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Short communication

Characterisation of the 5-HT receptor potentiating neurotransmission in rabbit bladder

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Abstract

We have investigated the effects of 5-hydroxytryptamine (5-HT) on electrically induced contractions of rabbit isolated bladder. Electrical field stimulation evoked twitch contractions which were potentiated by 5-HT (0.3–10 μM). The potentiating effect of 5-HT was inhibited by ondansetron (pA_2 9.2) and granisetron (pA_2 9.1) but not by methysergide or SB 204070 ((1-butyl-4-piperidinyl)methyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride). This suggests that the potentiating effect of micromolar concentrations of 5-HT on neuromuscular transmission in rabbit isolated bladder is mediated by 5-HT_3 receptors. The receptors involved in the response to lower concentrations of 5-HT, observed in some tissues, remain to be characterised.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); 5-HT_3 receptor; Urinary bladder; Electrical field stimulation; (Rabbit)

1. Introduction

It has been demonstrated that 5-hydroxytryptamine (5-HT) causes potentiation of contractions induced by electrical stimulation of urinary bladder of man (Corsi et al., 1991), mouse (Holt et al., 1986), guinea pig (Messori et al., 1995) and pig (Bushfield et al., 1996). Interestingly, a remarkable between-species variation with regard to the characteristics of the presynaptic 5-HT receptors involved in this effect has been reported. In the mouse, the potentiating effect is mediated by 5-HT_{1B} and 5-HT_2 receptors (Holt et al., 1986; Cleal et al., 1989), in the guinea pig by the 5-HT_{2A}, 5-HT_3 and 5-HT_4 subtypes (Messori et al., 1995) and in man by the 5-HT_4 receptor (Tonini et al., 1994). The 5-HT receptors in pig urinary bladder have not yet been characterised (Bushfield et al., 1996).

Previously, it has been shown that in the absence of electrical stimulation, contractions of the rabbit isolated urinary bladder to 5-HT are mediated mainly by presynaptic 5-HT_3 receptors (Chen, 1990). However, the effects of 5-HT on the electrically induced excitatory neuromuscular transmission in rabbit isolated bladder have not yet been described. The aim of the present study was therefore to reveal such an effect and to characterise the 5-HT receptors involved.

2. Materials and methods

2.1. Rabbit isolated urinary bladder preparation

Female New Zealand rabbits (approximately 18 weeks; ESD France) were killed by cervical dislocation and exsanguination and the urinary bladder was removed and placed in modified Krebs-Henseleit solution of the following (mM) composition: NaCl 114.0, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.7 and ascorbic acid 1.1. The bladder was cleared of surrounding adipose tissue and cut in strips (approximately 15 mm long and 4 mm wide) which were mounted between 2 platinum electrodes under 1 g isometric tension in 20 ml organ baths containing the modified Krebs-Henseleit solution (continuously gassed with 95% O_2 and 5% CO_2 and thermostatically controlled at 37°C). Tissue responses were measured using Grass FT03 isometric transducers.

2.2. Experimental protocol

After a 45 min stabilisation period, a calibration contraction (4.0 ± 0.3 g, $n = 43$) was obtained to 80 mM KCl

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in each tissue. Following a 30 min washout period, tissues were subjected to electrical field stimulation (Hugo Sachs Elektronik Stimulator I, Type 215/1). Trains of electrical pulses (20 Hz for 5 s, 0.3 ms pulse duration, 20 V) were delivered at 60-s intervals. Following stabilization of the electrically evoked contractions (approximately 30 min), a first 5-HT concentration-effect curve was obtained by cumulative additions as half-log unit concentration increments. Tissues were then washed for 30 min and incubated for 30 min with antagonist or vehicle before a second 5-HT concentration-effect curve was obtained. Only one concentration of antagonist was studied in each tissue. Because preliminary experiments had shown that the first and second 5-HT curve were not always superimposable in vehicle-treated tissues, it was not possible to evaluate the effects of the antagonists by comparing the first and second 5-HT curve in the same tissue. Therefore, the curves obtained following antagonist incubation were compared with the second 5-HT curve obtained in vehicle-treated tissues from the same animal.

2.3. Analysis

Individual agonist concentration-effect data were measured as increase of the amplitude of the electrically induced twitch contractions and were then expressed as percentage of the maximum increase. The midpoint locations (pEC_{50}) of individual concentration–effect curves were estimated by interpolation between the highest concentration that produced less than 50% effect and the lowest concentration that produced more than 50% effect. The effect of drug treatment on the pEC_{50} parameter was assessed by Student's non-paired t-test. Values of $P < 0.05$ were considered to be significant. From the pEC_{50} values obtained in the absence and presence of antagonist (B), pA_2 values were estimated using the equation $pA_2 = -\log_{10}[B] + \log_{10}(r - 1)$, where r is the concentration ratio.

