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5-HT2A receptor is the predominant receptor mediating contraction of the isolated porcine distal ureter to 5-HT in young and old animals

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ABSTRACT

Isolated ureteral strips develop spontaneous phasic contractile activity which is enhanced by 5-hydroxytryptamine (5-HT). The aim of this study was to identify the receptor subtype mediating these responses and to determine whether responses to 5-HT change with age. The frequency of contractions and the overall contractile activity (measured as the area under the curve, AUC) were recorded in strips of porcine distal ureter isolated from young (3 months) and old (2 years) pigs. Responses to 5-HT were examined in the absence and presence of selective 5-HT receptor subtype antagonists. Tissues from the younger animals elicited larger contractile responses to 5-HT ($5885 \pm 335\ g^{-1}$ s) than tissues from the older animals ($2787 \pm 317\ g^{-1}$ s, $P < 0.001$). The 5-HT_{2A} receptor antagonist ketanserin (10–100 nM) elicited rightward shifts of 5-HT concentration-response curves, antagonising AUC and frequency responses with high affinity in tissues from both age groups (pK_D values 8.4–8.8). The slopes of the corresponding Schild plots were not significantly different from unity, suggesting a competitive antagonism at a single receptor, except for frequency responses to 5-HT in the older animals. Antagonists selective for other 5-HT receptor subtypes: methiothepin (non-selective), RS-10221 (5-HT_{2C}), ondansetron (5-HT_3), GR-113808 (5-HT_4), SB699551 (5-HT_5), SB399885 (5-HT_6), SB269970 (5-HT_7)) had no effect on 5-HT-induced responses. The results suggest that the 5-HT_{2A} receptor subtype is the predominant receptor mediating 5-HT responses in ureteral tissues, being the sole mediator of responses in tissues from young animals, but with another receptor subtype also playing a minor role in the older animals.

1. Introduction

5-hydroxytryptamine (5-HT) is found throughout the body and influences smooth muscle activity in various tissues including the intestine, vasculature and urinary bladder (Kim and Camilleri, 2000; Klarskov and Horby-Petersen, 1986; Mohammad-Zadeh et al., 2008). The current classification of 5-HT receptors include 5-$HT_{1\ (1A,\ 1B,\ 1D,\ 1E,\ 1F)}$ which mediate responses via the inhibition of adenylate cyclase, 5-$HT_{2\ (2A,\ 2B,\ 2C)}$ which lead to activation of phospholipase C, 5-HT_3 which act as ligand-gated ion channels and 5-HT_4 which are positively coupled to adenylate cyclase. In addition, there are three subtypes that are not so well-defined, 5-HT_5 which mediate inhibition of adenylate cyclase and 5-HT_6 and 5-HT_7 receptors that lead to activation of adenylate cyclase (Roth, 1994).

While the mammalian urinary bladder and lower urinary tract have been shown to contract in response to 5-HT, in many studies in the human and pig (Todd and Mack, 1969, Ambache and Zar, 1970, Long and Nergardh, 1978, Sellers et al., 2000), relaxation has also been reported (Klarskov and Horby-Petersen, 1986). In the ureter however, in all species examined thus far, 5-HT has been found to produce an excitatory action only. In isolated strips of human ureter, 5-HT induces concentration-dependent contractions (Barnes, 2001; Gidener et al., 1999) that are antagonised by the 5-HT_{2A} receptor antagonist ketanserin and the mixed 5-HT_1/5-HT_2 receptor antagonist methysergide, but are not altered by 5-HT_3 and 5-HT_4 receptor antagonists (Gidener et al., 1995). In addition, the 5-HT_{2A} antagonist ritanserin inhibits contraction of the isolated pig intravesical ureter (Hernandez et al., 2003), whilst the 5-HT_{2A} agonist 2,5-dimethoxy-4-iodoamphetamine has been reported to increase the frequency of ureteral contractions in pig ureter in vivo in a dose-dependent manner (Hauser et al., 2002).

Ageing has been shown to increase the risk of ureteral calculus development (Hess, 2003). Whilst this could be related to changes in ureteral function, the effects of age on ureteral contractility are not yet known. Age-related changes in 5-HT-mediated contractile mechanisms have been observed in other systems, including the gastrointestinal system (Keating et al., 2015), and in the rat vas deferens the inhibitory effect of 5-HT_2 receptor stimulation was found to decrease with increasing age (Moritoki et al., 1986). The aim of this study was to

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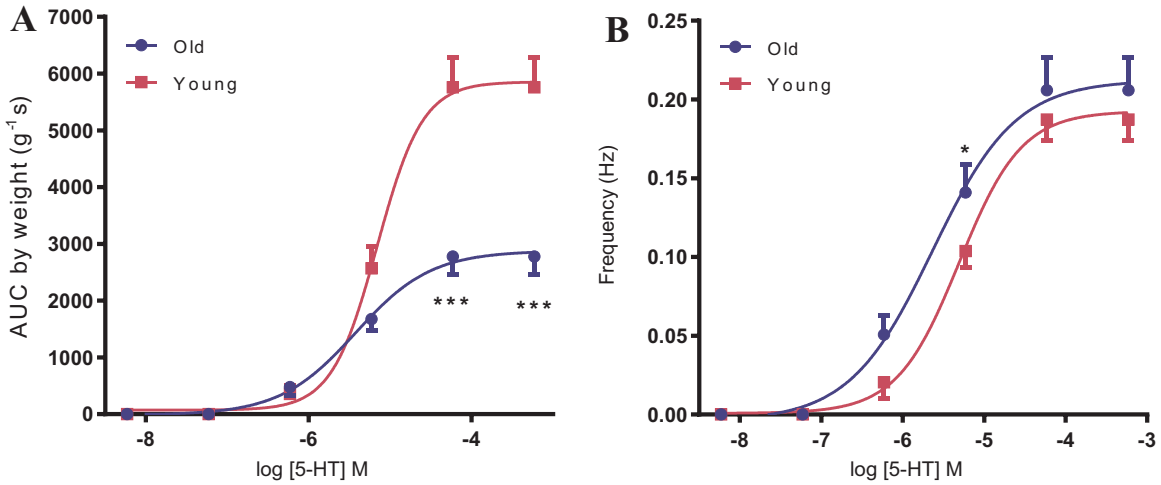

