Biochemical Pharmacology, 1962, Vol. 11, pp. 937–942. Pergamon Press Ltd., Printed in Great Britain.

# ANTI-CHOLINESTERASE ACTIVITY IN BIS- AND POLY-ONIUM NEUROMUSCULAR BLOCKING AGENTS

S. M. KIRPEKAR, J. J. LEWIS* and T. C. MUIR

Division of Experimental Pharmacology,
Institute of Physiology, The University, Glasgow

(*Received* 18 *April* 1962; *accepted* 18 *June* 1962)

**Abstract**—Anti-cholinesterase activity in a number of bis- and poly-onium neuromuscular blocking agents is affected by the inter-onium distance, alteration in the number and nature of the onium centres and changes in the size of the alkyl substituents on the onium centres. Anti-cholinesterase activity is not restricted to depolarizing drugs but is also a feature of non-depolarizing agents.

ANTI-CHE activity is a property of many quaternary ammonium compounds some of which also possess neuromuscular blocking activity.[1–6]

Depolarizing neuromuscular blocking agents, in general, possess more potent anti-ChE activity than non-depolarizing drugs and this is exerted against "true" to a greater extent than against "pseudo"—cholinesterase.[3, 6]

Although decamethonium is a moderately potent anti-ChE, the contribution of this property either to its effectiveness as a neuromuscular blocking agent or to the type of block produced has not been determined.[3] There remains the possibility, therefore, that in some neuromuscular blocking agents, especially those possessing depolarizing properties, anti-ChE activity may be sufficiently great to contribute towards the neuromuscular block. A series of synthetic bis- and poly-onium neuromuscular blocking agents[7–14] have therefore been investigated for anti-ChE activity in order to ascertain whether this was sufficiently great to be of significance in the neuromuscular blocking potency observed and, additionally, to determine the significance of alterations in chemical structure on anti-ChE activity.

## METHOD

The preparation of cholinesterase from rat brain and the measurement of anti-ChE activity was carried out by the modification of Ammon's method[15] described by Fenwick *et al.*[16] but using acetylcholine chloride instead of the bromide. Anti-ChE activity was calculated as the $PI_{50}$ value according to the method of Blaschko *et al.*.[17] The method employed is that for the preparation of "true" or "acetylcholinesterase".

## RESULTS

The results are shown in Tables 1 to 4, which also show the chemical structures and code numbers of the compounds and the qualitative nature of the neuromuscular block.[7–14]

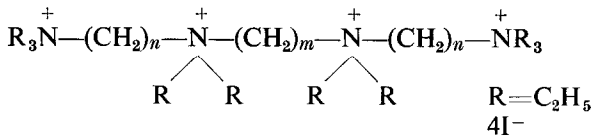
* Communications to be addressed to Mr. J. J. Lewis at the above address.

Anti-ChE activity varied with alterations in chemical structure and, in consequence, the results will be discussed on this basis.

## DISCUSSION

Anti-ChE potency was influenced by the following: alteration in the inter-onium distance, increase in the number of onium centres, alteration in the nature of the onium centres and changes in the size of the alkyl substituents on the onium centres.

TABLE 1. THE INFLUENCE OF INTER-ONIUM DISTANCE ON ACETYLCHOLINESTERASE ACTIVITY, INDICATED BY THE $PI_{50}$ VALUE, OF A SERIES OF TETRA-ETHONIUM ALIPHATIC NEUROMUSCULAR BLOCKING COMPOUNDS




| Code no. | $n$ | $m$ | Type of neuromuscular block produced | $PI_{50}$ |
|---|---|---|---|---|
| E36 | 6 | 6 | Non-depolarizing | 3·5 |
| E70 | 6 | 8 | Non-depolarizing | 2·3 |
| E71 | 8 | 6 | Non-depolarizing | 5·4 |
| E62 | 6 | 10 | Mainly non-depolarizing but with depolarizing features also | 4·1 |
| E72 | 8 | 8 | Mixed, but mainly non-depolarizing | 5·3 |
| E63 | 10 | 6 | Mainly depolarizing | 6·2 |

