5HT Increases Excitability of Nociceptor-Like Rat Dorsal Root Ganglion Neurons Via cAMP-Coupled TTX-Resistant Na\((+\) Channels

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Cardenas, Luz M., Carla G. Cardenas, and Reese S. Scroggs. 5HT increases excitability of nociceptor-like rat dorsal root ganglion neurons via cAMP-coupled TTX-resistant Na\((+\) channels. J Neurophysiol 86: 241–248, 2001. The physiological effects of 5HT receptor coupling to TTX-resistant Na\((+\) current, and the signaling pathway involved, was studied in a nociceptor-like subpopulation of rat dorsal root ganglion (DRG) cells (type 2), which can be identified by expression of a low-threshold, slowly inactivating A-current. The 5HT-mediated increase in TTX-resistant Na\((+\) current in type 2 DRG cells was mimicked and occluded by 10 \(\mu\)M forskolin. Superfusion of type 2 DRG cells on the outside with 1 mM 8-bromo-cAMP or chlorophyll-lithio-cAMP (CPT-cAMP) increased the Na\((+\) current, but less than 5HT itself. However, perfusion of the cells inside with 2 mM CPT-cAMP strongly increased the amplitude of control Na\((+\) currents and completely occluded the effect of 5HT. Thus it appears that the signaling pathway includes cAMP. The phosphodiesterase inhibitor 3-isobutyl-L-methylxanthine (200 \(\mu\)M) also mimicked the effect of 5HT on Na\((+\) current, suggesting tonic adenylyl cyclase activity. 5HT reduced the amount of current required to evoke action potentials in type 2 DRG cells, suggesting that 5HT may lower the threshold for activation of nociceptor peripheral receptors. The above data suggest that serotonergic modulation of TTX-resistant Na\((+\) channels through a cAMP-dependent signaling pathway in nociceptors may participate in the generation of hyperalgesia.

INTRODUCTION

Previous work has suggested that the release of serotonin (5HT) from platelets and other cells into a wound site may participate in generation of the attendant inflammation and hyperalgesia. A role for 5HT in the generation of inflammation was first suggested in experiments with rats, where preinjection of 5HT receptor antagonists reduced inflammation induced by subsequent injection of irritants such as carrageenan (Di Rosa et al. 1971; Doak and Sawynok 1997; Rowley and Benditt 1956; Vinegar et al. 1987). Also, it has been shown in rats and humans that injections or direct application of 5HT sensitizes nociceptors and produces hyperalgesia (Beck and Handwerker 1974; Douglas and Ritchie 1957; Herbert and Schmidt 1992; Jensen et al. 1990a,b; Neto 1978; Sicuteri et al. 1965; Taiwo and Levine 1992; Taiwo et al. 1992). Furthermore, co-injection of various pharmacological agents with 5HT into the foot pads of rats suggest that the reduction of the pain threshold by 5HT involves an increase in cAMP and cAMP-dependent protein kinase activity (Taiwo et al. 1992).

Although the reduction in pain threshold by 5HT appeared to involve a cAMP-dependent protein kinase in the above-mentioned in vivo rat model, the signaling pathway that couples 5HT receptors to TTX-resistant Na\((+\) channels in nociceptors has not been conclusively elucidated. For this purpose we employed electrophysiological experiments in a subpopulation of nociceptor-like DRG cells (type 2), in which we previously demonstrated that activation of 5HT\(_4\) receptors produces an increase in TTX-resistant Na\((+\) currents (Cardenas et al. 1995, 1997). The studies presented below suggest that 5HT receptors are indeed coupled to TTX-resistant Na\((+\) channels by a cAMP-dependent pathway in type 2 DRG cells, and that 5HT also produces an increase in excitability of type 2 DRG cells. Thus our results are consistent with the idea that 5HT, released into injured tissues, may be involved in the production of hyperalgesia via a cAMP-dependent increase in Na\((+\) conductance in nociceptors.

