5-HT$_7$ Receptors Mediate Direct Vascular Relaxation in Piglet Pulmonary Artery

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Introduction

Serotonin (5-hydroxytryptamine; 5-HT) produces its effects through a variety of membrane-bound receptors of which the subtypes 5-HT$_{1B}$, 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_7$ mediate vasoconstriction or vasorelaxation [1]. In pulmonary arteries of (adult) pigs, 5-HT acts as a highly potent agonist (pEC$_{50}$ 8.7) producing fast and endothelium-dependent relaxation via 5-HT$_{2B}$ receptor-mediated NO-release [2–4]. In contrast, 5-HT elicits slow relaxation in piglet pulmonary artery predominantly in an endothelium-independent way (Fig. 1). To clarify the question, which receptor is involved in direct relaxation to 5-HT in piglet pulmonary artery, we performed organ-bath experiments and second messenger studies.

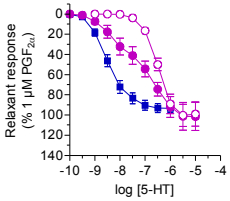

Fig. 1: Relaxant responses to 5-HT in pulmonary arteries of:
- ■ adult pig (+ endothelium)
- ● piglet (+ endothelium)
- ○ piglet (– endothelium)

Methods

Isolated organ bath studies: Endothelium-denuded rings of piglet (20 – 45 kg) pulmonary artery were mounted isometrically under an initial tension of 20 mN in organ baths filled with Krebs-Henseleit solution continuously aerated with 95% O_2/5% CO_2. All experiments were conducted in the presence of ketanserin (0.1 µM) to block 5-HT$_{2A}$ receptor-mediated contractions [4]. Following an equilibration period of 60 min, tissues were stimulated once with KCl (45 mM) and twice with PGF$_{2\alpha}$ (1 µM). The absence of endothelium was assessed by addition of bradykinin (10 nM). A cumulative E/[A] curve to 5-HT receptor agonists was recorded on each arterial ring following the second stimulation with PGF$_{2\alpha}$. Antagonists (or vehicle) were incubated for 30 min.

Second messenger studies: Following 30 min incubation of SB269970 (0.1 µM) or vehicle, the ring preparations were exposed to 5-CT (1 nM – 10 µM) for 20 min. The rings were quickly frozen in liquid nitrogen and homogenized in 0.1 M HCl solution with a Dismembrator. The homogenate was centrifuged at 2000 x g for 5 min. The cAMP in the extract was assayed using an enzyme immunoassay kit (cyclic AMP EIA Kit, BIOMOL, USA). The pellet was used for protein determination using bovine serum albumin as standard [5].

Results

Agonist studies

- 5-CT, 5-HT, 5-MeOT, and frovatriptan elicit slow relaxations in piglet pulmonary artery (Fig. 2). The rank order of agonist potencies (5-CT >> 5-HT > 5-MeOT >> frovatriptan) argues for an activation of 5-HT$_7$ receptors (Fig. 3; Tab. 1).
- Relaxant responses to 5-CT, 5-HT, 5-MeOT, and frovatriptan were antagonized by the 5-HT$_7$ receptor antagonist SB269970 (30 nM) (Tab. 1).

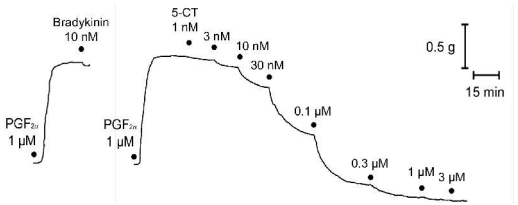

Fig. 2: 5-CT-induced relaxation in piglet pulmonary artery

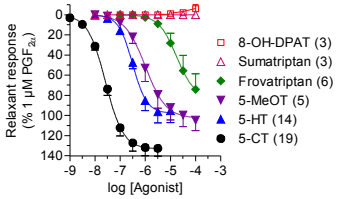

Fig. 3: Relaxant responses to 5-HT receptor agonists

Tab. 1: Potencies of 5-HT receptor agonists

	n	pEC$_{50}$	E$_{max}$ (%)	n	pK$_B$ (30 nM SB269970)
5-CT	19	7.60 ± 0.04	136 ± 8	7	8.59 ± 0.07
5-HT	14	6.49 ± 0.02	98 ± 11	6	8.65 ± 0.07
5-MeOT	5	5.92 ± 0.12	97 ± 11	4	8.82 ± 0.06
Frovatriptan	6	4.58 ± 0.14	91 ± 13	5	≈ 8.0
Sumatriptan	3	inactive	0	-	-
8-OH-DPAT	3	inactive	0	-	-

Antagonist studies

- Antagonist affinities against 5-CT fitted best with radioligand binding data at human and rat 5-HT$_7$ receptors (Tab. 2).