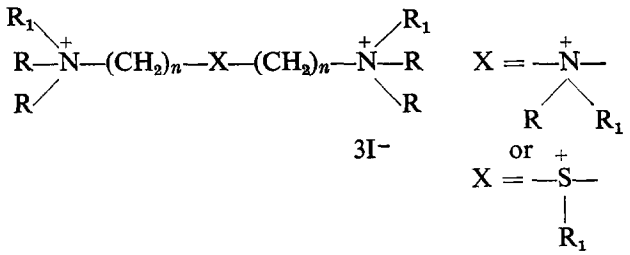
1. *Effect of increase in the number of onium centres on anti-ChE activity*

Increase in the number of onium centres does not decrease anti-ChE activity providing that the inter-onium distance remains constant. The slight increase in potency sometimes observed (for example, E24, E36 and E73, C10E and E31) is not comparable to the marked influence on potency produced by alteration of the inter-onium chain length and does not appear to be a major factor in determining the anti-ChE potency of these drugs.

2. *Effect of alteration in the inter-onium distance on anti-ChE activity*

An increase in the number of methylene groups from five (E93 and E94) to ten (E31) was accompanied by an increase in anti-ChE activity. A sharp rise in potency was observed with increase in the inter-onium chain length from six to eight methylene groups (E24 and E41; E27 and E40; E36 and E72). An increase in the chain length beyond ten methylene groups (E35, E17 and Ex) did not however enhance anti-ChE activity. Similar results were observed, using bis-onium derivatives (C6, C10, C11, C12, C13 and the fully ethylated analogue of decamethonium C10E) and are in agreement with those of Paton and Zaimis[3] who reported that, in a series of polymethylene bis-methonium compounds, anti-ChE activity increased until the compound containing twelve methylene groups was reached. The increase in anti-ChE activity with

TABLE 2. THE COMPARATIVE $PI_{50}$ VALUES OF A SERIES OF TRIS-ONIUM ALIPHATIC NEUROMUSCULAR BLOCKING COMPOUNDS SHOWING THE EFFECT OF ALTERATION IN THE ONIUM CENTRES, INTER-ONIUM CHAIN LENGTH AND THE ALKYL SUBSTITUENTS ON ANTI-ACETYLCHOLINESTERASE ACTIVITY