METHODS

Male rats (50–150 g; Sprague-Dawley purchased from Harlan) were rendered unconscious with methoxyflurane, decapitated, and dorsal root ganglion (DRG) from L\(_1\)–L\(_5\) lumbar regions were dissected out. The ganglia were incubated at 36 \(^\circ\)C for 1 h in Tyrode’s solution (composition below) containing 2 mg/ml collagenase (Sigma, Type 1A) and 5 mg/ml Dipase II (Boehringer Mannheim). Individual DRG cell bodies were isolated by trituration and adhered to the bottom of a 35-mm Petri dish.

The dish with adhered cells was continually superfused with Tyrode’s solution containing (in mM) 140 NaCl, 4 KCl, 2 MgCl\(_2\), 2 CaCl\(_2\), 10 glucose, and 10 HEPES, adjusted to pH 7.4 with NaOH. Solutions were changed around the cell under study using 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. 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.

Experiments were restricted to small-diameter DRG cells (average diameter, 25.2 \(\pm\) 4.2 \(\mu\)m, mean \(\pm\) SD) that were classified as type 2, based on the expression of an I\(_{\text{A-H}}\) like current (Fig. 1A) that was evoked on repolarization to \(-50\) mV after a 787-ms hyperpolarization to \(-90\) to \(-110\) mV (Cardenas et al. 1995, 1997, 1999). For detecting the presence or absence I\(_{\text{A-H}}\) DRG cells were superfused with Tyrode’s solution externally. To study Na\((+\) currents, the solution was changed to a Na\((+\) current isolation solution containing (in mM) 50 NaCl, 90

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tetraethylammonium chloride (TEA), 10 4-aminopyridine (4-AP), 2 BaCl₂, 0.1 CaCl₂, 0.4 CdCl₂, and 10 HEPES, pH 7.4 with HEPES. To study total outward K⁺ current the solution was changed to a K⁺ current isolation solution containing (in mM) 140 choline-Cl, 4 KCl, 2 MgCl₂, 2 COCl₂, 10 glucose, and 10 HEPES, pH 7.4 with tris base. For all experiments the patch electrodes were filled with a solution containing (in mM) 120 K-aspartate, 5 Na₂-ATP, 0.4 Na₂-GTP, 5 MgCl₂, 5 EGTA, 1.86 CaCl₂, and 20 HEPES. The cells were superfused with Tyrode’s solution (A–D), Na⁺ current isolation solution (E–G), or K⁺ current isolation solution (H).

Serotonin (Research Biochemicals) was dissolved in water as a 10-mM stock solution, which was stored frozen in small aliquots for up to 2 wk, and then diluted to 10 μM in the external solution on the day of the experiment. Forskolin and the inactive forskolin analogue, 1–9,deoxyforskolin, were dissolved in dimethylsulfoxide (DMSO) as 100-mM solutions and stored frozen for up to 2 days. The final concentration of DMSO in the external solution after dilution of forskolin or 1–9,deoxyforskolin was 0.1% vol/vol. 8-Bromo-cAMP, 8-chlorophenylthio-cAMP (CPT-cAMP), and 3-isobutyl-1-methylnitrate (IBMX; Research Biochemicals) were dissolved directly into the appropriate solution.

Currents and voltages were recorded in the whole cell 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). Data were leak subtracted using scaled current sweeps derived from small hyperpolarizing voltage commands that did not evoke voltage-gated currents. Electrodes were fabricated from soda lime capillary glass (Scientific Products, B4416-1) using a Narishige two-stage vertical puller, coated with silicone elastomer (Sylgard) to about 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 resistance was estimated from capacity transients before and after compensation, as described previously (Scroggs and Fox 1992). No data were included where series resistance resulted in greater than a 10-mV error in voltage commands.

