THE ACETYLCHOLINE-ATROPINE ANTAGONISM AS DETERMINED BY A SERIES OF pA VALUES*

M. B. GHARPURE

Medical College, Aurangabad

THOUGH Clark long time back (1926) showed that the acetylcholine-atropine antagonism is competitive in nature, there is no unanimity of opinion on this point. The author (1964) has summarized the varying view-points in this regard. Table I gives the findings of the various workers. This table has been reproduced from the author's previous article (1964). In that article, were reported the $pA_{5,000}$ and $pA_{25,000}$ values for the acetylcholine-atropine antagonism. That work was an attempt to settle the controversial point raised by the finding of Schild (1947) and Marshall (1955) that the acetylcholine-atropine antagonism is 'not-competitive' as judged from the pA_2 and pA_{10} values determined on the isolated guinea-pig ileum. The selection of the $pA_{5,000}$ and $pA_{25,000}$ values instead of pA_2 and pA_{10} values was not arbitrary. As explained in the above-referred article, the $pA_{5,000}$ and $pA_{25,000}$ values for the acetylcholine-atropine antagonism are roughly equivalent to the pA_2 and pA_{10} values for the histamine-atropine antagonism. However, the higher values determined did not clearly indicate that the acetylcholine-atropine antagonism is competitive.

The present work is an extension of the previous one. Six different pA values, namely, pA_2, pA_{11}, pA_{101}, $pA_{1,001}$ $pA_{10,001}$ and $pA_{100,010}$ have been determined. In the pA method, that concentration of the antagonist is determined which reduces the response of some multiple of the original concentration (nx) to the response of the original concentration (x) of the agonist. Thus, the antagonist is counteracting $nx - x$ or $(n-1)x$ concentration of the agonist. From this, it can be easily seen that each higher value is differing from the lower one by a factor of 10.

The two additional values, namely, the $pA_{5,001}$ and $pA_{25,001}$ have also been determined.

METHOD

Essentially, the method described in detail in the earlier article (1964) was followed. The slight variations made are mentioned below:

(1) *The Apparatus.*—The whole set-up was the one associated with the automatic biological assay apparatus. However, as the timer was out of order, the operations such as emptying the bath and filling it with proper solutions were carried out manually. This was done by using bull-dog clamps on the rubber tubings on the drug reservoirs.

TABLE I

Nature of acetylcholine-atropine antagonism

Authors	Nature of antagonism	Method	Test object
Clark, 1926	.. Competitive	Concentration-action curves :— Plots, (i) log x : log $y/100-y$ (ii) log [atropine] : log [A−ch]	Frogs :— (*a*) Isolated ventricular strip (*b*) Isolated rectus abdominis muscle
Timms, 1956	.. do.	log concentration-percentage action curves	Guinea-pig :—Isolated ileum
Schild, 1947	.. Not competitive	pA method	do.
Marshall, 1955	.. do.	do.	do.
Chen and Russell, 1950	.. Not competitive (*see* Fig. No. 3 on page 144)	Lineweaver-Burk curves	Anæsthetized dogs :—Blood pressure response
Matsumoto-and Kumoi, 1958	Not competitive [*see* Fig. 7 (III) on page 144]	do.	do.
Kirschner and Stone, 1951	.. Not competitive (*see* Fig. No. 3 on page 828)	do.	Frogs :—Isolated rectus abdominis muscle

(2) *Drug Solutions.*—The stock solutions of both atropine sulphate and acetylcholine chloride were prepared in distilled water. They were preserved in a refrigerator. The necessay dilutions were made in the Tyrode solution, fresh, every day.

*Paper presented at the 35th Annual Meeting of the Indian Academy of Sciences, Aurangabad, Dec. 1969.

(3) *The Tyrode Solution.*—The composition of Tyrode solution used was as follows :

NaCl 0·8% ; KCl 0·02% ; $CaCl_2$ 0·01% ; $MgCl_2$ 0·01% ; $NaHCO_3$ 0·07% (In place of 0·1%); NaH_2PO_4 0·015% (In place of 0·005%); Glucose 0·1%. The pH of this Tyrode was 7·4. With the changes made in the concentrations of $NaHCO_3$ and NaH_2PO_4, there was no need to adjust the pH to 7·4 every day.

