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THE A1A/L -ADRENOCEPTOR SUBTYPE MEDIATES CONTRACTION OF THE INTERNAL ANAL SPHINCTER TO NORADRENALINE

Hypothesis / aims of study

Contraction of the internal anal sphincter contributes 50-85% towards resting anal tone. Small clinical trials with phenylephrine suggest that α_1-adrenoceptor agonists could be used to increase IAS tone as a treatment for faecal incontinence. However, there are three subtypes of α_1-adrenoceptor, namely α_{1A}, α_{1B} and α_{1D}. It is possible that the α_{1A}-subtype may also exist in another form known as the α_{1L}-adrenoceptor which has a low affinity for prazosin. The aim of the present study was to determine the α_1-adrenoceptor subtypes present in the IAS, with a view to possibly finding a more specific therapeutic target for the treatment of faecal incontinence.

Study design, materials and methods

Circular muscle strips minus the epithelium were taken from porcine IAS. Tissues were mounted in 30ml tissue baths containing Krebs bicarbonate solution and gassed with 95% O_2 and 5%CO_2. Resting tension on the tissue was set at 1g and the tissues allowed to stabilize for 30 minutes. Tissues were then incubated with an α-adrenoceptor antagonist for a further 30 minutes. BMY7378 (3, 10, 30μM), RS100329 (3, 10, 30nM), 5MU (3, 10, 30nM), Prazosin (1, 3, 10nM) and RS17053 (10, 30, 100μM) were the antagonists used. A cumulative concentration-response curve to noradrenaline was then obtained in either the absence of any antagonist or in the presence of a single concentration of one of the antagonists. Only one concentration-response curve was obtained on each tissue. All experiments were performed in the presence of cocaine (10μM) and corticosterone (10μM) to inhibit amine uptake and propanolol (1 μM) to antagonize β-adrenoceptors. EC_{50} values could then be determined from the curves, and the affinity of the antagonists calculated. In separate experiments, concentration-response curves were constructed to A61603, an α-adrenoceptor agonist with a high potency at α_{1A}-adrenoceptors.

Results

Noradrenaline produced concentration-dependent contraction of the IAS and the addition of antagonists caused parallel rightward displacements of the concentration-response curves. The Schild slope, affinity estimates (pK_B) and pA_2 values for each antagonist are outlined in the table below.

Drug	**n**	**pK_B**	***Slope***	**pA_2**
BMY7378	13	6.17	1.17 ± 0.30	6.2
RS100329	13	9.26	0.70 ± 0.38	9.5
5MU	14	8.56	0.97 ± 0.03	8.6
Prazosin	15	8.65	0.46 ± 0.44	8.7
RS17053	9	5.32	1.53 ± 0.86	5.3

RS100329 and 5MU both demonstrated a high affinity in the tissue, with pK_B values of 9.26 and 8.56 respectively. BMY7378 was found to have a lower affinity value of 6.17. The Schild slopes for these antagonists were similar to unity, with slopes of 0.70±0.38, 0.97±0.03 and 1.17±0.3 for RS100329, 5MU and BMY7378 respectively. Relatively low affinity values of 5.3 and 8.7 were obtained for RS17053 and prazosin, but the corresponding Schild slopes were not equal to unity. There was no significant change in the maximum response with the addition of any antagonist. The concentration-response curves for noradrenaline and A61603 in the absence of any antagonist indicate that A61603 has a higher potency than noradrenaline in IAS.

Interpretation of results

The α_{1A}-adrenoceptor selective agonist A61603 was 90-fold more potent than noradrenaline, suggesting that the α_{1A}-receptor is the main adrenoceptor present in the IAS. The antagonist data support this conclusion, with the α_{1A}–adrenoceptor selective antagonists 5-methylurapidil and RS100329 having high affinities in this tissue and ruling out the possible involvement of the α_{1B}-adrenoceptor. The low affinity of the α_{1D}-receptor selective antagonist BMY7378 also ruled out the involvement of the α_{1D}-adrenoceptors. Schild plots for these antagonists had slopes of unity, which suggest that the tissue contains only one α_1-adrenoceptor subtype, which must therefore be the α_{1A}-adrenoceptor. The low potency of prazosin and RS17053 suggests that it is the α_{1L}-form of this tissue that predominates in this tissue.

Concluding message

The pharmacological characteristics of the α_1-adrenoceptor in the IAS indicate the presence of the α_{1A}–adrenoceptor subtype. The low potency of prazosin and RS17053 suggests that it is the low affinity form of this receptor (α_{1L}) that mediates contraction. The identification of the α_1-adrenoceptor may provide a more specific therapeutic target for future treatments of incontinence.

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