When analyzing the effects of drugs on Na⁺ current amplitude, it was often necessary to take into consideration the rate of Na⁺ current rundown or runup, which varied from cell to cell. Estimates of the effects of drugs on Na⁺ current amplitude were made from plots of current versus time. To estimate what the amplitude of the current would have been at various times if no drug had been added, a line was drawn through 5–10 control data points collected immediately before drug addition. This line reflected the slope of the control data and was extended beyond the control data to positions in the plot adjacent to data points reflecting current amplitude in the presence of drug. Then the y-coordinates of the line at these positions were used as the control current amplitudes.

Statistical analysis was performed using the nonparametric Mann-Whitney analysis (Systat, SPSS), which is a nonparametric analogue of the two-sample t-test; or the nonparametric Wilcoxon signed-rank test (Systat, SPSS), which is a nonparametric analogue of the paired t-test. All statistical analyses were carried out on raw data in the form of picoamperes or millivolts. Unless otherwise stated, the data in the RESULTS section are written as the means ± SE.
RESULTS

Characteristics of type 2 DRG cells and effects of 5HT on Na\(^+\) and K\(^+\) currents

The present study was restricted to type 2 DRG cells, which can be identified by their expression of a low-threshold A-current (Fig. 1A) (Cardenas et al. 1995, 1997). As illustrated in Fig. 1, B–D, type 2 DRG cells have several properties associated with nociceptors including sensitivity to capsaicin, long-duration action potentials with a Ca\(^{2+}\)-dependent shoulder on the falling limb, small diameter (average, 25.2 ± 4.2 \(\mu\)m, mean ± SD), and expression of a surface antigen (Galβ1–4 GlcNAc-R), which has been previously shown to be expressed by a subpopulation of DRG sensory neurons that terminate in the substantia gelatinosa (Cardenas et al. 1997; Del Mar and Scroggs 1996; Dodd and Jessell 1985).

At holding potentials of −60 mV or less negative, the whole cell Na\(^+\) current in type 2 DRG cells is predominately TTX resistant (Fig. 1E) (Cardenas et al. 1997). In the present study the TTX-resistant Na\(^+\) current in type 2 DRG cells was observed to exhibit pronounced use dependence when activated by 23-ms depolarizing voltage commands at a frequency of 1 Hz (Fig. 1F). In five type 2 DRG cells tested (holding potential, −40 mV), the peak Na\(^+\) current was significantly reduced by an average of 46 ± 4.5% (mean ± SE) from a control amplitude of 426 ± 79 pA after 14 s of 1-Hz stimulation (Wilcoxon signed-rank test, \(P = 0.043\)).

Cardenas et al. (1997) reported that the TTX-resistant Na\(^+\) current in type 2 DRG cells was increased by activation of 5HT\(_{4}\) receptors (Fig. 1G). Further study indicated that 5HT had little effect on outward K\(^+\) current in type 2 DRG cells, which was predominately a 4-AP–sensitive A-like current (Fig. 1H).

In five cells tested, the outward K\(^+\) current averaged 5.91 ± 0.49 nA after 11–15 min of exposure to 10 \(\mu\)M 5HT, compared with 5.95 ± 0.49 nA under control conditions. Thus 5HT appears to be selective for Na\(^+\) currents versus voltage-dependent K\(^+\) currents in type 2 DRG cells.

Involvement of cAMP in the 5HT-induced increase in TTX-resistant Na\(^+\) current

Forskolin, a stimulator of adenylyl cyclase, increased the amplitude of TTX-resistant Na\(^+\) currents in type 2 DRG cells, similar to 5HT (Fig. 2, A and B). In five type 2 DRG cells tested, 10 \(\mu\)M forskolin increased Na\(^+\) current by an average of 41 ± 14%, which was significantly greater than the average 6 ± 3% increase observed in the presence of 10 \(\mu\)M 1–9-dideoxyforskolin, an inactive analogue of forskolin (Wilcoxon signed-rank test, \(P = 0.043\); Fig. 2C).

