady state inhibition by 5HT was observed after blockade of N and L channels with nímophine and CTX, suggesting the possibility that non-N/L channel(s) participate in steady state inhibition. Thus it seems possible that N channels also play a role in steady state inhibition.

Several observations support the idea that the 5HT-induced kinetic slowing was voltage sensitive, whereas the steady state inhibition was not. 5HT-induced kinetic slowing was reversed by prepulses to +70 mV. In addition, when kinetic slowing was abolished by selective blockade of N-type Ca\(^{2+}\) channels, prepulses no longer reversed inhibition of Ca\(^{2+}\) channel current by 5HT. Thus the voltage sensitivity of 5HT-induced inhibition depended on the presence of kinetic slowing. Conversely, prepulses had little effect on steady state inhibition, which was especially obvious when kinetic slowing was blocked with CTX. Together these data suggest that 5HT\(_{1A}\) receptors couple negatively to Ca\(^{2+}\) channels via two pathways: a membrane-delimited pathway, which couples to N channels and actuates voltage-sensitive kinetic slowing, and a pathway dependent on a cytosolic diffusible component and free intracellular Ca\(^{2+}\) that couples to L channels and actuates steady state inhibition.

The normal physiological role of the 5HT\(_{1A}\) receptor coupling to Ca\(^{2+}\) channels in DRG cell bodies is not clear. It is possible that neuroactive substances are released in the DRG. Several reports have been made of sparsely distributed synapses in DRG including varicosities containing norepinephrine and acetylcholine (Kayahara et al. 1981; Kummer 1994). In addition, serotonergic fibers have been observed in dorsal roots, suggesting the possibility of serotonin release in the DRG (Di Carlo 1983). Also, it is possible that the DRG cell bodies themselves release neuroactive substances, similar to sympathetic and parasympathetic neuronal cell bodies (Johnson and Pilar 1980; Suetake et al. 1981). Thus it is possible that various neurotransmitter-ion channel systems are expressed by subpopulations of DRG cell bodies to transduce this putative chemical input into changes in metabolism, growth, and excitability.

Under physiological conditions, where Ca\(^{2+}\) channels are triggered to open by action potentials, the inhibition of Ca\(^{2+}\) entry into type 1 DRG cell bodies by 5HT or other neuroactive agents might be exaggerated due to the simultaneous reduction in action potential duration. The change in Ca\(^{2+}\) channel activity produced by 5HT may reduce Ca\(^{2+}\) entry by two independent mechanisms, a direct decrease in the permeability of the membrane to Ca\(^{2+}\) during a depolarization and a secondary reduction resulting from shortening of the action potential duration. Previous studies have demonstrated that decreasing the duration of action potentials, used as depolarizing stimulus in voltage-clamp experiments, can decrease the amount of Ca\(^{2+}\) entry into neurons independently of any direct change in channel open probability (McCobb and Beam 1991; Penington et al. 1992; Scroggs and Fox 1992b). On the other hand, a neurotransmitter-induced reduction of Ca\(^{2+}\) channel opening probability will reduce Ca\(^{2+}\) entry evoked by a constant depolarizing stimulus (Bean 1991). Thus the reduction in Ca\(^{2+}\) entry due to 5HT-induced action potential narrowing could be expected to be additive to the reduction in Ca\(^{2+}\) entry produced by 5HT-induced inhibition of Ca\(^{2+}\) channel opening. Such a scenario has been demonstrated previously regarding 5HT inhibition of Ca\(^{2+}\) entry during action potentials in dorsal raphe neurons (Penington et al. 1992).

It is unknown what purpose is served by having parallel signaling pathways coupling 5HT receptors to Ca\(^{2+}\) channels in the type 1 DRG cells. The answer may lie in the fact that the two pathways are distinctly different regarding key characteristics such as coupling to Ca\(^{2+}\) channel subtypes, susceptibility to reversal by strong positive voltage, and participation of different molecules in the signaling pathway. These differences may permit various physiological input to selectively inactivate one or the other pathway. For example, one might expect rapid trains of action potentials to selectively reduce modulation via the membrane-delimited pathway by relieving the voltage-sensitive kinetic slowing produced by 5HT. Also, the voltage-sensitive kinetic slowing predominately involved N channels, whereas L-channel inhibition participated mainly in steady state inhibition. Thus due to the fact that N channels become inactivated at more negative membrane potentials than L channels (Fox et al. 1987a,b), depolarization or hyperpolarization of the membrane potential could be expected to alter the participation of the membrane-delimited pathway versus the diffusible cytosolic pathway. Finally, it is likely that different molecules are involved in each pathway, as evidenced by the dependence of the diffusible cytosolic pathway on free intracellular Ca\(^{2+}\) and the other various different characteristics of the two pathways. Participation of different molecules could lead to variations in the modulation of the two pathways by activation of other receptors. For example, activation of protein kinase C has been observed to reduce neurotransmitter-induced inhibition of Ca\(^{2+}\) currents via voltage-sensitive pathways in several neuronal types (Swarz 1993). Possibly activation of other second messenger pathways through synaptic activity or prolonged exposure to 5HT also might inactivate selectively one or the other pathways coupling 5HT\(_{1A}\) receptors to Ca\(^{2+}\) channels.

Selective inactivation or enhancement of the membrane-delimited pathway versus the cytosolic diffusible pathway might have important consequences because the two pathways are coupled to different Ca\(^{2+}\) channel subtypes, have different overall time courses, and most likely differ regarding the timing of inhibition of Ca\(^{2+}\) entry relative to the rise and fall of the action potential. The coupling of the membrane-delimited and the cytosolic diffusible pathways to different Ca\(^{2+}\) channel subtypes may be particularly important because different Ca\(^{2+}\) channel types have been shown to be variably coupled to the release of different neurotransmitters (Waterman 1996).

If 5HT inhibits Ca\(^{2+}\) currents in the afferent terminals of type 1 DRG cells, which resemble nociceptors, the release of 5HT into the spinal cord could be expected to reduce greatly neurotransmitter release from them. This possibly could explain the antinociceptive effect of 5HT release into the spinal cord in some cases (Yaksh and Wilson 1979). Regarding such cases, the antinociceptive effects of 5HT
also could be susceptible to impinging signals, which alter firing rates, change membrane potential, or activate alternate signaling pathways. A point of interest here is the possibility that L channels, which are not typically found at synapses, might be involved in neurotransmitter release from some types of nociceptors. This might render certain types of pain susceptible to blockade by dihydropyridines.

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