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Effect of atropine on the rat anococcygeus muscle

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The rat anococcygeus muscle, which is densely innervated with adrenergic neurons (Gillespie 1972), is preferred to the vas deferens for postjunctional studies because of the controversy that surrounds the motor transmitter in the vas deferens. There is little cholinergic innervation of this tissue as neither motor nor inhibitory responses to nerve stimulation are influenced by atropine (Gillespie 1980). When atropine was used to block the possible chlolinergic contribution it was found that responses to noradrenaline were also reduced. The effects of atropine on the anococcygeus muscle of the rat are now described.

Methods

Male albino rats, 250–300 g, were killed by a head-blow and bled. The two anococcygeus muscles were dissected according to Gillespie (1972). Each muscle was suspended in a 10 ml organ bath under a resting tension of 0·5–0·6 g. The aerated bathing fluid maintained at 36 °C had the composition (mM): NaCl 137, KCl 2·4, $CaCl_2$ 1·8, $MgCl_2$ 1·0, NaH_2PO_4 0·2, $NaHCO_3$ 11·9 and glucose 5·5. The muscle was left to equilibrate for 1 h during which the bathing fluid was replaced every 15 min. Contractions were magnified 5×. Acetylcholine (ACh), carbachol (CCh) and 5-hydroxytryptamine (5-HT) were allowed to act for 2 min whilst noradrenaline (NA) was allowed to act for 90 s before being washed out. Physostigmine was added 5 min before atropine and 20 min before the agonists. Phentolamine and methysergide bimaleate were added 15 min before agonists. Antagonism was characterized as pA_2 values according to Arunlakshana & Schild (1959) and was regarded as competitive when the slope of the regression of the Schild plot was close to unity.

Results were expressed as mean ± s.e. of n observations and were regarded as significant, on Student's *t*-test analysis, when $P < 0.05$.

The drugs used were: acetylcholine chloride (Sigma), carbamoylcholine chloride (carbachol; BDH), noradrenaline (BDH), 5-hydroxytryptamine creatinine sulphate (BDH), atropine sulphate (BDH), physostigmine salicylate (Burroughs-Wellcome), phentolamine mesylate (Ciba-Geigy) and methysergide bimaleate (Sandoz).

Results

Effect of agonists. ACh ($4·4 \times 10^{-5}$–$1·4 \times 10^{-3}$ M), carbachol ($1·76 \times 10^{-6}$–$5·6 \times 10^{-5}$ M), noradrenaline ($1·2 \times 10^{-6}$–$3·8 \times 10^{-5}$ M) and 5-HT (5×10^{-6}–$3·2 \times 10^{-4}$ M) produced concentration-related contractions of the tissue. Tachyphylaxis was not observed in the presence of the agonists. ACh, carbachol, 5-HT and noradrenaline had the following -log EC50 valves: 3·60 ± 0·14, 6·15 ± 0·18, 4·25 ± 0·12 and 5·60 ± 0·22 respectively.

Effect of atropine, methysergide and phentolamine. Atropine ($1·15 \times 10^{-9}$–$1·15 \times 10^{-8}$ M) produced a concentration-dependent reduction in the effects of ACh and carbachol. The A–S plot gave pA_2 values of 11·37 ± 0·20 and 9·04 ± 0·13 for ACh and carbachol respectively. The slope of the Schild regression line was significantly less than unity ($P < 0·1$) for atropine-ACh interaction, however, the slope was 0·95 ± 0·01 for atropine-CCh carbachol interaction. This value was not significantly different from unity ($P > 0·05$). Because of the apparent discrepancy in the pA_2 and slope of atropine-ACh dose-ratio −1 plot, this interaction was repeated in the presence of physostigmine ($7·50 \times 10^{-8}$ M). When atropine gave a pA_2 value of 8·93 ± 0·11 against ACh. The slope of the A–S plot was 1·14 indicating competitive antagonism (Fig. 1). This pA_2 value was not significantly different from the value of 9·04 ± 0·13 obtained for atropine-carbachol interaction indicating a common muscarinic receptor.