In another series of experiments, we tested the ability of forskolin and 5HT to occlude the effects of one another. In five type 2 DRG cells where 10 \(\mu\)M forskolin was added first, the TTX-resistant Na\(^+\) current was increased by an average of 40 ± 11% over a time period averaging 6.3 min. Subsequent addition of 10 \(\mu\)M 5HT for an additional 2 min, in the continued presence of forskolin, did not produce an additional increase in Na\(^+\) current amplitude (−0.3 ± 2%), when the drug effects were estimated from plots of current amplitude versus time (Fig. 3, A–C; see METHODS). Conversely, in five type 2 DRG cells where 10 \(\mu\)M 5HT was added first, the TTX-resistant Na\(^+\) current was increased by an average of 59 ± 15% over a time period averaging 6.2 min, compared with an additional change of −5 ± 3% when 10 \(\mu\)M forskolin was subsequently applied in the continued presence of 5HT for an additional 3 min (Fig. 3, D–F). These data suggest that forskolin and 5HT may share the same mechanism of action.

We also examined the action of cAMP analogues on Na\(^+\) current in type 2 DRG cells. These compounds increased Na\(^+\) current, but not as effectively as 5HT itself. In six cells tested, 1 mM 8-bromo-cAMP slowly increased the TTX-resistant Na\(^+\) current by an average of 29 ± 14% over a time period of 4–5 min. After wash out of the effects of 8-bromo-cAMP in the same six cells, a subsequent application of 10 \(\mu\)M 5HT increased Na\(^+\) current by 48 ± 14% (Fig. 4, A–C). We also detected increases in Na\(^+\) current during application of 1 \(\mu\)M or 1 mM chloropropylthio-cAMP (CPT-cAMP), which amounted to approximately 9 and 46%, respectively, of sub-

FIG. 2. Effect of forskolin on TTX-resistant Na\(^+\) currents in a type 2 DRG cell. A: plot of peak Na\(^+\) current vs. time in a type 2 DRG cell. Na\(^+\) currents were evoked every 10 s using a test command to +10 mV from a holding potential of −40 mV. The 1st arrow indicates the initiation of superfusion with 10 \(\mu\)M 1–9, dideoxyforskolin, which was continued until the 2nd arrow, when the solution was switched to one containing 10 \(\mu\)M forskolin. The break in the middle of the plot is due to the construction of a current-voltage curve. B: individual Na\(^+\) current sweeps taken from the same experiment depicted in A, immediately before addition of forskolin (1–9,dideoxy Forskolin) and at the peak effect of forskolin (Forskolin). C: bar graph summarizing the effect of 1–9,dideoxyforskolin (10 \(\mu\)M) or forskolin (10 \(\mu\)M) on Na\(^+\) current amplitude in 5 type 2 DRG cells. The bars represent the average percent increase in Na\(^+\) current. The error bars indicate SE. The numbers above the error bars indicate the number of cells in each group. * Significantly different from the effect of 1–9,dideoxyforskolin. Solutions were the same as those in Fig. 1, E–G.
amplitude currents recorded without CPT-cAMP in the pipette (average amplification of control Na$^+$ cell, and the complete occlusion of the effects of 5HT and 100 pA). The above-described partial mimicry of the G protein (injected through the patch electrode in current-clamp mode) in 5 type 2 DRG cells. The 1st bar (Forskolin) represents the average percent increase in Na$^+$ current produced by 10 μM forskolin, while the 2nd bar (5HT + Forskolin) represents the additional average percent change in Na$^+$ current amplitude produced by a subsequent application of 10 μM 5HT + forskolin. D: plot of Na$^+$ current amplitude vs. time, showing the effects of 5HT, followed by the effects of co-application of 5HT + forskolin. E: current traces recorded during the experiment depicted in C, under control conditions, after 7.8 min of treatment with forskolin, and 3 min after co-application of forskolin + 5HT. F: bar graph summarizing experiment (C) in 5 type 2 DRG cells. The 1st bar (5HT) represents the average percent increase in Na$^+$ current amplitude produced by 10 μM 5HT, while the 2nd bar (5HT + Forskolin) represents the additional average percent change in Na$^+$ current amplitude produced by co-application of 10 μM forskolin + 5HT. Solutions were the same as those in Fig. 1, E–G.

