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SPECIAL REPORT

5-Hydroxyindole slows desensitization of the 5-HT_3 receptor-mediated ion current in N1E-115 neuroblastoma cells

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Effects of 5-hydroxyindole (5-OHi) on 5-HT_3 receptor-operated ion current were investigated in voltage-clamped N1E-115 neuroblastoma cells. In the presence of 1 mM 5-OHi, the amplitudes of inward currents induced by the agonists 5-hydroxytryptamine (5-HT), 2-methyl-5-HT and dopamine were enhanced and desensitization of the responses was markedly slowed down. The results indicate that 5-OHi selectively modifies the desensitization of the 5-HT_3 receptor-mediated ion current.

Keywords: 5-Hydroxytryptamine; 5-HT_3 receptor; desensitization; 5-hydroxyindole; N1E-115 mouse neuroblastoma; whole-cell voltage clamp

Introduction Cells of the murine neuroblastoma clone N1E-115 express 5-hydroxytryptamine 5-HT_3 receptors (Hoyer & Neijt, 1988). The fundamental physiological and pharmacological properties of the 5-HT_3 receptor-operated ion current in whole-cell voltage-clamped neuroblastoma cells have been described in detail (Neijt *et al.*, 1986; 1988; 1989; Yakel & Jackson, 1988; Lambert *et al.*, 1989; Yang, 1990). The 5-HT_3 receptor appears to be a member of the ligand-gated ion channel family (Maricq *et al.*, 1991). Receptor occupation by 5-HT leads to a rapid increase of membrane inward current, followed by a decrease. The latter has been demonstrated to depend on agonist concentration and is caused by desensitization, which persists in the presence of the agonist. Desensitization is completely reversed after removal of the agonist (Neijt *et al.*, 1989). In N1E-115 cells, responses similar to those to 5-HT are evoked by the selective 5-HT_3 receptor agonist, 2-methyl-5-HT (2-Me-5-HT) and dopamine (Neijt *et al.*, 1988). Dopamine acts as a partial 5-HT_3 agonist. The response to dopamine in N1E-115 cells is completely blocked by the selective 5-HT_3 receptor antagonist, ICS 205–930 (Neijt *et al.*, 1986), and the agonists 5-HT and dopamine show full cross-desensitization (Neijt *et al.*, 1988). This demonstrates that the response to dopamine in N1E-115 cells is solely due to 5-HT_3 receptor activation.

We have investigated the effects of extracellular 5-hydroxyindole (5-OHi) in N1E-115 cells and show that this 5-HT moiety slows agonist-induced desensitization of the 5-HT_3 receptor-mediated inward current.

Methods Cell culture and electrophysiological techniques were identical to those previously described (Neijt *et al.*, 1989). Experiments were performed on dibutyryladenosine 3':5'-cyclic monophosphate (db-cyclic AMP) differentiated cells of passages 31–42 of the murine neuroblastoma clone N1E-115 (Amano *et al.*, 1972) under whole-cell voltage clamp, using the suction pipette technique (Lee *et al.*, 1978; Neijt *et al.*, 1989). Cells were voltage-clamped at a holding potential of −70 mV. Cells were continuously superfused with external solution and ion currents were evoked by changing to agonist- and, optionally, 5-OHi-containing external solution (Neijt *et al.*, 1989). 5-OHi was dissolved in dimethylsulphoxide as a 1 M stock solution, stored frozen at −20°C, and freshly thawed prior to the experiments. The composition of external and pipette solutions was the same as previously described (Neijt *et al.*, 1989). Cells were repeatedly exposed to agonist after an interval of at least 100 s in order to allow complete recovery from desensitization. All experiments were performed at room temperature (20–24°C). Values are presented as mean ± s.d.