Atropine ($2·30 \times 10^{-7}$–$9·20 \times 10^{-7}$ M) produced parallel shifts of the concentration-response curves of noradrenaline and 5-HT to the right. With noradrenaline, the maximum was significantly enhanced with increasing concentrations of atropine. The pA_2 values obtained for atropine were 6·52 ± 0·06 (slope = 1·19)

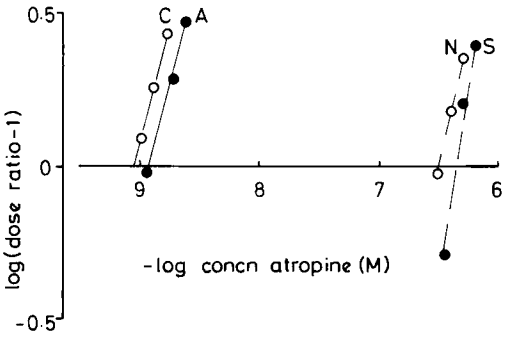

FIG. 1. Schild plot of the antagonism produced by atropine against carbachol (C), ACh (A), noradrenaline (N) and 5-hydroxytryptamine 5-HT. The pA_2 values obtained were 9·04 ± 0·13 (slope = 0·95 ± 0·01) (carbachol); 8·93 ± 0·11 (slope = 1·17) (ACh); 6·52 ± 0·06 (slope = 1·19) (noradrenaline) and 6·45 ± 0·10 (slope = 1·00) (5-HT).

and 6·45 ± 0·10 (slope = 1·0) against noradrenaline and 5-HT respectively indicating competitive antagonism. Methysergide ($1·6 \times 10^{-7}$–$1·3 \times 10^{-6}$ M) reduced the responses of the tissue to low concentrations of 5-HT without effect on the submaximal and maximal concentrations. The pA_2 value for this interaction was 6·40 ± 0·22. However, the slope was significantly different from unity (slope = 0·64). On the other hand, methysergide (up to $3·2 \times 10^{-6}$ M) failed to influence the effect of ACh. Noradrenaline-induced contractions were enhanced by methysergide.

Phentolamine ($6·25 \times 10^{-8}$–$1·0 \times 10^{-6}$ M) produced parallel rightward shifts of the curves of noradrenaline and 5-HT but did not modify the effect of ACh. The pA_2 values of phentolamine against noradrenaline and 5-HT were 6·85 ± 0·16 (slope = 1·03) and 6·72 ± 0·15 (slope = 1·10) respectively.

The difference is pA_2 values of phentolamine and atropine against noradrenaline and 5-HT was not statistically significant ($P > 0·05$).

Discussion

The present results suggest that the rat anococcygeus muscle has a complex network of pharmacological receptors. The agonists used behaved as true agonists because the differences in the attainable maximal responses were not statistically significant ($P > 0·05$) (Ariens & van Rossum 1957). However, this is only true of ACh in the presence of physostigmine. Without the anticholinesterase, the antagonism was non-competitive and the pA_2 value was significantly higher than the value obtained in its presence. This is probably due to the fact that the rat anococcygeus muscle has a high cholinesterase activity (1·65 ± 0·13 μmol min^{-1} g^{-1} tissue) (Smith & Spriggs 1979). This would reduce the effective concentration of ACh in the vicinity of the receptors hence the antagonistic action of atropine is enhanced or exaggerated. This significant difference in pA_2 values is not believed to be due to two different muscarinic receptors for ACh and carbachol.

Atropine gave similar pA_2 values against NA and 5-HT indicating that the mechanisms involved in their actions may be similar. 5-HT and noradrenaline share similar receptors in the rabbit ear artery (Apperley et al 1976; Tayo 1981). However, the receptors involved in those studies were α-noradrenoceptors. 5-HT has been well documented to act neuronally on muscarinic receptors in many tissues. However, this kind of action has not been shown with noradrenaline. If this effect of atropine is on muscarinic receptors then they must be different from those subserving the effects of ACh and carbachol in this tissue. The carbachol receptors seem to be similar to muscarinic receptors encountered in most tissues (pA_2 values of atropine-ACh interaction range from 8·8 (guinea-pig bronchi), 8·9 (rat intestine) to 9·0 (guinea-pig ileum) (see Bowman & Rand 1980). It is possible that the antagonism by atropine of noradrenaline may be unspecific at these relatively high concentrations. This antagonistic effect of atropine was concentration-related. That the pA_2 values of atropine against noradrenaline and 5-HT are not different statistically, coupled with the competitive nature of the antagonism, suggest an action on receptors which in this case might be α-noradrenoceptors. In support of this was the finding of no statistically significant difference in the pA_2 values of atropine and phentolamine against noradrenaline and 5-HT. After submitting this paper, Abraham et al (1981) reported that atropine produced a dose-related decrease in blood pressure in normotensive rats at doses greater than those needed to block the muscarinic effect. This hypotensive action of atropine was resistant to ganglion blockers and β-blockers but was abolished by α-blockade. In addition, these authors found that atropine inhibited the pressor response to α-adrenoceptor agonists dose-dependently and concluded that atropine, at higher doses, blocked α-noradrenoceptors.

In conclusion, atropine appears to block α-noradrenoceptors in concentrations greater than those required to block muscarinic receptors in the rat anococcygeus muscle.

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