We tested the idea that the cAMP analogues were not reaching adequately high concentrations inside the DRG cells when added to the outside, by including the cAMP analogues in the pipette solution. As illustrated in Fig. 4, D–F, inclusion of 2 mM CPT-cAMP in the pipette solution completely occluded the effects of 10 μM 5HT on Na$^+$ current (average increase = $\pm 2\%$, $n = 6$). In 10 control cells tested during the same time period, 10 μM 5HT increased the Na$^+$ current by 41 ± 7% (Mann-Whitney analysis, $P = 0.007$). In addition, the control Na$^+$ currents recorded before the addition of 5HT were significantly larger when CPT-cAMP was included in the pipette (average amplitude = 1,229 ± 330 pA) than control currents recorded without CPT-cAMP in the pipette (average amplitude = 382 ± 75 pA. Mann-Whitney analysis, $P = 0.003$; Fig. 4G). The above-described partial mimicry of the effects of 5HT by cAMP analogues added to the outside of the cell, and the complete occlusion of the effects of 5HT and amplification of control Na$^+$ currents by cAMP analogues added directly to the pipette solution suggests that cAMP is involved in coupling of the 5HT receptors to TTX-resistant Na$^+$ channels in type 2 DRG cells.

A final observation regarding the role of adenyl cyclase in the modulation of TTX-resistant Na$^+$ current in type 2 DRG cells was that the nonselective phosphodiesterase inhibitor IBMX also produced an increase in Na$^+$ current. In 11 type 2 DRG cells studied, superfusion with 200 μM IBMX increased Na$^+$ current by an average of 26 ± 4% over a 2-min time period (Fig. 5, A and B). This effect of IBMX on Na$^+$ currents fits with the idea that there was some basal activity of adenylyl cyclase in type 2 DRG cells under the conditions of our experiments.

**Physiological role of 5HT-induced increase in sodium current**

The effects of 5HT on the threshold for evoking action potentials in type 2 DRG cells was studied by determining the magnitude of 23-ms depolarizing current pulses (increased in 10-pA increments) required to evoke action potentials, before and during exposure to 5HT. In five type 2 DRG cells studied, 5HT produced a significant decrease in the amount of current (injected through the patch electrode in current-clamp mode) that was required to evoke action potentials (Fig. 6, A and B). Under control conditions the threshold current pulse required to evoke an action potential averaged 327 ± 49 pA. However, after exposure to 5HT for several minutes, the threshold for action potential generation was reduced to an average of 230 ± 49 pA (Wilcoxon signed-rank test, $P = 0.043$). There was also a small but significant increase in action potential amplitude in the above five cells, from 120 ± 4 mV under control conditions to 127 ± 3 mV after exposure to 5HT (Wilcoxon signed-rank test, $P = 0.043$; Fig. 6, A and C). These data suggest that 5HT increases the excitability of type 2 DRG cells, an effect that is likely caused by the increase in Na$^+$ current produced by 5HT.
We also tested the effects of 5HT on the frequency of action potential firing in type 2 DRG cells subjected to 300-ms long supramaximal depolarizing current pulses. The firing frequency of type 2 DRG cells ranged from an average of about 35 to 50 Hz in response to current pulses ranging from 0.5 to 1 nA, and was not significantly affected by 10 μM 5HT (Fig. 6, D–F). There was also a decrease in action potential amplitude over the course of trains of action potentials (Fig. 6, D and E), possibly resulting from the use dependence of the TTX-resistant Na\(^+\) current in type 2 DRG cells. This also was not significantly affected by 5HT.