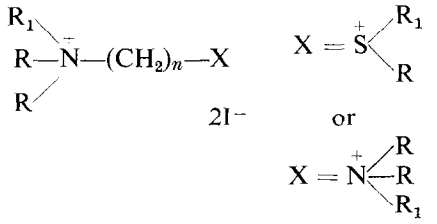

| Code no. | R | $R_1$ | $n$ | X | Type of block produced | $PI_{50}$ |
|---|---|---|---|---|---|---|
| E24 | $C_2H_5$ | $C_2H_5$ | 6 | N | Non-depolarizing | 2·3 |
| E41 | $C_2H_5$ | $C_2H_5$ | 8 | N | Non-depolarizing | 6·0 |
| E31 | $C_2H_5$ | $C_2H_5$ | 10 | N | Depolarizing | 5·5 |
| E74 | $C_2H_5$ | $CH_3$ | 6 | N | Non-depolarizing | 3·5 |
| E75 | $C_2H_5$ | $n$—$C_3H_7$ | 6 | N | Non-depolarizing | 3·5 |
| E76 | $C_2H_5$ | $n$—$C_4H_9$ | 6 | N | Non-depolarizing | 3·6 |
| E82 | $n$—$C_3H_7$ | $n$—$C_3H_7$ | 6 | N | Non-depolarizing | 3·5 |
| E83 | $n$—$C_3H_7$ | $C_2H_5$ | 6 | N | Non-depolarizing | 3·5 |
| E84 | $n$—$C_3H_7$ | $CH_3$ | 6 | N | Non-depolarizing | 3·7 |
| E93 | $C_2H_5$ | $CH_3$ | 5 | N | Non-depolarizing | Nil (at M/200 conc.) |
| E94 | $C_2H_5$ | $C_2H_5$ | 5 | N | Non-depolarizing | Nil (at M/200 conc.) |
| E27 | $C_2H_5$ | $C_2H_5$ | 6 | S | Non-depolarizing | 3·3 |
| E40 | $C_2H_5$ | $C_2H_5$ | 8 | S | Non-depolarizing | 5·7 |
| E30 | $C_2H_5$ | $C_2H_5$ | 10 | S | Mixed | 5·9 |
| E18 | $CH_3$ | $CH_3$ | 6 | S | Non-depolarizing | 3·5 |
| E61 | $CH_3$ | $C_2H_5$ | 6 | S | Non-depolarizing | 3·4 |
| E60 | $C_2H_5$ | $CH_3$ | 6 | S | Non-depolarizing | 3·4 |
| E64 | $CH_3$ | $n$—$C_4H_9$ | 6 | S | Non-depolarizing | 4·2 |
| E65 | $C_2H_5$ | $n$—$C_3H_7$ | 6 | S | Non-depolarizing | 4·2 |

increase in inter-onium distance reported in the present investigation was unaffected by the size of the onium substituents or the nature of the onium centres themselves.

3. *Effect of alteration in the nature of the onium centres on anti-ChE activity*

In the N,N,N (E24, E41 and E31) and N,S,N (E27, E40 and E30) ethonium series, it is clear that replacement of a quaternary nitrogen by a tertiary sulphur atom produced no dramatic alteration in anti-ChE activity. Similar results were observed in the

TABLE 3. THE COMPARATIVE $PI_{50}$ VALUES OF A SERIES OF BIS-ONIUM ALIPHATIC NEUROMUSCULAR BLOCKING AGENTS, SHOWING THE EFFECT OF ALTERATION OF THE INTER-ONIUM CHAIN LENGTH, THE ALKYL ONIUM SUBSTITUENTS AND THE ONIUM CENTRES ON ANTI-ACETYLCHOLINESTERASE ACTIVITY



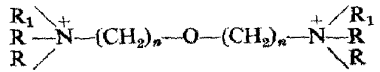
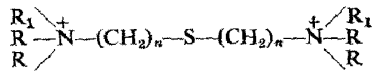
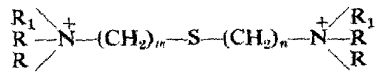
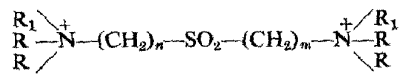
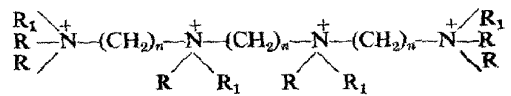
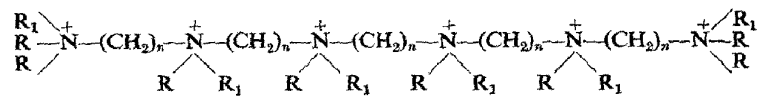
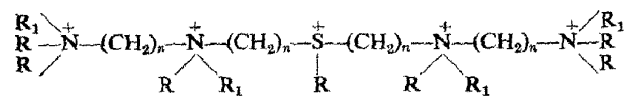

