Br. J. Pharmac. (1976), **57**, 395–398

THE USE OF DIFFERENT AGONISTS IN ANTAGONIST AFFINITY CONSTANT ESTIMATIONS

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1 The affinities of 4 muscarinic antagonists were estimated on intact pieces of guinea-pig ileum using the agonists carbachol and pentyl trimethylammonium both in separate experiments and in the same experiment.

2 The apparent affinities were slightly but consistently higher when estimated from the responses produced by pentyl trimethylammonium than when estimated from the responses produced by carbachol.

3 This difference was greatly reduced or abolished if totally denervated logitudinal muscle strips were used rather than intact pieces of ileum. It is therefore suggested that the difference is due to the presence of receptors in the ganglionic layer.

4 To explain the difference in apparent affinity of the antagonists these receptors can not be identical to the muscarinic receptors on the smooth muscle.

5 In addition they can not be nicotinic ganglionic receptors as the difference did not appear to be affected by the presence or absence of hexamethonium.

Introduction

Abramson, Barlow, Mustafa & Stephenson (1969) compared the affinity constants of a number of muscarinic antagonists on guinea-pig ileum using different agonists. The largest difference they observed was for the one comparison made between carbachol and pentyl trimethylammonium (pentyl TMA) but the difference was not statistically significant. However real differences in mean log affinity as large as 0.1 log units might not have been evident in their experiments. The possibility that there is a real, albeit small, difference in apparent affinity has now been investigated.

Methods

Intact pieces of guinea-pig ileum or logitudinal muscle strips were prepared as described by Edinburgh Staff (1968) and Paton & Rang (1965) respectively. Strips were only considered to be totally denervated if they failed to respond to the ganglion stimulant, pentyl TMA ($NH_2 . C_6H_4 . CH_2 . CH_2 . N^+Me_3I^-$; Barlow & Franks, 1971). Such strips could only be obtained from animals weighing between 500 and 600 g. Otherwise animals weighing between 150 and 400 g were used. The extent to which Auerbach's plexus remained attached was estimated after staining with methylene blue.

The preparations were suspended in an organ bath containing Tyrode solution at 36°C and through which air was bubbled. The Tyrode solution was of the following composition (mM): Na^+ 149.2, K^+ 2.7, Mg^{2+} 1.1, Ca^{2+} 1.8, Cl^- 143.2, HCO_3^- 11.9, $H_2PO_4^-$ 0.4, SO_4^{2-} 1.1, glucose 5.6 and hexamethonium bromide 0.276 (unless specified otherwise). The responses produced by carbachol or pentyl TMA were recorded isotonically with a differential transformer. When intact pieces of ileum were used a weight of between 0.5 and 0.8 g was used to load the lever; with muscle strips, 0.2 to 0.6 g was used. Responses were produced throughout each experiment every 90 s, the agonist was removed after 17 s and a second wash applied after another 30 seconds. The apparatus was described by Abramson *et al.* (1969).

The affinity constants of the antagonists were measured using either carbachol or pentyl TMA as described by Edinburgh Staff (1968): Responses were produced by two concentrations of agonist, one double the other, used alternately. The high-low response sequence was maintained in the presence of the antagonist with adjusted concentrations of agonist. Affinity constant estimations were also made using both agonists in the same experiment. In these a high-low response sequence was maintained using the four possible combinations of the four agonist solutions in an order determined by a Latin square.

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The drugs used were kindly provided by R.B. Barlow except where specified otherwise: benziloyl tropine methyliodide (BTrMe), carbaminoylcholine chloride (carbachol) (BDH Chemicals), diphenylacetoxyethyldimethylethyl ammonium iodide (desoxylachesine), diphenylhydroxyacetoxyethyldimethylethylammonium bromide (lachesine), hexamethonium bromide (Koch-Light), *n*-pentyl triethylammonium iodide (pentyl TEA), *n*-pentyl trimethylammonium iodide (pentyl TMA).