Fig. 1. Concentration-response curves to 5-HT in distal ureteral tissues from old and young pigs. Responses are expressed as AUC by weight in g^{-1} s (a) and frequency in Hz (b). Results are presented as mean ± S.E.M of 8 preparations for each group. (*P < 0.05, ***P < 0.001 vs corresponding value for young).

identify the receptor subtype mediating responses to 5-HT in the porcine ureter and to determine whether age has any effect on these responses.

2. Materials and methods

2.1. Tissue preparation

Fresh bladders with ureters attached were obtained from a local abattoir and immediately immersed in ice-cold Krebs-bicarbonate solution (4 °C) composed of NaCl (188.4 mM), $NaHCO_3$ (24.9 mM), glucose (11.7 mM), $CaCl_2$ (1.9 mM), $MgSO_4$ (1.2 mM) and KH_2PO_4 (1.2 mM). Tissues were obtained from 4 month old (young) and 3 year old (old) female pigs. The ureters were detached from the bladders and peri-ureteric fat was removed. The distal ureter was determined as the region 5 cm from the entrance to the bladder. The tissues were dissected into 4 mm long tissue strip sections. The tissue strips were mounted longitudinally under 1 g tension in 8 ml organ baths (EZ-baths, Global Towns, CA) containing Krebs-bicarbonate solution, which was maintained at 37 °C and continuously gassed with 95% O_2 and 5% CO_2. Isometric tension developed by the tissues was recorded to PC via a Powerlab recording system and Labchart software (ADInstruments, Castle Hill, Australia).

2.2. Contractile responses to 5-HT and α-methyl-5-HT

Complete cumulative concentration-response curves to 5-HT or α-methyl-5-HT (5-HT_2 agonist) were obtained in isolated ureteral tissues from both age groups in the absence and presence of the selective 5-HT_{2A} receptor antagonist ketanserin (10–100 nM). Additionally, concentration-response curves to 5-HT were obtained in the absence and presence of the non-selective 5-HT antagonist methiothepin (10 nM), 5-HT_{2C} antagonist RS-10221 (30 nM), 5-HT_3 antagonist ondansetron (30 nM), 5-HT_4 antagonist GR-113808 (100 nM), 5-HT_5 antagonist SB699551 (10 nM), 5-HT_6 antagonist SB399885 (100 nM) and 5-HT_7 antagonist SB269970 (10 nM). Concentrations producing a selective antagonism for a particular receptor were chosen based on affinity values obtained from the literature.

Only one concentration-response curve was obtained in each tissue. Preparations from the same ureter were compared in each individual experiment, with at least two preparations acting as controls without the addition of any antagonist.

2.3. Data analysis

GraphPad PRISM software was used for statistical analyses. Mean (± S.E.M.) increases in area under the curve (AUC) relative to weight in grams per second (g^{-1} s) and frequency responses in Hertz (Hz) were calculated. Mean (± S.E.M.) maximum responses and –logEC50 (pEC50) values were also recorded for each concentration-response curve. Paired Student's *t*-tests were performed on studies with two groups, whilst two-way ANOVA was used for studies involving more than two groups.

Dissociation constant estimates (apparent pK_B) for antagonists were determined from the equation: pK_B = log (CR-1)-log[B], where CR is the concentration ratio (ratio of the EC_{50} values) in the absence and presence of the antagonist obtained with a concentration [B] of antagonist. Schild plots were also constructed (Arunlakshana and Schild, 1959) where data from several antagonist concentrations was available.

2.4. Drugs

The following drugs were used: 5-HT hydrochloride (Abcam), α-methyl-5-HT maleate (Tocris), methiothepin mesylate salt (Sigma-Aldrich), ketanserin (+)-tartrate salt (Sigma-Aldrich), RS-102221 (Tocris), ondansetron hydrochloride dehydrate (Sigma-Aldrich), GR-113808 (Tocris), SB 699551 (Sigma-Aldrich), SB-399885 hydrochloride (Sigma-Aldrich) and SB-269970 hydrochloride (Sigma-Aldrich). All drugs were dissolved in distilled water before being added to the bathing solution.

3. Results

3.1. The effect of age on 5-HT-induced contractile responses

When subjected to increasing concentrations of 5-HT, or the 5-HT_2 selective agonist α-methyl-5HT, all ureteral strips from both age groups developed bursts of phasic contractions and overall contractile activity increased when measured as area under the curve and expressed relative to tissue weight (AUC, g^{-1} s) (Fig. 1). Increasing concentrations of 5-HT also resulted in an increase in frequency of phasic activity, which was measured in Hz (Fig. 1). The potency (pEC_{50}) of 5-HT was similar for tissues from both age groups (Table 2). However, maximal contractile responses measured as AUC were smaller in tissues from the older animals (2787 ± 317 g^{-1} s, P < 0.001, unpaired Student's *t*-test), compared to the younger animals (5885 ± 335 g^{-1} s, Fig. 1A), while maximum frequency responses were similar in the two age groups (Fig. 1B).