| Code no. | R | $R_1$ | $n$ | X | Type of neuromuscular block produced | $PI_{50}$ |
|---|---|---|---|---|---|---|
| C6 | $CH_3$ | $CH_3$ | 6 | $N^+(CH_3)_3$ | Non-depolarizing | 3·0 |
| C10 | $CH_3$ | $CH_3$ | 10 | $N^+(CH_3)_3$ | Depolarizing | 5·1 |
| C10E | $C_2H_5$ | $C_2H_5$ | 10 | $N^+(C_2H_5)_3$ | Non-depolarizing | 5·1 |
| C11 | $CH_3$ | $CH_3$ | 11 | $N^+(CH_3)_3$ | Mainly depolarizing | 5·4 |
| C12 | $CH_3$ | $CH_3$ | 12 | $N^+(CH_3)_3$ | Mixed depolarizing and non-depolarizing | 5·5 |
| C13 | $CH_3$ | $CH_3$ | 13 | $N^+(CH_3)_3$ | As above | 5·7 |
| I | $CH_3$ | $C_2H_5$ | 8 | $S^+(CH_3C_2H_5)$ | Mainly depolarizing | 2·9 |
| II | $CH_3$ | $CH_3$ | 10 | $S^+(CH_3)_2$ | Depolarizing | 4·9 |
| III | $CH_3$ | $CH_3$ | 8 | $S^+(CH_3)_2$ | Depolarizing | 4·0 |

bis-onium compounds II and decamethonium. These results parallel observations made during investigations on the neuromuscular blocking potency of these drugs. The compounds E17 and Ex, in which the sulphur atom of each is uncharged, were, as expected, more potent than the corresponding N,S,N-sulphonium derivatives, emphasizing the importance of inter-onium distance in determining the anti-ChE activity of these compounds.

4. *Effect of changes in the size of the alkyl substituents on the onium centres on anti-ChE activity*

Increase in the alkyl group size is less important than increase in the inter-onium distance, but in both the N,N,N-(E74, E24, E75, E76 and E82) and N,S,N-(E18, E27, E60, E61, E64 and E65) tris-onium series, the presence of more bulky substituents

TABLE 4. THE COMPARATIVE ANTI-ACETYLCHOLINESTERASE ACTIVITY, MEASURED AS THE $PI_{50}$ VALUES, OF A NUMBER OF BIS- AND POLYONIUM ALIPHATIC NEUROMUSCULAR BLOCKING AGENTS

| Name or code no. | Basic chemical structure | $R_1$ | R | $n$ | Anion | Type of neuromuscular block produced | $PI_{50}$ |
|---|---|---|---|---|---|---|---|
| Oxydi-pentonium |  | $CH_3$ | $CH_3$ | 5 | $2Cl^-$ | Depolarizing | 5·4 |
| E17* |  | $CH_3$ | $CH_3$ | 6 | $2I^-$ | Non-depolarizing | 5·6 |
| Ex* |  | $CH_3$ | $CH_3$ | 10 | $2I^-$ | Mainly *non*-depolarizing | 5·9 |
| E35* |  | $C_2H_5$ | $C_2H_5$ | 6 | $2I^-$ | Non-depolarizing | 5·8 |
| E36 |  | $C_2H_5$ | $C_2H_5$ | 6 | $4I^-$ | Non-depolarizing | 3·5 |
| E73 |  | $C_2H_5$ | $C_2H_5$ | 6 | $6I^-$ | Non-depolarizing | 3·5 |
| E58 |  | $C_2H_5$ | $C_2H_5$ | 6 | $5I^-$ | Non-depolarizing | 3·3 |
| Neostigmine | | | | | | | 7·1 |
| Eserine | | | | | | | 7·0 |

* Sulphur atom carries no positive charge.

was generally associated with a slight increase in potency. In each series, the ethonium derivatives E24 and E27 were the least potent.

*5. General conclusions*

The association of depolarizing neuromuscular blocking activity with a comparatively high degree of anti-ChE activity has been confirmed in many cases. Relatively high anti-ChE activity was found both in depolarizing and also in some predominantly non-depolarizing compounds (for example E40, E41, E71, E72 and C10E) suggesting that anti-ChE activity was not contributing significantly to the *type* of block produced. As non-depolarizing compounds may possess anti-ChE activity, it follows that the acetylcholinesterase and the cholinergic receptor surfaces are probably not identical. It is, however, possible that the cholinergic receptor and the acetylcholinesterase surface may be closely related, being located on similar surfaces but not at precisely the same points. Thus the structure of the cholinesterase enzyme surface proposed by Cohen and his associates[6] appears particularly relevant.