Results

The results obtained in the present work are given in Tables II and III. In Table IV are reproduced some previous results. Table V compares some of the pA values from the two series worked out in the present work.

Table II

The pA values for the acetylcholine-atropine antagonism

Test Object : Isolated guinea-pig ileum.
Antagonism determined at 14 minutes.
+ = Competitive ; x = 'not competitive'.
Test for competition :
$n = (10 + 10) - 2 = 18$; P = 0·05 ; $t = 2·10$.
Expected difference berween two consecutive values for competitive antagonism : 1·0 of a pA unit.

pA_x	pA_2	pA_{11}	pA_{101}	$pA_{1,001}$	$pA_{10,001}$	$pA_{100,001}$
pA value	8·82	8·06	7·07	6·01	4·90	3·94
St. dev.	0·189	0·085	0·143	0·211	0·111	0·086
n	10	10	10	10	10	10
Difference		0·76	0·99	1·06	1·11	0·96
+ or x		x	+	+	+	+
t		3·660	0·1901	0·7446	1·457	0·8993

Table III

The pA values for the acetylcholine-atropine antagonism

Test Object : Isolated guinea-pig ileum.
Antagonism determined at 14 minutes.
+ = Competitive ; x = 'not competitive'.
Test for competition :
$n = (4 + 5) - 2 = 7$; P = 0·05 ; $t = 2·36$
Expected difference between the two values for competitive antagonism : 0·7 of a pA unit.

pA_x	$pA_{5,001}$	$pA_{25,001}$
pA value	5·24	4·41
St. dev.	0·187	0·092
n	4	5
Difference	0·83	
+ or x	+	
t	1·275	

Table IV

The pA values for the acetylcholine-atropine antagonism

Test Object : Isolated guinea-pig ileum.
Antagonism determined at 14 minutes.
+ = Competitive ; x = 'not competitive'.
Test for competition :
$n = (4 + 4) - 2 = 6$; P = 0·05 ; $t = 2·45$.
Expected difference between the two values for competitive antagonism : 0·7 of a pA unit.

pA_x	$pA_{5,000}$	$pA_{25,000}$
pA value	5·44	4·62
St. dev.	0·042*	0·012
n	4	4
difference	0·82	
+ or x	†	
t	5·381	

* In the previous paper (1964) 0·012 was wrongly printed in place of 0·042.

† The difference is significantly higher than the expected difference of 0·7 of a pA unit. In the previous paper (1964) this was interpreted to indicate competitive antagonism.

Table V

The pA values for the acetylcholine-atropine antagonism

Test Object : Isolated guinea-pig ileum.
Antagonism determined at 14 minutes.
+ = Competitive ; x = 'non competitive'.
Comparison of some pA values from the two series

pA_x	$pA_{1,001}$	$pA_{5,001}$	$pA_{10,001}$	$pA_{25,001}$	$pA_{100,001}$
pA value	6·01	5·21	4·90	4·41	3·94
St. dev.	0·211	0·187	0·111	0·092	0·086
n	10	4	10	5	10
Difference		0·77	0·34	0·49	0·47
Expected difference		0·7	0·3	0·4	0·6
+ or x		+	+	+	x
t found out		0·6184	0·3909	1·686	2·680
t from the table		2·18	2·18	2·16	2·16

Discussion

The results given in Table II very clearly indicate that while the antagonism is 'not competitive at the level of pA_2–pA_{11}, it is competitive from the level of pA_{11}–pA_{101} to that of $pA_{10,001}$–$pA_{100,001}$. As there are six different pA values available, five tests for the nature of antagonism can be applied by comparing two consecutive pA values. The four out of these five tests make out the acetylcholine-atropine antagonism as competitive and only one makes it out as 'not competitive'. Thus, there is an overwhelming evidence in favour of the acetylcholine-atropine antagonism

being competitive in nature as tested by the Schild's pA method on the isolated guinea-pig ileum. The comparison of the pA_2 and pA_{11} values shows that the antagonism is not competitive. This is in agreement with the findings of Schild (1947) and Marshall (1955). Why a compound like atropine which antagonizes acetylcholine competitively even at the level of its $pA_{10,001}$–$pA_{100,001}$ values does not do so at the lower level of its pA_2–pA_{10} (or pA_2–pA_{11}) values is difficult to understand. This is as yet an unsolved problem. The present work was not designed to find an explanation to this apparent discrepancy.