Results

The affinity constants K_{aff} of 3 antagonists were estimated using pentyl TMA and carbachol in

Table 1 Mean values of log affinity constant (Log K_{aff}) obtained for 3 muscarinic antagonists using intact pieces of guinea-pig ileum. Estimates were made from responses to carbachol or pentyl TMA, the agonists being used in separate experiments

Antagonist	*Range of Antagonist Concentrations M*	*Mean Log K_{aff} ± S.E.M.*	
		Carbachol	*Pentyl TMA*
BTrME	$6–200 \times 10^{-10}$	10.129 ± 0.044 (27)	
	$10–39 \times 10^{-10}$		10.206 ± 0.068 (9)
Lachesine	$0.5–2 \times 10^{-8}$	8.817 ± 0.027 (26)	8.963 ± 0.011 (5)
Pentyl TEA	$0.3–8 \times 10^{-4}$	4.558 ± 0.016 (24)	4.593 ± 0.029 (5)

(*n*) = number of estimations made. Benziloyl tropine methyliodide (BTrMe), *n*-pentyl triethylammonium iodide (pentyl TEA).
According to Student's *t*-test or Mann–Whitney U test, the probability of observing these differences is > 0.2 in each case if the null hypothesis were true.

Table 2 Values of log affinity constant (Log K_{aff}) estimated from responses of intact pieces of guinea-pig ileum to carbachol and pentyl TMA, both agonists being used in each experiment

Antagonist	*Antagonist Concentration M*	*Log K_{aff}*		
		Carbachol	*Pentyl TMA*	*Difference*
BTrMe	10×10^{-10}	10.000	10.079	+0.079
		9.778	9.963	+0.125
		10.146	10.230	+0.084
Pentyl TEA	2.5×10^{-4}	4.611	4.667	+0.056
	5×10^{-4}	4.588	4.806	+0.218
	15×10^{-4}	4.444	4.597	+0.153
Desoxylachesine	13×10^{-7}	7.378	7.549	+0.171
		7.509	7.671	+0.162
		7.549	7.618	+0.069
		7.549	7.567	+0.018
	26×10^{-7}	7.477	7.732	+0.255
		7.544	7.576	+0.032
		7.642	7.823	+0.181
		7.681	7.699	+0.017
	40×10^{-7}	7.735	7.844	+0.109
		7.691	7.884	+0.203
		7.688	7.833	+0.145
		7.639	7.803	+0.164
	50×10^{-7}	7.834	7.946	+0.112
		7.491	7.742	+0.251
		7.604	7.721	+0.117
		7.534	7.835	+0.301

Benziloyl tropine methyliodide (BTrMe), *n*-pentyl triethylammonium iodide (pentyl TEA).
According to the sign test the probability of observing 22 differences all in the same direction is < 0.0001 if the null hypothesis were true.

Table 3 Values of log affinity constant (Log K_{aff}) of desoxylachesine (5×10^{-7}M) estimated on intact pieces of ileum from the dose-ratios in the absence and then in the presence of hexamethonium (2.76×10^{-4}M)

No Hexamethonium			*Hexamethonium*		
Log K_{aff}			*Log K_{aff}*		
Carbachol	*Pentyl TMA*	*Difference*	*Carbachol*	*Pentyl TMA*	*Difference*
7.292	7.881	+0.589	7.193	7.790	+0.597
7.589	7.719	+0.130	7.362	7.688	+0.326
7.584	7.619	+0.035	7.428	7.511	+0.083
7.668	7.740	+0.070	7.571	7.619	+0.048
		mean +0.206			mean +0.263

separate experiments (Table 1), the antagonists being chosen to cover as wide a range of potency as possible. As found by Abramson *et al.* (1969), although the mean value of K_{aff} obtained using pentyl TMA is for each antagonist higher than that obtained using carbachol, the difference is too small to be statistically significant.

