are obtained when phenethylamines and imidazolines are used as agonists (Sanders & others, 1975).

These studies were supported by a grant from the United States Public Health Service, N.I.H. Grant No 9M-17859.

November 4, 1976

REFERENCES

ARIËNS, E. J. & SIMONIS, A. M. (1964). *J. Pharm. Pharmac.*, **16**, 137–157.
BARSOUM, G. S. & GADDUM, J. H. (1935). *J. Physiol. (Lond.)*, **85**, 1–14.
EASSON, L. H. & STEDMAN, E. (1933). *Biochem. J.*, **27**, 1257–1266.
KALSNER, S. (1970). *Life Sci.*, **9**, 961–974.
KALSNER, S. (1973). *Br. J. Pharmac.*, **47**, 386–397.
MUJIC, M. & VAN ROSSUM, J. M. (1965). *Archs int. Pharmacodyn. Thér.*, **155**, 432–448.
PATIL, P. N., BURKMAN, A. M., YAMAUCHI, D. & HETEY, S. (1972). *J. Pharm. Pharmac.*, **25**, 221–228.
PATIL, P. N., MILLER, D. D. & TRENDELENBURG, U. (1974). *Pharmac. Rev.*, **26**, 323–392.
RUFFOLO, R. R., MILLER, D. D., FOWBLE, J. & PATIL, P. N. (1976a). *Pharmacologist*, **18**, 138.
RUFFOLO, R. R., FOWBLE, J. W., MILLER, D. D. & PATIL, P. N. (1976b). *Proc. natn. Acad. Sci., U.S.A.*, **73**, 2730–2734.
SANDERS, J., MILLER, D. D. & PATIL, P. N. (1975). *J. Pharmac. exp. Ther.*, **195**, 362–371.
SCHILD, H. O. (1973). *Drug Receptors*, p. 29. Editor: Rang, H. P. London: MacMillan.
WAUD, D. R. (1968). *Pharmac. Rev.*, **20**, 49–88.

Neuromuscular action of the anticholinesterase RX72601 in the frog

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RX72601 (*cis*-2-(3-hydroxyphenyl)-1-pyrrolidinocyclohexane methobromide) is a member of a series of phenylcyclohexamines which has anticholinesterase properties *in vitro* (Dettmar, Lewis & others, 1974). Metcalf & Dettmar (1975) reported that after injection into anaesthetized animals, RX72601 inhibited acetylcholinesterase in arterial blood and this would explain the antagonism of non-depolarization neuromuscular blockade which they observed in several animal species.

I have used intracellular recording, which is a precise technique for evaluating drug action on neuromuscular transmission (Riker & Okamoto, 1969) to provide information on the mode of action of the drug and to ascertain if a pre-junctional action increasing transmitter release is also present, which would account in part for its anti-curare property.

Experiments were made on frog (*Rana temporaria*) isolated sartorius nerve muscle preparations at 17–20° employing the usual techniques for intracellular recording (Fatt & Katz, 1951) with glass capillary microelectrodes filled with 3 M KCl and resistances 5–10 Mohms, connected through a cathode follower to a Tektronix Type 502A dual beam oscilloscope. Simultaneous d.c. recording was made on paper using an ink-writer Mingograf 34, at low amplification for membrane potentials and a.c. recording at higher amplification for miniature endplate potentials (m.e.p.p.s.) and endplate potentials (e.p.p.s.). The frequency response of the recording system was flat from 5–500 Hz (−3db at Hz and 700 Hz). The composition of the standard hysiological solution was (mM): NaCl, 103; KCl, 1; aCl_2, 1·8; $NaHCO_3$, 1·2.

The electrode was inserted at an endplate and when the membrane potential was stable m.e.p.p.s. were recorded as controls. With the electrode still in position the effect of RX72601 on m.e.p.p.s. was tested either by adding a solution of the drug to the bath fluid or exchanging the bath fluid for one containing the drug and recording m.e.p.p.s. for periods up to 5 min. The bath fluid was then replaced by normal Ringer solution and further records taken. To avoid cumulative effects, in each experiment the effect of one drug concentration only was recorded from a single muscle fibre. Concentrations of RX72601 (2×10^{-8}M and over) increased m.e.p.p. amplitude without altering discharge frequency or the resting membrane potential (Fig. 1a and b). From 4 experiments the mean % increase in amplitude of m.e.p.p.s. (with s.d.) induced by RX72601 (2×10^{-8}M) was 90(18). At high concentrations of 1×10^{-6}M membrane depolarization was not > 5 mV.