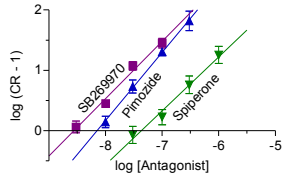

Fig. 4: Schild plots for SB269970, pimozide and spiperone

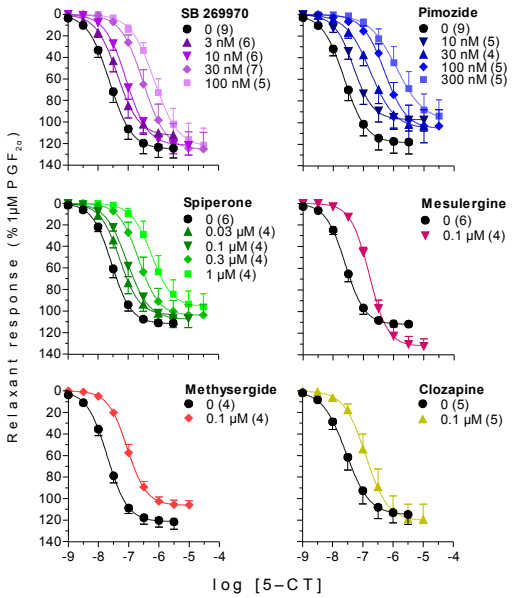

Fig. 5: Inhibition of the relaxant response to 5-CT by a series of antagonists

Tab. 2: Antagonist affinities against 5-CT compared to published pK$_i$ at cloned human [6 – 10] and rat 5-HT receptors [11 – 13]

	piglet pulmonary artery			radioligand binding affinities					
	n	pK$_B$	slope	h5-HT$_{2B}$	h5-ht$_{5A}$	r5-ht$_5$	h5-ht$_6$	r5-HT$_7$	h5-HT$_7$
SB 269970	24	8.52 ± 0.04	0.95 ± 0.07	5.0	7.2		5.2		8.9
Pimozide	19	8.26 ± 0.05	1.13 ± 0.10			7.2		9.3	
Spiperone	16	7.32 ± 0.06	0.85 ± 0.11	6.3	5.6	5.8		8.0	7.6
Mesulergine	4	7.74 ± 0.09	-	8.5	< 6.0	5.8	5.4	8.2	8.1
Methysergide	4	7.55 ± 0.09	-	8.8	7.6	6.4	6.8	7.9	7.6
Clozapine	5	7.50 ± 0.08	-	8.2		8.2	8.0	7.9	7.7
r^2				0.37	0.22	0.55	0.55	0.88	0.96
P				> 0.05	> 0.05	> 0.05	> 0.05	0.02	0.003

Second messenger studies

- 5-CT-induced relaxation in piglet pulmonary artery is associated with a concentration-dependent 2-fold increase in cAMP (pEC$_{50}$ 7.3) that was surmountably antagonized by SB269970 (100 nM) (Fig. 6).

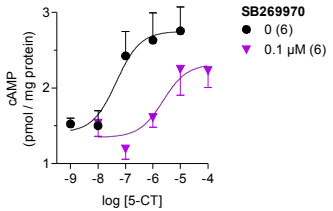

Fig. 6: cAMP levels following application of 5-CT in the absence and presence of SB269950

Conclusions

- Relaxant responses to 5-HT receptor agonists in piglet pulmonary artery are mediated by smooth muscle 5-HT$_7$ receptors.
- This is in contrast to pulmonary arteries of adult pigs, where only an endothelium-dependent relaxation via activation of 5-HT$_{2B}$ receptors has been observed [3,4].

References

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