**DISCUSSION**

We have previously provided evidence that the receptor mediating the 5HT-induced increase in TTX-resistant Na\(^+\) current in type 2 DRG cells belongs to the 5HT\(_4\) category (Cardenas et al. 1997). The present study provides evidence that, in type 2 DRG cells, 5HT receptors couple to TTX-resistant Na\(^+\) channels via a cAMP-dependent signaling pathway. The effects of 5HT on Na\(^+\) currents were mimicked and occluded by forskolin, which is known to increase the activity of adenyl cyclase. Although membrane-permeant cAMP analogues did not appear to fully mimic the effects of 5HT when added to the outside the type 2 DRG cells, their application directly to the inside of the cells by inclusion in the pipette solution potently occluded the effects of 5HT and increased...
control Na⁺ current amplitude. These data are consistent with the idea that cAMP is a link in the signaling pathway coupling 5HT₄ receptors to TTX-resistant Na⁺ channels in type 2 DRG cells. However, maximal up-regulation of the TTX-resistant Na⁺ channels by one agent could possibly occlude the effects of another, even if they acted through different signaling pathways.

Gold et al. (1998) reported that low concentrations of cAMP analogues were more effective than high concentrations at increasing TTX-resistant Na⁺ currents in cultured rat DRG neurons. However, we did not find evidence for the “bell-shaped dose-response curve” for cAMP analogues referred to in this previous study (Gold et al. 1998), as 1 μM CPT-cAMP was not more effective than 1 mM at increasing TTX-resistant Na⁺ currents in type 2 DRG cells. Perhaps this discrepancy points to some differences in the signaling pathways coupling TTX-resistant Na⁺ channels to receptors in cultured DRG neurons (Gold et al. 1998), versus the subpopulation of acutely isolated DRG cell bodies used in the present study. Similarly, different types of TTX-resistant Na⁺ channels may be expressed in the two different preparations (see Rush et al. 1998).

The idea that 5HT₄ receptors couple positively to TTX-resistant Na⁺ currents by a cAMP-dependent pathway in type 2 DRG cells is consistent with several previous studies. There are numerous reports demonstrating that 5HT₄ receptor activation can lead to activation of adenylyl cyclase and an increase in cAMP levels (Bockaert et al. 1992; Boess and Martin 1994; Dumuis et al. 1988; Fagni et al. 1992; Ford et al. 1992; Kaumann et al. 1991). Also, there are several reports that TTX-resistant Na⁺ currents are up-regulated by a cAMP-dependent pathway in subpopulations of cultured DRG cells (England et al. 1998; Gold et al. 1996, 1998). Finally, Fitzgerald et al. (1999) demonstrated that cAMP-dependent phosphorylation within the intracellular I-II loop of cloned TTX-resistant Na⁺ channels (SNS) resulted in an increase in Na⁺ currents conducted through these channels. Our observation that IBMX increased TTX-resistant Na⁺ currents in type 2 DRG cells is consistent with the idea of tonic adenylyl cyclase activity under control conditions in our experiments. Basal protein kinase A–dependent phosphorylation of TTX-resistant Na⁺ channels could allow for down-regulation as well as up-regulation of nociceptor excitability.