To observe the effect of the drug on e.p.p.s., neuromuscular transmission was blocked by adding a solution of tubocurarine chloride to the bath fluid and e.p.p.s. were elicited in response to nerve stimulation at 1 Hz. A solution of RX72601 was then added to the bath fluid and further records from the same endplate showed increased amplitude of e.p.p.s. (Fig. 1c and d). 12 experiments were undertaken to provide an estimate of the mean acetylcholine quantum content of e.p.p.s., by analysis of their amplitude variance using a single muscle fibre recording in each experiment. The formula used to determine mean acetylcholine quantum content (m) of e.p.p.s. was: $m = (\text{mean e.p.p.})^2/(\text{e.p.p. variance})$. At least 100 e.p.p.s. were recorded as controls and in the

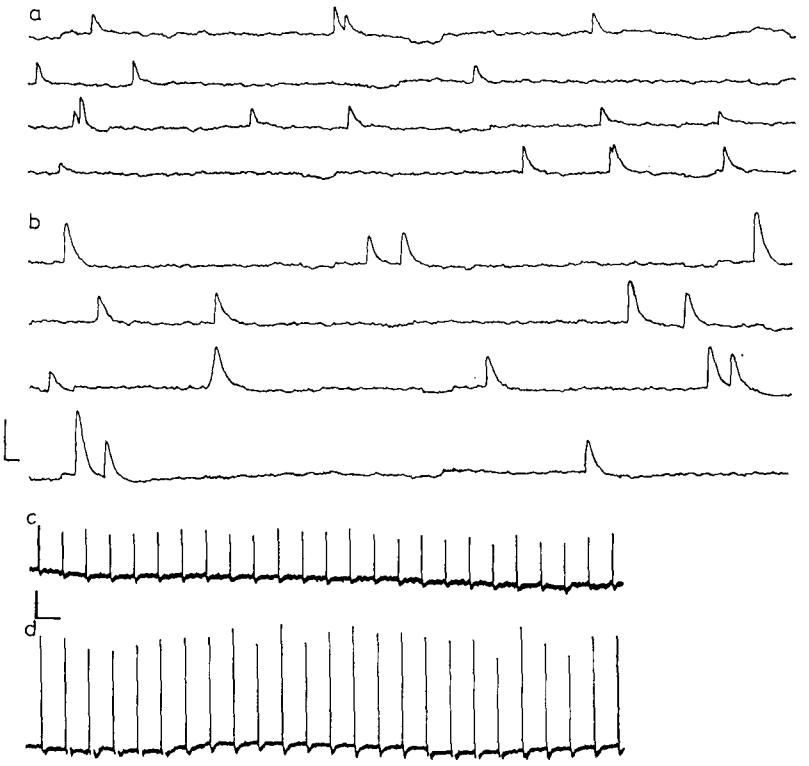

FIG. 1. The effects of RX72601 on m.e.p.p. and e.p.p. amplitude. (a) Control m.e.p.p.s. recorded intracellularly from one endplate of a frog sartorius muscle fibre (membrane potential 80 mV). (b) m.e.p.p.s. recorded from the same endplate in the presence of RX72601 (2×10^{-8}M). (c) Control e.p.p.s. recorded intracellularly from one endplate of a curarized frog sartorius muscle fibre (membrane potential 83 mV), with nerve stimulation 1 Hz. (d) e.p.p.s. recorded from the same endplate in the presence of RX72601 (2×10^{-8}M). Calibrations: m.e.p.p.s., 1mV and 20mS: e.p.p.s., 1mV and 1S.

presence of RX72601, with nerve stimulation rate of 1 Hz. The sizes of the e.p.p.s. were corrected for non-linearity of the endplate response and the resting membrane potential of the muscle fibre using the method of Elmqvist & Quastel (1965). The control value (with s.e.m.) for the mean acetylcholine quantum content was 130(14) and in the presence of the drug 158(22). There is no significant difference between these values (Student's *t*-test, $P < 0{\cdot}1$).

Since RX72601 did not alter transmitter release, the increased amplitudes of both m.e.p.p.s. and e.p.p.s. probably reflects inhibition of cholinesterase. Although multiple sites for drug action are present at the neuromuscular junction it seems likely that the anticholinesterase action of RX72601 is of prime importance in the facilitation of neuromuscular transmission reported here and would explain the anticurare actions of this drug observed by Metcalf & Dettmar (1975).

I am grateful to Mr C. E. Lythe for technical assistance and to Reckitt and Colman for the gift of RX72601.

January 24, 1977

REFERENCES

DETTMAR, P. W., LEWIS, J. W., METCALF, G., READHEAD, M. J., SMITH, M. H., GILLETT, G. B., HEDGES, A. & RICHENS, A. R. (1974). *J. Pharm. Pharmac.*, **26**, 134–135.

ELMQVIST, D. & QUASTEL, D. M. J. (1965). *J. Physiol., Lond.*, **178**, 505–529.

FATT, P. & KATZ, B. (1951). *Ibid.*, **115**, 320–370.

METCALF, G. & DETTMAR, P. W. (1975). *Br. J. Anaesth.*, **47**, 451–456.

RIKER, W. F., JR. & OKAMOTO, M. (1969). *A. Rev. Pharmac.*, **9**, 173–208.