Affinity constants were therefore estimated using both agonists in the same experiment (Table 2). In each of 22 experiments the affinity constant estimated from the pentyl TMA responses was higher than that calculated from the carbachol responses. This difference is statistically significant, $P < 0.0001$ by sign test. Desoxylachesine was used instead of lachesine in these experiments because it is in the same potency range as lachesine but its onset and offset of action is appreciably faster.

As hexamethonium had been present in all the experiments covered by Tables 1–2, its removal might have been expected to affect the difference in the affinity constants if the difference were due to nicotinic receptors in the ganglionic layer. The apparent affinity of desoxylachesine was therefore measured in the absence of hexamethonium and with hexamethonium present throughout in its normal concentration of 2.76×10^{-4}M (Table 3). The difference in apparent

Table 4 The dose-ratio produced by hexamethonium (2.76×10^{-4}M) on intact pieces of ileum using carbachol and pentyl TMA in the same experiment

Dose-ratio		
Carbachol	*Pentyl TMA*	*Difference*
1.20	1.30	+0.10
1.43	1.02	−0.41
1.22	1.07	−0.15
1.02	1.09	+0.07
		mean −0.10

Table 5 The effect of partial or total denervation on the difference in the log of the affinity constant (Log K_{aff}) when estimated from responses to carbachol and from responses to pentyl TMA, both agonists being used in the same experiment. (a) Longitudinal muscle strips with less than 30% of the area covered by the nerve plexus. (b) Totally denervated longitudinal muscle strips

Antagonist	*Antagonist Concentration M*	*Log K_{aff}* *Carbachol*	*Pentyl TMA*	*Difference*
(a) Desoxylachesine	50×10^{-7}	7.563	7.631	+0.068
		7.428	7.486	+0.058
		7.567	7.609	+0.042
		7.458	7.560	+0.102
				mean +0.068
(b) Pentyl TEA	2.5×10^{-4}	4.526	4.530	+0.004
		4.651	4.653	+0.002
	15×10^{-4}	4.456	4.473	+0.017
		4.621	4.596	−0.025
		4.444	4.444	+0.000
				mean −0.004

Comparable figures with intact pieces of ileum are given in Table 2. The Mann–Whitney U test was used to test whether the groups were drawn from the same population. The probabilities were 0.018 ($n_1 = 3$, $n_2 = 5$) and 0.014 ($n_1 = n_2 = 4$) for pentyl TEA and desoxylachesine if the null hypothesis were true.

affinity did not appear to be affected by the absence of hexamethonium. The dose-ratio produced by this concentration of hexamethonium itself was also measured using carbachol and pentyl TMA in the same experiment (Table 4). The dose-ratio estimated from the pentyl TMA responses were not consistently different from those estimated from the carbachol responses. It is therefore unlikely that the difference in apparent affinity can be explained in terms of nicotinic ganglionic receptors.

If the difference in apparent affinity were due to non-nicotinic receptors in the ganglia, the difference would not be observed in totally denervated muscle strips. Using such preparations the difference in apparent affinity was found to be greatly reduced or abolished (Table 5). It therefore seems likely that the difference in apparent affinity of an antagonist when estimated from responses to carbachol and pentyl TMA can be attributed to the presence of Auerbach's plexus.

Discussion

By using two agonists in the same experiment it has been possible to show that there is a real but small difference in the apparent affinity of an antagonist when measured using carbachol as compared with pentyl TMA. As this difference was greatly reduced or abolished when denervated preparations were used and was not affected by hexamethonium it must be due to the presence of non-nicotinic receptors in Auerbach's plexus although the exact site and mode of action can not be established from these experiments. These ganglionic receptors may also account for the small difference in affinity noted by Barlow & Tubby (1974) when using the agonists 3,3-dimethylbutyl-acetate and carbachol and that observed by Furchgott & Bursztyn (1967) and Waud (1969) when measuring the affinities of partial agonists using different methods.

F. Roberts is grateful for a training grant from the M.R.C.

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(Received November 28, 1975)