The increase in amplitude of voltage-activated Na⁺ currents and resultant increase in excitability produced by 5HT in type 2 DRG cells observed in the present study is consistent with a possible role of 5HT in hyperalgesic pain. In humans, serotonin (5HT) in the blood is bound to intracytoplasmic granules mostly in platelets, in the amount of 0.1–0.2 μg/ml, and is released by factors such as thromboxane, generated during the severing of blood vessel walls (Guyton 1981; Levy 1974). Thus based on the amount normally present in bound form, release of the 5HT in response to injury could potentially result in concentrations up to 1 μM. Possibly higher concentrations of 5HT could be reached at a wound site, due to localized aggregation of platelets. This would likely result in a strong activation of 5HT receptors on exposed nociceptive nerve endings, since most 5HT receptors exhibit pKi values in the nanomolar range for serotonin (Boess and Martin 1994).
There is considerable circumstantial evidence that type 2 DRG cell bodies belong to nociceptive sensory neurons. The type 2 DRG cell bodies (as classified based on the expression of a low-threshold A-current) consistently express several characteristics of nociceptors including small-diameter cell bodies, long-duration action potentials, TTX-resistant Na⁺ current, sensitivity to capsaicin, and expression of a carbohydrate surface antigen (Galβ1–4 GlcNAc-R), which has been previously shown to be expressed by a subpopulation of DRG sensory neurons that terminate in the substantia gelatinosa (Cardenas et al. 1995, 1997; Del Mar and Scroggs 1996; Djouhri et al. 1998; Dodd and Jessell 1985; Harper and Lawson 1985a,b; Holzer 1991; Light and Perl 1976, 1979; Villiere and McLachlan 1996). If 5HT produces a decrease in action potential threshold at the peripheral sensory receptors of the corresponding sensory neurons, then one might expect that after exposure to 5HT released during an injury, less stimulus would be required to generate signals in these neurons, thus lowering the threshold for pain. Such an effect of 5HT could partly underlie the sensitization of nociceptors and hyperalgesia produced by 5HT in humans and other mammals (Beck and Handwerker 1974; Douglas and Ritchie 1957; Herbert and Schmidt 1992; Jensen et al. 1990a,b; Neto 1978; Sicuteri et al. 1965; Taiwo and Levine 1992; Taiwo et al. 1992).

However, 5HT may participate in the production of hyperalgesia by activating multiple 5HT-receptor subtypes, which may be variably expressed by different types of nociceptors. For example, in rodents, 5HT₁₄ receptors appeared to be involved in the mediation of pain resulting from noxious heat, intraperitoneal injection of acetic acid, and inflammation of the paw (Doak and Sawynok 1997; Espejo and Gil 1998). On the other hand, hyperalgesia induced by injections of 5HT into the foot pads of rats appeared to be mediated by an adenyl cyclase coupled receptor with a pharmacological profile similar to 5HT₁₅ or 5HT₇ receptors, rather than 5HT₁₄ receptors (Taiwo and Levine 1992; Taiwo et al. 1992). In these latter studies, the degree of hyperalgesia induced by 5HT was assessed by applying a linearly increasing force to the bottom of the rats hindpaw and measuring changes in the force needed to elicit foot withdrawal. Thus it is possible that high-threshold mechanoreceptors were an important component in the above foot withdrawal response, and that type 2 DRG cell bodies with 5HT₁₄ receptors represent some other class of nociceptors that are not strongly activated by noxious mechanical pressure. There is some direct evidence for the existence of sensory neurons, with characteristics different from type 2 DRG cell bodies, that could possibly explain the observations reported in the Taiwo and Levine (1992) and Taiwo et al. (1992) studies. Cardenas et al. (1999) described subpopulations of nontype 2 DRG cell bodies that express adenyl cyclase–coupled 5HT receptors with a pharmacological profile similar to 5HT₁₅ receptors. 5HT produced an increase in excitability in these DRG cells by increasing I₅. However, whether these subpopulations of DRG cells represent nociceptors, or would be activated by noxious mechanical stimulus is unknown.

Rush et al. (1998) provided biophysical evidence for three types of TTX-resistant Na⁺ channels in sensory neurons. One of these three channel types exhibited a pronounced use dependence, similar to the TTX-resistant Na⁺ channels expressed by type 2 DRG cells. The physiological role of this use dependence is not clear; it may be an artifact of the nonphysiological 23-ms depolarizing voltage commands used to elicit the response. In any case, use dependence, and yet undiscovered idiosyncrasies of TTX-resistant Na⁺ channels, may prove useful for identifying channel subtypes. This may help us learn from future experiments whether different types of nociceptors express different types of TTX-resistant Na⁺ channels that vary in their physiological roles and mechanisms of modulation by inflammatory mediators.

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References


