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INFLUENCE OF PROPRANOLOL TREATMENT ON α_1- AND ß-ADRENOCEPTORS IN THE GUINEA-PIG HEART

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(^{3}H)Prazosin and (^{125}I)iodo-2-(ß-(4-hydroxyphenyl)ethylaminomethyl)tetralone - (^{125}I)IHEAT - binding sites in male guinea-pig heart ventricular membrane preparations are shown to display characteristics of α_1-adrenoceptors. The rank order of potency in displacing the radioligands was in the series of antagonists: prazosin = IHEAT > phenoxybenzamine > phentolamine ≅ urapidil > yohimbine. K_D- and B_{max} - values (± S.E.M.) for (^{3}H)prazosin and (^{125}I) IHEAT, determined by equilibrium binding assay, were: 46.6±4.3 pmol/l, 10.3±0.65 fmoles/mg protein (n=9) and 53.2±6.2 pmol/l, 8.4±0.97 fmoles/mg (n=9), respectively.
Treatment of male guinea-pigs (300-400 g, n=9) with an average of 81.3 mg propranolol/kg/day over 12 days performed via the drinking-water reduced (^{3}H)prazosin binding by 27 % ($P<0.005$) with K_D-values unchanged, whereas ß-adrenoceptors - (^{3}H)dihydroalprenolol binding - in the same preparation were uneffected.

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Identification of 5HT-M-receptor subtypes by a new class of drugs

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5HT-M-receptors are widely distributed in the peripheral nervous system. The synthesis of a new class of competitive antagonists has allowed comparisons to be made between 5HT-M-receptors in different tissues. pA_2-values of three selective 5HT-antagonists were determined against 5HT and 2-CH_3-5HT, a selective 5HT-M-agonist, in the isolated rabbit vagus nerve, the rabbit heart perfused by the Langendorff technique and the longitudinal muscle of guinea pig ileum. The antagonists used were (3α-homotropanyl)-1-methyl-5-fluoro-indole-3-carboxylic acid ester (A), (N-desmethyl-3α-homotropanyl)-1H-indole-3-carboxylic acid ester (B), and ICS 205-930: (3α-tropanyl)1H-indole-3-carboxylic acid ester (C), pA_2-values ± S.E. and slopes from Schild plot analysis are given below for n=3-9 experiments.

AGONIST	VAGUS	HEART	ILEUM
(A) 5HT	13.1±0.8(0.76)	10.1±0.1(1.17)	9.1±0.1(0.91)
2-CH_3-5HT	13.6±0.8(0.77)		
(B) 5HT	8.9±0.2(0.82)	9.8±0.1(1.26)	7.7±0.2(0.98)
2-CH_3-5HT		9.7±0.6(1.16)	
(C) 5HT	10.2±0.2(0.95)	10.6±0.2(0.92)	7.8±0.1(1.22)
2-CH_3-5HT			8.03±0.1(0.93)

The pA_2-values determined with 5HT and 2-CH_3-5HT as agonists were not statistically different from each other in a given tissue, indicating that the pharmacological effects obtained were the consequence of 5HT-M-receptor activation. The fact that affinity constants differed significantly between tissues suggests the existence of at least three 5HT-M-receptor subtypes.

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CHARACTERIZATION OF SEROTONIN (5-HT) RECEPTORS IN THE ISOLATED GUINEA-PIG ILEUM

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2-[^{125}I]LSD labels a binding site in membranes of guinea-pig ileum that is different from the 5-HT_2 binding site (labelled with the same ligand) in rat brain membranes. Under appropriate conditions 5-HT can either relax or contract the isolated guinea-pig ileum. Contraction is best measured on strips of longitudinal muscle. Relaxation can be quantified using the ileum with intact mucosa, precontracted with 1 µM histamine. The antagonistic potency (pA_2-values) of 8 5-HT antagonists with respect to inhibition of relaxation, was assessed from Schild-plots. Contraction induced via activation of 5-HT-D receptors, was obtained after desensitization of 5-HT-M receptors. Inhibition of responses to 1 µM 5-HT was determined for 13 antagonists (pIC_{50}). The antagonistic potencies obtained in both pharmacological tests were compared with pK_D-values derived from binding assays in ileum and brain:

	ileum membr.	brain membr. (5-HT_2)	
relaxation (pA_2)	r=0.87 p<.01	r=0.79 p<.01	n=8
contraction (pIC_{50})	r=0.76 p<.01	r=0.87 p<<.001	n=13

pD_2-values of 5-HT and 11 analogues were determined for contraction of the rat aorta in the presence of 1 µM prazosin (a 5-HT_2 model) and guinea-pig ileum relaxation. No correlation was found (r=0.20). These results suggest that the 5-HT-D receptor mediating contraction is a 5-HT_2 receptor. The receptor mediating relaxation is not a 5-HT_2 receptor and may be identified in a binding assay with 2-[^{125}I]LSD on membranes of guinea-pig ileum.

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CHARACTERIZATION OF MULTIPLE SEROTONIN (5-HT) RECOGNITION SITES IN RAT AND PIG BRAIN MEMBRANES BY RADIOLIGAND BINDING

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5-HT1 binding sites, which were originally defined by high affinity for [^{3}H]5-HT can be further subdivided. 5-HT1A, 5-HT1B and 5-HT1C sites were labelled with [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin), [^{125}I]CYP ((-)[^{125}I]iodocyanopindolol) in the presence of 30 µM isoprenaline and [^{3}H]mesulergine respectively. Non specific binding was defined by 10 µM 5-HT. Table 1 illustrates the affinity values (pK_D±S.E.M. n > 3) of a series of selective compounds for the 5-HT1 and 5-HT2 sites (labelled by [^{3}H]ketanserin in rat cortex membranes).

	5-HT1A	5-HT1B	5-HT1C	5-HT2
5-HT	8.5±0.1	7.6±0.1	7.5±0.1	5.5±0.4
8-OH-DPAT	8.7±0.3	4.2±0.1	5.1±0.2	5.2±0.0
(-)21-009*	7.9±0.2	9.4±0.3	5.3±0.2	5.5±0.3
mesulergine	6.2±0.1	4.9±0.1	8.6±0.1	8.4±0.1
pirenperone	5.9±0.1	5.2±0.1	7.2±0.1	8.8±0.0

* 21-009 = 4[3-ter-butyl-amino-2-hydroxy- propoxy]-indol-2-carbonic acid isopropylester.

5-HT1A sites are identical in rat and pig cortex. 5-HT1B sites show high affinity for some indole ß-blockers and could be detected in rat cortex only. 5-HT1C sites are strongly concentrated in pig choroid plexus but identical sites can be demonstrated in rat cortex.
The three 5-HT_1 sites are clearly different from one another and from 5-HT2 receptors as illustrated by their pharmacological profile determined with 22 serotonergic compounds. The functional significance of these sites will be discussed.

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