Results The superfusion of N1E-115 cells under whole-cell voltage clamp ($V_m = -70$ mV) with external solution containing a maximally effective concentration of 10 μM 5-HT evoked a characteristic, transient 5-HT_3 receptor-mediated inward current (Figure 1a(i)). Superfusion with external solution containing 1 mM 5-OHi evoked no detectable electrophysiological response (not shown). However, the response evoked by superfusion with 10 μM 5-HT in the presence of 1 mM 5-OHi was greatly modified. The peak amplitude was enhanced and the desensitization was slowed down as compared to those of the control 5-HT response (Figure 1a(ii)). The same effects were observed irrespective of whether cells were already pre-exposed to 5-OHi or not, suggesting a rapid action. The effects of 5-OHi were rapidly reversed on washing with external solution (Figure 1a(iii)). Amplitude and desensitization of the 5-HT-induced inward current were hardly affected at a concentration of 10 μM 5-OHi (not shown). Similar reversible effects of 5-OHi were observed on inward currents evoked by superfusion with 50 μM of the selective 5-HT_3 agonist, 2-Me-5-HT (Figure 1b) or with 1 mM of the partial 5-HT_3 agonist, dopamine (Figure 1c). Responses in Figures 1b and 1c have been scaled to Figure 1a for matching control response amplitudes in order to demonstrate clearly the effects of 5-OHi. Average peak amplitudes of the 50 μM 2-Me-5-HT- and the 1 mM dopamine-induced control inward currents were 75 ± 7% ($n = 4$) and 35 ± 7% ($n = 17$) of that of the 10 μM 5-HT control responses in the same cells. To quantify the effects, the peak inward currents in the presence of 5-OHi and the fitted exponential time constants of desensitization have been normalized to those of control responses obtained from the same cells. The data presented in Table 1 show that 5-OHi enhanced the peak amplitude of inward currents induced by superfusion with the various 5-HT_3 receptor agonists by 30–94% and slowed desensitization 2.5–5 fold.

Discussion The mechanism of desensitization of the 5-HT_3 receptor-mediated ion current is still unresolved. The rate of desensitization has been shown to increase with agonist concentration in a sigmoid manner and it has been suggested that at saturating agonist concentration, desensitization is rate-limited by some intrinsic conformational change of the agonist receptor-ion channel complex (Neijt *et al.*, 1989). The present results show that 5-OHi, a moiety of the 5-HT molecule, dramatically slows the desensitization of inward

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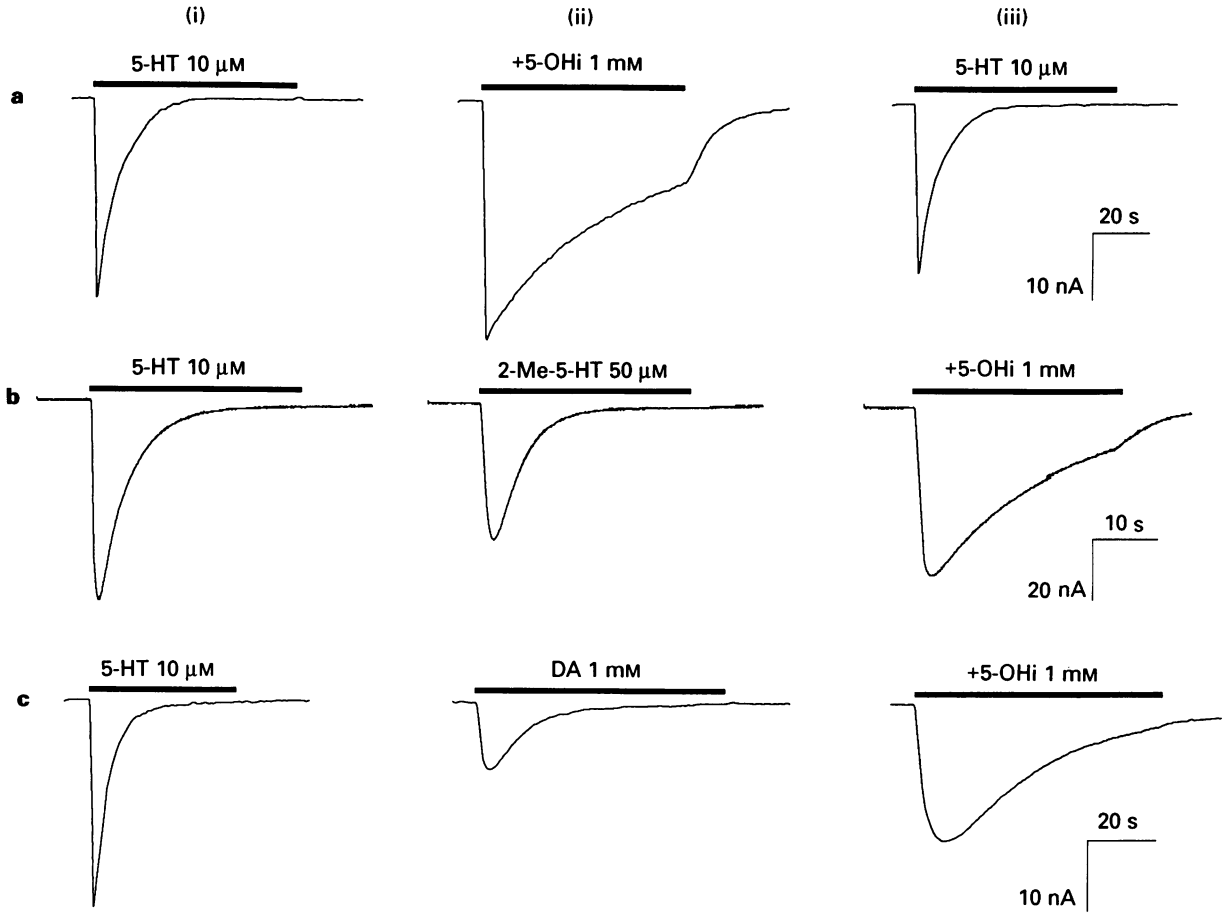

