RESEARCH PAPERS

PHARMACOLOGICAL BLOCKING ACTIONS OF ATROPINE AND CERTAIN ATROPINE-LIKE COMPOUNDS

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Atropine is known to antagonise the responses of the guinea-pig ileum to 5-hydroxytryptamine (HT), when it is used in higher concentrations than those which antagonise the responses to acetylcholine (ACh). This has been explained by the theory that HT acts on nerve cells, and that atropine counters this effect by blocking the ACh liberated by these cells. An alternative theory is that atropine acts on both ACh and HT receptors. If the latter were so, some other drug might have a more specific action on ACh receptors than atropine itself; it was in the hope of finding such a drug that these experiments were done.

The drugs studied were four synthetic compounds supplied through the courtesy of Dr. A. C. White of the Wellcome Research Laboratories. They had been shown to be similar to atropine by various tests. Some notes of the comparative activities of each are given below.

Quantitative studies were made of the antagonism between atropine and HT, and also between atropine and ACh, and atropine and histamine. Similar experiments were made with the atropine-like compounds. In each case the pA_{10}, at equilibrium, of each drug/antagonist pair was found.

The Atropine-like Compounds

3:3-*Diphenyl propan*-3-*ol diethylamide methiodide* (186C47). This is "Compound 22" of White, Green and Hudson[1]. Comparisons by these workers of 186C47 and atropine showed that both had very similar activities against carbachol, pilocarpine and histamine. 186C47 was 0·7 to 1·0 times as active as atropine in tests on the same strip of isolated rabbit ileum, stimulated by carbachol or pilocarpine. In various tests, including one on mydriasis and another on histamine-induced asthma, the activity of 186C47 was found to be not less than half of the activity of atropine. (Information supplied by Dr. White.)

3-*Pyrrolidino*-1-*phenyl*-1-cyclo*hexyl propan*-1-*ol hydrochloride*. (54C50; procyclidine, Kemadrin.)[2] In the antagonism of ACh spasm in the isolated guinea-pig ileum, 54C50 was about 0·14 times as active as atropine. It was active also against carbachol in the guinea-pig ileum, and against both these activating drugs in the rabbit ileum. In higher concentrations, 54C50 could abolish contractions due to histamine and to barium. In tests involving salivation and mydriasis, the activity of

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54C50 was about 0·04 times that of atropine, and its effect was of shorter duration. (Information supplied by Dr. White.)

3-*Pyrrolidino*-1-*phenyl*-1-cyclo*hexyl propan*-1-*ol methiodide* (377C50, methiodide of procyclidine). This methiodide, 377C50, had a somewhat greater atropine-like activity than 54C50. It had about half the potency of atropine against ACh on isolated guinea-pig ileum. Also, in a mydriasis test in mice, it was about half as active as atropine. (Information supplied by Dr. White.)

1 : 1-*Diphenyl* 3-*piperidino propan*-1-*carbonamide hydrobromide* (182C52). The atropine-like activity of 182C52, and of the base from which it is derived, were equal, on a molar basis in a test of mydriasis in mice; being about 0·7 times as active as atropine. The base was about 1·5 times as active as atropine in the antagonism of ACh on isolated guinea-pig ileum. (Information supplied by Dr. White.)

Other investigations on this base were made by Schaumann and Linder[3]; it is their "compound 9980." They stated that it was about 0·16 times as effective as atropine in the reduction of the response of the isolated guinea-pig colon to histamine, but in a test of salivation in kittens, it was twice as active as atropine.

Experimental Method

Small guinea-pigs (150 g.), were fasted overnight, and killed by a blow on the head. Portions (1·5 to 2 cm. long) of the ileum, were taken from within 10 cm. of the cæcum, and were suspended in Tyrode's solution at 36° to 37° C. The organ bath was closely connected by a two-way tap to either of two warming coils and reservoirs. Regular responses were obtained to a sub-maximal dose of activator, while the tissue was bathed by Tyrode's solution. Then, without interruption of the dosage regimen, Tyrode's solution containing a certain concentration of an antagonist was allowed to fill the bath, and a 10-fold dose of activator was given at regular intervals. Responses were recorded with a light frontal writing lever, having a magnification of 10 to 15 times.

