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Activation of 5-HT_3 receptor subtypes causes rapid excitation of rabbit parasympathetic neurones

T. Akasu, H. Hasuo & [1]T. Tokimasa

Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan

Intracellular recordings were made from parasympathetic neurones of the rabbit vesical pelvic ganglia maintained *in vitro*. 5-Hydroxytryptamine (5-HT) caused a membrane depolarization which was antagonized by ICS 205–930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester) but not by methysergide. ICS 205–930 caused a parallel shift to the right of the dose-response curve for 5-HT. These results suggest that the 5-HT_3 receptor is involved in the membrane depolarization.

Introduction 5-Hydroxytryptamine (5-HT) has a potent depolarizing action on visceral primary afferent neurones (Wallis *et al.*, 1982; Higashi & Nishi, 1982), sympathetic neurones (Wallis & Woodward, 1974; Wallis & North, 1978) and neurones within the enteric division (Hirst & Silinsky, 1975; Wood & Mayer, 1979; Johnson *et al.*, 1980). Since the discovery of selective antagonists for some 5-HT receptor subtypes (Fozard, 1984; Richardson *et al.*, 1985; Richardson & Engel, 1986; Round & Wallis, 1986), it has become possible to correlate the ionic mechanism of the action of 5-HT with the particular receptor subtypes. We noticed during the course of experiments on rabbit vesical pelvic ganglia (parasympathetic neurones) that 5-HT also causes a rapid membrane depolarization. We therefore aimed to determine the receptor subtypes involved by using the selective antagonist ICS 205–930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester).

Methods Intracellular recordings were made from neurones in pelvic ganglia isolated from anaesthetized rabbit urinary bladder wall. The preparation was continuously perfused with preheated (37°C at the recording site) Krebs solution that contained (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $NaHPO_4$ 1.2, $MgCl_2$ 1.2, $NaHCO_3$ 25, glucose 11 saturated with 5% CO_2/95% O_2. When the concentration of calcium ions in Krebs solution was reduced from 2.5 to 0.25 mM, magnesium ions were increased from 1.2 to 6.0 mM. This solution is referred to as the low calcium solution throughout this paper. Glass-microelectrodes contained 3 M KCl and had resistances of 40–80 MΩ. Drugs were applied by changing the superfusion solution; the time required for new solutions to clear the dead space in the perfusion system was about 20 s. 5-HT was also applied from drug-containing (0.1–10 μM) broken-tip micropipettes (diameter about 10 μm) placed about 30 μm away from the neurones from which the recordings were made. Drugs used were 5-hydroxytryptamine dihydrochloride (Sigma), ICS 205–930 (Sandoz), methysergide hydrochloride (Sigma), and tetrodotoxin (Sankyo).

Results 5-HT caused membrane depolarizations when applied by brief pressure ejection pulse (20 kN m^{-2} for 50 ms) onto the soma membrane. The depolarization reached its peak within 50–100 ms and passed off within 5–20 s. Figure 1 shows an example. The depolarization was associated with a decreased membrane resistance (Figure 1) and the ionic mechanism was essentially the same as that observed in the superior cervical ganglion (Wallis & North, 1978) and the nodose ganglion (Higashi & Nishi, 1982) of the rabbit. The depolarization was not eliminated in a solution which contained tetrodotoxin (TTX, 1 μM) or in a TTX-containing low calcium Krebs solution, where synaptic transmissions are blocked (Gallagher *et al.*, 1982) (Figure 1a). These results suggested that 5-HT directly depolarizes the post-ganglionic neurones. Methysergide (10 μM) caused no inhibitory action on the 5-HT depolarization (Figure 1b).

ICS 205–930 (0.01–1 nM) was found to be a potent antagonist of the 5-HT receptors in parasympathetic neurones in urinary bladder wall. Figure 1c shows an example of a recording in which ICS 205–930 (1 nM) markedly depressed the 5-HT-induced depolarization. ICS 205–930 had no significant effects on the resting membrane properties. In five experiments, ICS 205–930 (1 nM) produced a 82 ± 6% depression of the depolarization evoked by 5-HT (Figure 1c). The effect of this antagonist is concentration-dependent, with reduction in the amplitude of 5-HT response apparent at a concentration of 10 pM. Figure 1d shows the effect of ICS 205–930 (100 pM) on the dose-response relationship of 5-HT obtained from a single neurone. ICS 205–930 shifted the dose-response curve to the right without any significant reduction in the slope