Figure 1 Effects of 5-hydroxyindole (5-OHi) on 5-HT_3 receptor-mediated inward current in N1E-115 cells voltage clamped at −70 mV. (a) Superfusion with external solution containing 10 μM 5-HT evokes a transient inward current, which rapidly desensitizes in the continued presence of 5-HT (a(i)). In external solution containing 1 mM 5-OHi the peak inward current induced by the same concentration of 5-HT is enhanced and the desensitization is markedly slowed (a(ii)). These effects of 5-OHi are rapidly reversed within 100 s of washing with external solution (a(iii)). (b), (c) Desensitization of inward currents induced by the selective 5-HT_3 receptor agonist 2-methyl-5-HT (b(ii)) and the partial 5-HT_3 receptor agonist dopamine (c(ii)) is also slowed and the peak amplitude of the inward currents is also enhanced in the presence of 5-OHi (b(iii) and c(iii)). Responses in (a), (b) and (c) are from three different cells. The responses of the different cells have been scaled for matching peak amplitudes of control inward currents evoked with 10 μM 5-HT.

Table 1 Effects of 1 mM 5-hydroxyindole on 5-hydroxytryptamine 5-HT_3 receptor-mediated inward currents in N1E-115 cells

Agonist	*Relative peak amplitude*	*Relative time constant*	n
5-HT	1.30 ± 0.10	3.27 ± 0.76	19
2-Me-5-HT	1.35 ± 0.10	4.75 ± 0.39	3
Dopamine	1.94 ± 0.15	2.73 ± 0.23	3

Peak amplitudes and time constants of desensitization are relative to the values obtained from control responses with the respective agonists in the same cell. Values represent mean ± s.d. obtained from *n* different cells.

currents induced by 5-HT_3 receptor agonists in N1E-115 cells. No effect of 5-OHi on the activation of inward current was observed. Simultaneously with the slowing of desensitization the amplitude of the inward current was enhanced. From the results in Table 1 there does not appear to be any relationship between the effects of 5-OHi on the amplitude and desensitization of currents evoked by 5-HT, 2-Me-5-HT and dopamine. Since all three agonists mediate their effects by 5-HT_3 receptors in N1E-115 cells, the independence of the effects of 5-OHi on amplitude and desensitization is most likely due to agonist-dependent differences in the coupling between receptor activation and desensitization.

The availability of a tool to modulate selectively the desensitization of the 5-HT_3 receptor-operated ion channel opens new perspectives for investigation of the mechanism of a functional process that is shared with other ligand-gated ion channels. In addition, the potential existence of endogenous modulators of desensitization becomes highly interesting from a pharmacological viewpoint. Although it is conceivable from the structural analogy between 5-HT and 5-OHi that the latter is able to interact with the agonist recognition site of the 5-HT_3 receptor, the precise nature of the interaction of 5-OHi remains to be established and is currently being investigated.

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