When the pilot experiments were done for the measurement of the pA_{10} of atropine and HT, the responses to the 10-fold dose in the presence of atropine diminished rapidly. If the response at a particular time, say 10 minutes, after the introduction of the atropine was measured in relation to the unantagonised response in each experiment, there were inconsistencies in the results. It appeared that certain portions of the ileum were especially liable to develop tachyphylaxis to stimulation by HT. Tachyphylaxis of this tissue to this drug has been noted elsewhere[4,5]. If dosing with HT was continued, there was some recovery of the responses, perhaps because the atropine had reached its full effect, and tachyphylaxis was no longer having its influence. In later experiments with higher concentrations of atropine, and in all the experiments with the atropine-like compounds, the antagonist rapidly reached its full effect, and the responses to HT rapidly reached an equilibrium level without tachyphylaxis.

The height of the steady response to the 10-fold dose was expressed as

a percentage of the response to the single doses of activating drug before the introduction of the antagonist. (See Fig. 1.) The regression line was calculated, and the pA_{10} determined.

This index (pA_{10}), was different from that of Schild[6] only in that Schild expressed the height of all responses in terms of the maximal response obtainable in the absence of antagonist, whereas here responses are measured in terms of a steady sub-maximal response (see Reuse[7]).

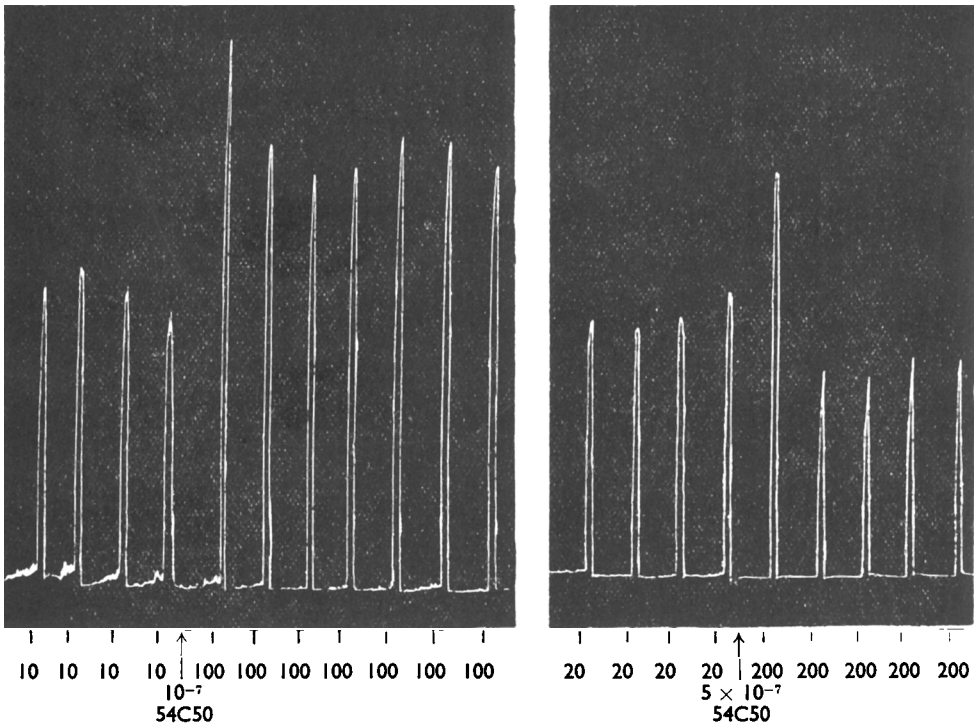


FIG. 1. Guinea-pig ileum in Tyrode's solution at 37° C., 2 ml. bath. First tracing: Responses to 10 ng. HT; from ↑, 10^{-7} 54C50 present in Tyrode's solution until the end of the experiment and the doses of HT are 100 ng.

Second tracing:
Fresh portion of ileum in Tyrode's solution. Responses to 20 mg. HT; from ↑, 5×10^{-7} 54C50 present in Tyrode's solution and the doses of HT are 200 ng. Concentration corresponding to pA_{10} lies between these two values.

Also, Schild's index was always qualified by the length of contact of the antagonist and the tissue, but here dosing was continued until the response reached a steady level (equilibrium response in 20 to 25 minutes).

Despite the difference in method, the effective concentrations for atropine/acetylcholine, and for atropine/histamine antagonism are of the same order.