In addition to the postulated presence of anionic and esteratic sites on the enzyme surface,[18] Cohen and his associates proposed the existence of non-specific anionic sites capable of interacting with drugs possessing a cationic head without inducing depolarization, although the receptor might be blocked. This view would seem to accommodate the anti-ChE activity of non-depolarizing neuromuscular blocking agents and to account for the anticholinesterase activity of certain of the drugs reported in the present investigation. The affinity of a neuromuscular blocking compound for such non-specific sites would consequently be determined by the chemical and electronic configuration of the molecule in a manner analogous to, but not identical with its combination with the cholinergic receptor.

*Acknowledgements*—We are indebted to Messrs. A. P. Ritchie and P. Leitch for technical assistance and to Dr. H. Morren, Brussels, Dr. R. B. Barlow, Edinburgh and Mr. D. Brown, Beecham's Ltd., Betchworth, Surrey, respectively, for gifts of oxydipentonium, decaethonium and compounds I, II and III. The remaining compounds were synthesized by Professor J. B. Stenlake, Glasgow, and his colleagues whose kindness is gratefully acknowledged.

## REFERENCES

1. G. BRISCOE, *Lancet* i, 469 (1936).
2. A. ROSENBLUETH, B. D. LINDSLEY and R. S. MORISON, *Amer. J. Physiol.* **115**, 53 (1936).
3. W. D. M. PATON and E. J. ZAIMIS, *Brit. J. Pharmacol.* **4**, 381 (1949).
4. W. F. RIKER, *Pharmacol. Rev.* **5**, 1 (1953).
5. A. TODRICK, *Brit. J. Pharmacol.* **9**, 76 (1954).
6. J. A. COHEN, M. C. P. J. WARRINGA and I. INDORF, *Acta physiol. pharmacol. néerl.* **4**, 187 (1955).
7. D. EDWARDS, J. J. LEWIS, J. B. STENLAKE and M. S. ZOHA, *J. Pharm. Lond.* **9**, 1004 (1957).
8. D. EDWARDS, J. J. LEWIS, J. B. STENLAKE and M. S. ZOHA, *J. Pharm., Lond.* **10**, 106T (1958a).
9. D. EDWARDS, J. J. LEWIS, J. B. STENLAKE and M. S. ZOHA, *J. Pharm., Lond.* **10**, 122T (1958b).
10. D. EDWARDS, J. J. LEWIS, J. B. STENLAKE and F. STOTHERS, *J. Pharm., Lond.* **11**, 87T (1959).
11. D. EDWARDS, J. B. STENLAKE, J. J. LEWIS and F. STOTHERS, *J. Med. pharm. Chem.* **3**, 369 (1961).
12. S. LEVIS, S. PREAT and J. DAUBY, *Arch. int. Pharmacodyn.* **93**, 46 (1953).
13. F. MACL. CAREY, D. EDWARDS, J. J. LEWIS and J. B. STENLAKE, *J. Pharm., Lond.* **11**, 70T (1959).
14. T. C. MUIR and J. J. LEWIS, *J. Pharm., Lond.* **11**, 91T (1959).
15. R. AMMON, *Pflüg. Arch. ges. Physiol.* **233**, 486 (1933).
16. M. L. FENWICK, J. R. BARRON and W. A. WATSON, *Biochem. J.* **65**, 58 (1957).
17. H. BLASCHKO, E. BÜLBRING and T. C. CHOU, *Brit. J. Pharmacol.* **4**, 29 (1949).
18. D. NACHMANSOHN and I. B. WILSON, *Advanc. Enzymol.* **12**, 259 (1951).