[1] Author for correspondence

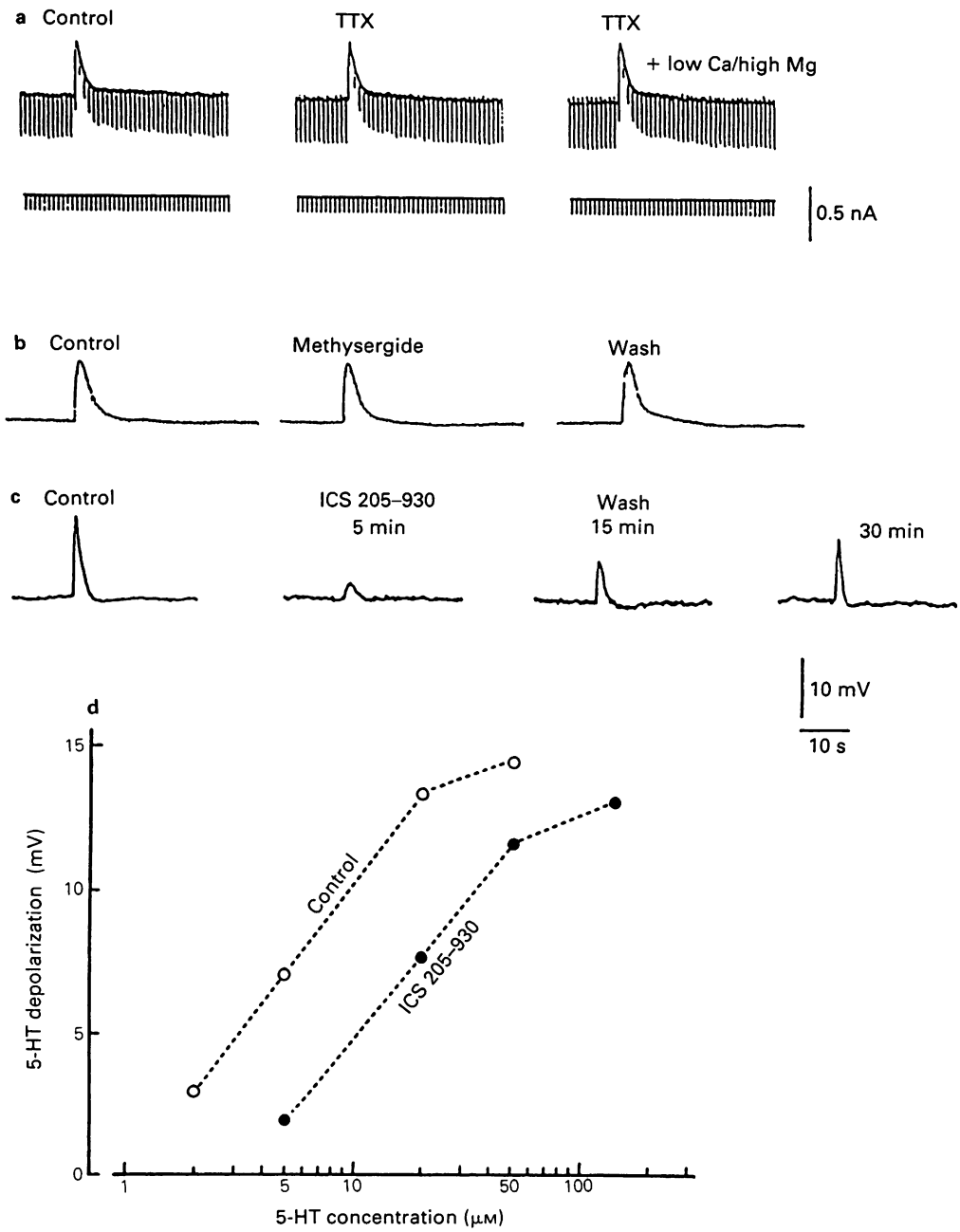

Figure 1 (a) Effects of tetrodotoxin (TTX 1 μM) and low Ca (0.25 mM)/high Mg (6 mM) on the depolarization evoked by 5-hydroxytryptamine (5-HT). Downward deflections are electrotonic potentials (upper traces) produced by applying hyperpolarizing current pulses (lower traces) with durations of 100 ms through the recording microelectrode. (b) Lack of effect of methysergide (10 μM) on the 5-HT-induced depolarization. Methysergide was applied in the superfusing solution for 10 min. (c) The inhibitory effect of ICS 205–930 ([3α-tropanyl]-1H-indole-3-carboxylic acid ester) at a concentration of 1 nM on the 5-HT depolarization. In (a), (b) and (c) 5-HT (1 μM) was applied by brief pressure pulse (20 kN m^{-2}; 50 ms) through a micropipette. (d) Effect of ICS 205–930 (100 pM) on the depolarization evoked by 5-HT, obtained from a single parasympathetic neurone. The 5-HT was applied by superfusion for 1 min intervals.

(Figure 1d), compatible with competitive antagonism. Depolarizations induced by acetylcholine were not antagonized by ICS 205–930 (10 nM).

Discussion We have found that 5-HT depolarizes the parasympathetic neurones in rabbit vesical pelvic ganglia. Actions of 5-HT on neuronal function were well analysed in visceral primary afferent nerve, sympathetic ganglia and enteric plexus (Watanabe & Koketsu, 1973; Wallis & Woodward, 1974; Wallis & North, 1978; Wood & Mayer, 1979; Round & Wallis, 1986). Recently, neuronal elements containing 5-HT were found to exist in mudpuppy parasympathetic cardiac ganglia (Neel & Parsons, 1986). It has been reported that 5-HT releases a non-cholinergic, non-adrenergic, probably purinergic neurotransmitter (Burnstock *et al.*, 1978) from postganglionic parasympathetic nerve fibres in urinary bladder through 5-HT M-receptors (now called 5-HT_3) (Saxena *et al.*, 1985). We, therefore, propose that 5-HT may increase the excitability of parasympathetic neurones in the urinary bladder wall, by acting on a 5-HT_3 receptor.

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