The effects of atropine and the atropine-like compounds on the responses to HT were compared in a short series of experiments with their effects

on responses to nicotine. Doses of nicotine and HT, which caused similar steady responses were found, and then given alternately throughout the rest of the experiment. A particular concentration of one of the antagonists was introduced, and allowed to remain until constant responses to the constant doses were obtained (8 to 20 minutes). The antagonist was then removed, and the doses of the activating drugs were continued until recovery from the inhibition was complete.

TABLE I

COMPARISON OF AUTHOR'S RESULTS WITH THOSE OF SCHILD[6]

	Schild's pA_{10} at 14 minutes	Author's results pA_{10} equilibrium
Atropine/ acetylcholine	8·05	8·59
Atropine/ histamine	4·60	4·88

RESULTS OF THE ESTIMATIONS ON ATROPINE AND THE ATROPINE-LIKE COMPOUNDS

For each of the antagonists, the pA_{10} was found for HT, as well as for acetylcholine and histamine.

The equilibrium response to the 10-fold dose in the presence of a given concentration of antagonist, was compared with the response to the single dose in the absence of the antagonist. All the estimates were made on guinea-pig ileum.

Effect of Atropine

Under the experimental conditions which were used here, fairly high concentrations of atropine (10^{-7}), were necessary to cause inhibition of the response of the isolated guinea-pig ileum to the 10-fold dose of HT. In fact, the atropine concentration corresponding to pA_{10} for HT, was more than 100 times greater than that for ACh. For histamine, the atropine concentration was more than 10 times greater than that for HT.

Attempts to measure the effects of atropine, in concentrations less than 10^{-7}, on the 10-fold dose of HT failed because of apparent tachyphylaxis, (see method).

In a single experiment, where responses to HT and nicotine were matched, atropine (10^{-6}) was introduced into the Tyrode's solution. The responses to both nicotine and HT were similarly reduced and recovered in parallel on removal of the atropine.

Effect of the atropine-like compounds

All the four atropine-like compounds were more active in the antagonism of ACh responses than in the antagonism of HT responses. Their activity against histamine was much less (see Table II).

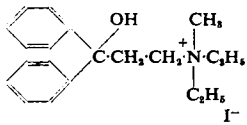
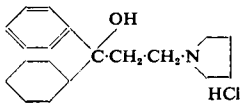
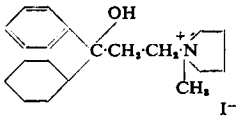
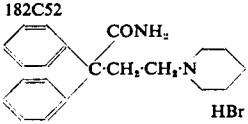
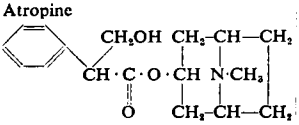
If the dose ratio is calculated by dividing the dose in the presence of the antagonist, by the dose having a similar effect in its absence, then in the presence of 186C47 (10^{-7}), the dose ratio for HT was ten, while that for ACh was fifty, and that for histamine was one[8].

The pA_{10}'s of the two quaternary compounds, 186C47 and 377C50, for HT are almost the same. These values are only a little less than the

pA_{10}'s of these compounds for ACh. The other two antagonists, 54C50 and 182C52, both show a greater difference between the pA_{10} for ACh and that for HT—but whereas with 54C50 (Fig. 2) there is a wide scatter of the estimates of effect, with 182C52 (Fig. 3) the estimates lie close to the calculated lines.

TABLE II

pA_{10} ESTIMATES FOR ATROPINE AND THE ATROPINE-LIKE COMPOUNDS

	Acetyl-choline	5-Hydroxy-tryptamine	Histamine
186C47 	7·87 (3)	7·43 (10)	4·97 (3)
54C50 	6·80 (4)	5·81 (11)	5·22 (3)
377C50 	7·86 (4)	7·25 (8)	4·63 (2)
182C52 	8·61 (4)	7·28 (8)	5·0 (2)
Atropine 	8·60 (5)	6·31 (6)	4·88 (2)

The figure in brackets indicates the number of experiments on which each pA_{10} is based.

The concentration-effect curves for histamine in the presence of 186C47 and 54C50 are shown in Figure 2. No antagonism was recorded for histamine responses by 377C50 in concentrations less than $10^{-4\cdot6}$ (Fig. 2). Spontaneous responses were recorded in the presence of 10^{-4} molar 182C52, and this made estimation of the pA_{10} impossible.