2.4. Compounds

Compounds were obtained from the following sources: atropine sulfate, 5-hydroxytryptamine hydrochloride (5-HT), 5-methoxytryptamine hydrochloride and α,β-methylene-ATP: Sigma; tetrodotoxin: R.B.I.; granisetron hydrochloride: gift from SmithKline Beecham Pharmaceuticals; methysergide maleate: gift from Sandoz; ondansetron hydrochloride, SB 204070 ((1-butyl-4-piperidinyl)methyl 8-amino-7-chloro-1,4-benzodioxan-5-carboxylate hydrochloride) and GR 113808 ([1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl] methyl 1-methyl-1*H*-indole-3-carboxylate): Synthélabo Recherche. SB 204070 and methysergide were dissolved in 40% and absolute ethanol, respectively. All other compounds were dissolved in distilled water.

3. Results

3.1. Contractile response to electrical field stimulation

Electrical field stimulation (20 Hz for 5 s every 60 s, 0.3 ms pulse duration, 20 V) evoked submaximal twitch contractions (49.6 ± 6.0% compared to the response to 80 mM KCl, $n = 43$) of rabbit urinary bladder strips (Fig. 1). These contracile responses were blocked completely by 3 μM tetrodotoxin (Fig. 1A) and 1 μM atropine (Fig. 1B). In contrast, the electrically stimulated contractions were not affected by 30 min treatment with 30 μM α,β-methylene-ATP (data not shown).

3.2. Effects of 5-HT and 5-methoxytryptamine on the response to electrical field stimulation

Cumulative addition of 5-HT (0.3–10 μM) produced a significant, concentration-dependent increase in the amplitude of the twitch contractions (Fig. 1C) with an associated pEC_{50} value of 6.24 ± 0.04 ($n = 20$). In some tissues, a small response (approximately 10–20% compared to the

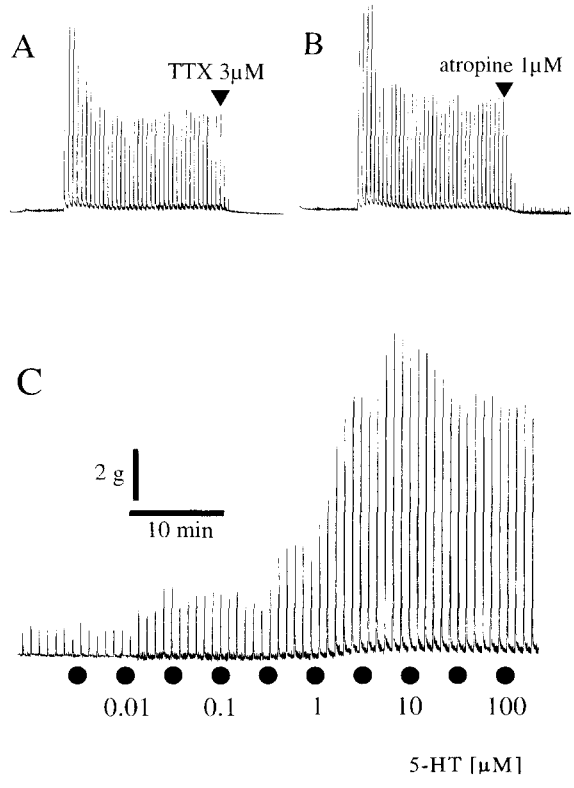

Fig. 1. Representative experimental tracings showing the effects of (A) 3 μM tetrodotoxin (TTX), (B) 1 μM atropine and (C) 3 nM–100 μM 5-hydroxytryptamine (administered using a cumulative dosing regimen at half-log unit concentration increments, ●) on the contractile responses of rabbit isolated urinary bladder strips to electrical field stimulation 20 Hz for 5 s every 60 s, 0.3 ms pulse duration, 20 V).

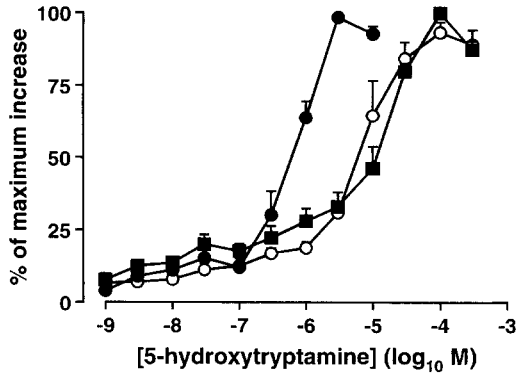

Fig. 2. Concentration-effect curves for the potentiation by 5-hydroxytryptamine of the contractile responses of rabbit isolated urinary bladder strips to electrical field stimulation in the absence (●, $n = 8$) and presence of 10 nM ondansetron (○, $n = 4$) and 10 nM granisetron (■, $n = 4$). Effects are expressed as percentage of the maximum increase of the amplitude of the electrically induced twitch contractions. Error bars indicate S.E.M.

maximum effect) was also observed with lower concentrations (1–100 nM) of 5-HT (Fig. 1C and Fig. 2), but we could not detect a consistent, concentration-effect relationship for this first phase in all tissues. 5-HT produced no effect in tissues which were pretreated with tetrodotoxin (3 μM) or atropine (1 μM; data not shown).