The author (1964) for the first time determined such high values as $pA_{5,000}$ and $pA_{25,000}$ in the study of drug antagonism. In the present work, the highest pA value determined is even higher than the one previously determined, namely, $pA_{25,000}$. One possible objection to testing very high concentrations of an antagonist is that at high concentrations, it may exert a non-specific action. This is a distinct theoretical possibility to be borne in mind. However, it must be proved to apply in a given instance. In the case of a truly competitive and highly specific compound, it may not apply. From the present work, it appears that such is the case with atropine for its acetylcholine antagonism.

In the present work, pA_2, pA_{11}, pA_{101}, $pA_{1,001}$, $pA_{10,001}$ and $pA_{100,001}$ values have been determined and not pA_2, pA_{10}, pA_{100}, $pA_{1,000}$, $pA_{10,000}$ and $pA_{100,000}$. This change is only a finer refinement in the pA method. The expected differences between pA_2 and pA_{10} and pA_2 and pA_{11} for competitive antagonism are respectively 0·9542 and 1·0000 of a pA unit. Handling the integral is easier than the fraction. Hence the change was made.

The $pA_{5,000}$ and $pA_{25,000}$ values determined previously had not unequivocally indicated that the acetylcholine-atropine antagonism is competitive. Therefore, along with the above-referred series of pA values, these values were repeated. Actually, however, the values determined were $pA_{5,001}$ and $pA_{25,001}$ to be in line with the first series of the pA values.

The results of this work are given in Table III. For the sake of comparison, the previously determined $pA_{5,000}$ and $pA_{25,000}$ values are reproduced in Table IV. The comparison of the $pA_{5,001}$ and $pA_{25,001}$ values shows that the antagonism is competitive. However, the comparison of the $pA_{5,000}$ and $pA_{25,000}$ values had not clearly indicated the presence of competition. The difference between the two values was significantly higher than the expected difference of 0·7 of a pA unit. This was provisionally interpreted as indicating competitive antagonism. The difference in the two sets of results emphasizes that four observations may not be adequate. The smaller the interval between the pA values compared, greater should be the number of observations. In the present work, though only four observations have been made for $pA_{5,001}$ and $pA_{25,001}$ values, for the series of six values between pA_2 and $pA_{100,001}$, ten observations have been made for each pA value. By interposing the $pA_{5,001}$ and $pA_{25,001}$, values in the first series of the pA values some more comparisons and therefore some more tests for the nature of the antagonism are available. Table V gives these comparisons. Three out of the four tests available make out the antagonism as competitive. This is an added support to the conclusion arrived at from the present work that the acetylcholine-atropine antagonism is competitive in nature as tested on the isolated guinea-pig ileum, by the pA method. The fact that one of the tests is falling out of line may be explained, as discussed above, on the basis of five determinations for $pA_{25,001}$ values being not adequate. It is to be noted that $pA_{100,001}$ value differs from the $pA_{25,001}$ by a factor of 4.

ACKNOWLEDGEMENT

The technical assistance of Mr. N. A. Utpat, Mr. V. G. Hirve and Miss Jaidesh Chandhok is gratefully acknowledged. Thanks are due to Mr. Abdul Hameed for typing the manuscript.

1. Chen, G. and Russell, D., *Jour. Pharmacol.*, **1950, 99, 401.**
2. Clark, A. J., *Jour. Physiol.*, 1926, **61, 547.**
3. Gharpure, M. B, "An assessment of three methods for the experimental study of during antagonism," *Thesis* for the M.Sc. degree of St. Andrews University (U.K.), **1961.**
4. —, *Ind. Jour. Med. Res.*, **1964, 52, 194.**
5. Kirschner, K. B. and Stone, W. E., *Jour. Gen. Physiol.*, **1951, 34, 821.**
6. Marshall, P. B., *Brit. Jour. Pharmacol.*, **1955, 10, 354.**
7. Matsumoto, H. and Kumoi, T., *Kobe Jour. Med. Sci.*, **1958, 4, 139.**
8. Schild, H. O., *Brit. Jour. Pharmacol.*, 1947, **2**, 189.
9. Timms, A. R., *Ibid.*, **1956, 11, 273.**