The effect of these atropine-like compounds on responses to HT and to nicotine were found to be very similar. Matching responses to nicotine and HT were equally reduced by a given concentration of any one of these compounds, and, on removal of the antagonist, the responses to both drugs recovered in parallel.

In most cases, the small spontaneous contractions occurring while Tyrode's solution was the bathing fluid, were reduced, or eliminated, by the antagonist solution.

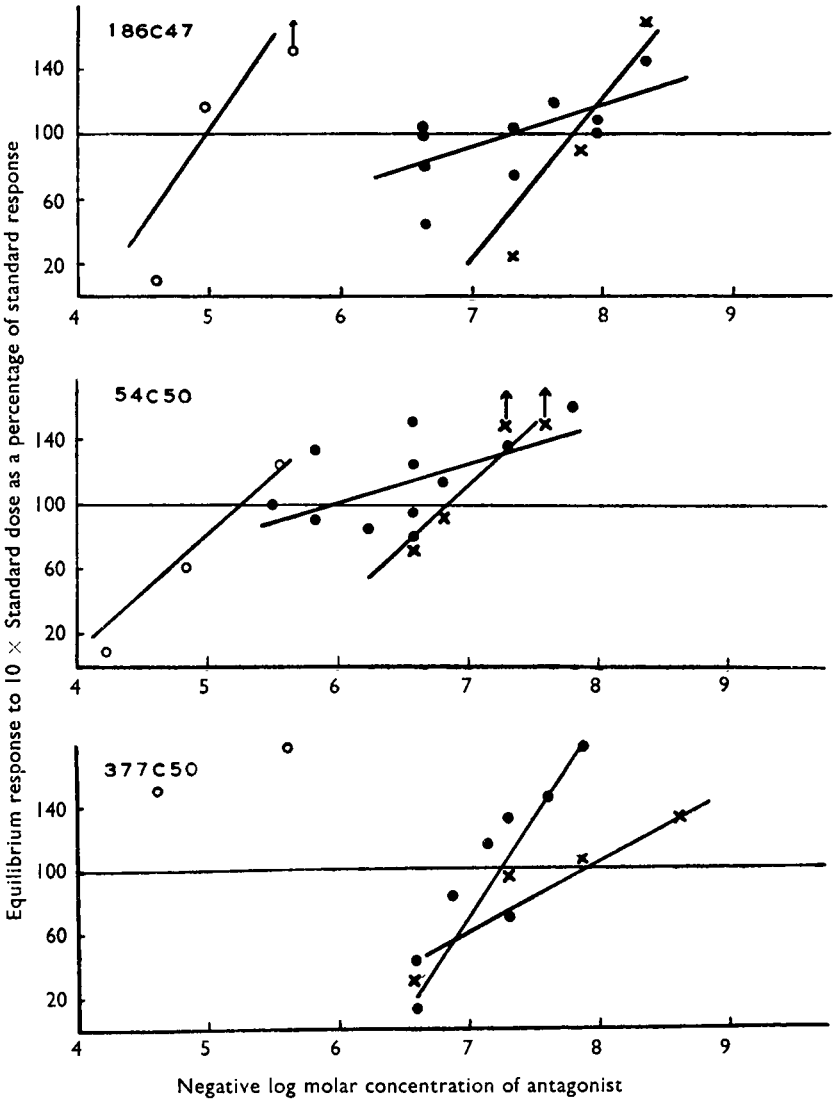


FIG. 2. The graphs show the calculated regression lines for various activator/antagonist pairs.

○ Histamine. ● 5-Hydroxytryptamine. × Acetylcholine.

DISCUSSION

Atropine was found to be more than 100 times more effective against ACh than it was against HT; and, more than 10 times more active against this drug than against histamine. These results agree with those reported by Rapport and Koelle[9].