In contrast to 5-HT, the selective 5-HT_4 receptor agonist, 5-methoxytryptamine (Craig et al., 1990), produced only a small potentiation (18.3 ± 13.5%, $n = 3$) of the electrically induced contractions at the highest concentration tested (0.3 mM).

3.3. Antagonism of the potentiating effect of 5-HT

The selective 5-HT_3 receptor antagonists, ondansetron (10 nM) and granisetron (10 nM), had no effect on the basal contractions, but produced parallel rightward shifts of the 5-HT (0.3–10 μM) concentration-effect curve with associated pA_2 values of 9.20 ± 0.16 ($n = 4$) and 9.08 ± 0.21 ($n = 4$), respectively (Fig. 2). In contrast, the 5-HT_1/5-HT_2 receptor antagonist, methysergide (1 μM) and the 5-HT_4 receptors antagonist, SB 204070 (Wardle et al., 1994), tested at 10 nM, had no significant effect on the 5-HT response ($pEC_{50} = 6.25 \pm 0.07$ and 6.15 ± 0.12 in the absence and presence of methysergide, respectively, $n = 3$, $P > 0.2$; $pEC_{50} = 6.28 \pm 0.07$ and 6.17 ± 0.05 in the absence and presence of SB 204070, respectively, $n = 6$, $P > 0.2$). Furthermore, GR 113808, a selective 5-HT_4 receptor antagonist which has recently been shown to antagonise the potentiating effect of 5-HT on neuromuscular transmission in human urinary bladder with nanomolar potency (Tonini et al., 1994), produced a rightward shift of the 5-HT concentration-effect curve only at the highest concentration tested (1 μM) with an associated pA_2 value of 6.30 ± 0.07 ($n = 3$).

Because the response to low concentrations (1–100 nM) of 5-HT was not observed in all control tissues (see above), it was not possible to conclude whether the antagonists had an effect on this response.

4. Discussion

In this study, we have demonstrated that 5-HT potentiates the contractions induced by electrical stimulation of the isolated urinary bladder of rabbit, consistent with results obtained in other species (see Section Section 1). In the presence of tetrodotoxin or atropine, the electrically induced contractions were abolished and subsequent addition of 5-HT produced no effect. This suggests that in rabbit, like in man (Corsi et al., 1991; Tonini et al., 1994), the potentiation by 5-HT in the isolated bladder is mediated via neuronal release of acetylcholine.

The response to 5-HT was characterised by two phases. The second, low-potency phase was observed in all tissues and was blocked by selective concentrations (10 nM) of granisetron and ondansetron. This suggests that 5-HT_3 receptors play a major role in the effect of micromolar concentrations of 5-HT, which has also been proposed to be the case in the guinea pig isolated bladder by Messori et al. (1995). The fact that these authors found a ~ 100-fold lower potency for granisetron (pK_B 7.1–7.3) than we did (pA_2 9.1) may be due to differences between rabbit and guinea pig 5-HT_3 receptors (see Hoyer et al., 1994). Further evidence for the involvement of 5-HT_3 receptors was obtained with GR 113808, since this selective 5-HT_4 antagonist blocked the low-potency 5-HT response with an associated pA_2 value (6.3) which is almost identical to its reported affinity for 5-HT_3 receptors (pK_i 6.0; Gale et al., 1994).

On the basis of the present data it is not possible to conclude whether or not the high potency phase in the rabbit bladder is mediated by the same receptors (5-HT_{2A} and 5-HT_4 receptors) that are involved in the response to low concentrations of 5-HT in the guinea pig bladder (Messori et al., 1995). However, considering the low potency and efficacy of 5-methoxytryptamine, it is unlikely that 5-HT_4 receptors play a significant role in the rabbit.

In conclusion, this study has shown that in rabbit isolated bladder, contractile responses to electrical field stimulation are mediated mainly by neuronal release of acetylcholine. Micromolar concentrations of 5-HT produce potentiation of these contractions via an action at 5-HT_3 receptors. These data extend the study of Chen (1990), who showed that presynaptic 5-HT_3 receptors mediate contractions of rabbit bladder to 5-HT in the absence of electrical stimulation. The receptors involved in the less pronounced potentiating effects of lower concentrations of 5-HT remain to be characterised.

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