Graphical comparison (Figs. 2 and 3) of the effect of the various

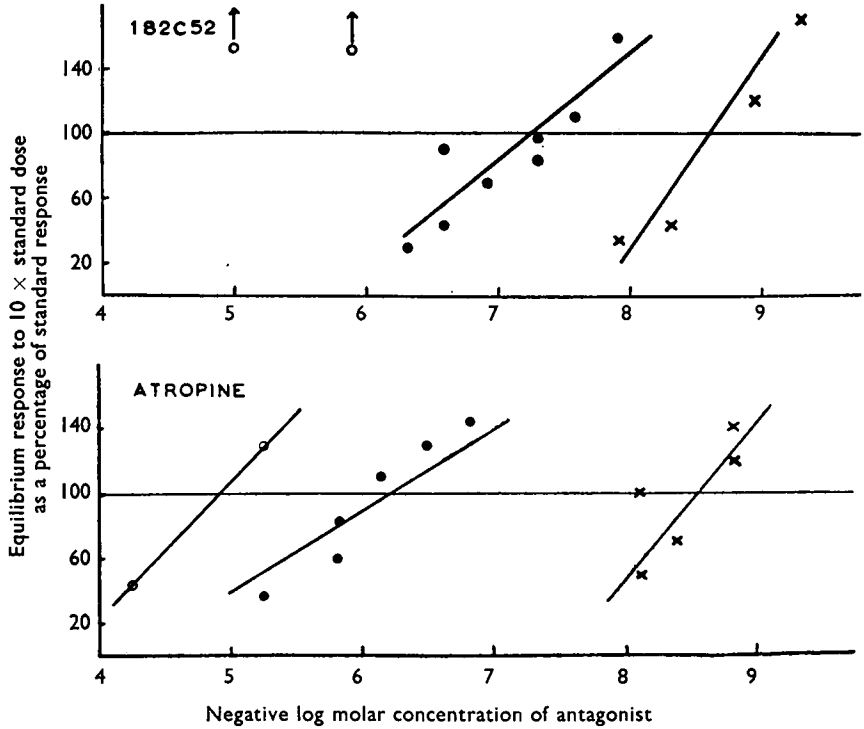


FIG. 3. The graphs show the calculated regression lines for the various activator antagonist pairs.

○ Histamine. ● 5-Hydroxytryptamine. × Acetylcholine.

atropine-like compounds and those of atropine on the spasmogenic drugs illustrated the following points.

(*a*) Widely different concentrations of both atropine and 182C52 were required to antagonise ACh, HT and histamine.

(*b*) Although the pA_{10} values of 54C50 antagonism of ACh and HT were themselves separated by one unit, the wide scatter of the observations reduces the significance of these estimates.

(*c*) More significance may be attached to the pA_{10} values of 186C47 and 377C50 for ACh and HT although they were separated by less than 0·5 units because the results of the individual estimations were less scattered.

(*d*) The activity of 182C52 on ACh responses was very slightly greater than that of atropine against ACh. The corresponding activity of the other compounds was less than that of atropine.

(*e*) 186C47, 377C50 and 182C52 were all considerably more active against HT responses than was atropine, 54C50 was less active.

(*f*) The activities of the compounds, where measurement was made, were of the same order with reference to the histamine response.

From these results, it was interesting to note that though 186C47, 377C50 and 182C52 are not more active anti-acetylcholines than atropine,

they are all more active anti-hydroxytryptamines than atropine. This suggests that they are not preventing HT spasm by antagonism of the effects of ACh, released by the HT stimulation. This conclusion could however be reversed were it shown that these drugs penetrate more readily to the site of released ACh action than does atropine. Alternatively, they may have a more specific blocking effect than has atropine on HT receptors.

The difference between the activities of 54C50 and 377C50 may be attributed to the quaternary ammonium radicle in the latter; 186C47, whose activity closely follows that of 377C50, also bears a quaternary ammonium grouping. This radicle cannot, however, be essential for activity in molecules of this type, for it is absent from the most active member of the series, 182C52. The nitrogen atom in this compound is contained in a piperidine ring. This latter compound has, also, an amide group in the position in which the other compounds carry an hydroxyl group.

Summary

1. The quantitative comparison of anti-acetylcholine and anti-hydroxytryptamine activities of atropine and certain atropine-like compounds, supplied through the courtesy of the Wellcome Research Laboratories, showed that three of them had more anti-hydroxytryptamine effect than has atropine.

2. All the compounds were, however more potent anti-acetylcholine than anti-hydroxytryptamine agents.

I wish to thank Professor J. H. Gaddum for the guidance and advice he gave throughout this study.

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