PRELIMINARY ASSESSMENT OF RX 72601, A NEW ANTICHOLINESTERASE IN MAN: REVERSAL OF COMPETITIVE NEUROMUSCULAR BLOCKADE

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1 The effect of RX 72601, neostigmine plus atropine, or saline on neuromuscular blockade with (+)-tubocurarine was studied in a double blind trial in four healthy male volunteers.

2 The methods used to assess neuromuscular function were voluntary grip strength and indirectly evoked muscle twitches. The muscarinic action was assessed by measuring intestinal motility with a pressure sensitive radio pill, and auscultation of the abdomen. Blood pressure and pulse rate were also recorded. The degree of acetylcholinesterase inhibition achieved in whole blood was determined before and at intervals after the administration of the anticholinesterases.

3 Both RX 72601 (0.66-0.83 mg) and neostigmine (2.5 mg) completely reversed the neuromuscular blockade produced by tubocurarine, but the time course of reversal differed. RX 72601 (0.66-0.83 mg) produced similar inhibition of acetylcholinesterase in whole blood to neostigmine (2.5 mg). The doses of RX 72601 used caused minimal stimulation at muscarinic sites as evidenced by the limited effect on pulse rate and intestinal activity.

4 It was concluded that RX 72601 could be safely used to reverse the effects of neuromuscular blockade without the need for premedication with atropine.

Introduction

In a previous study of substituted cyclohexylamines RX 67668, *cis*-2-phenyl-1-(*N*-pyrrolidinyl) cyclohexane hydrochloride, was shown to possess anticholinesterase properties which were particularly obvious at nicotinic sites (Doxey, Metcalf, Smith & Whittle, 1972). After preliminary testing in man RX 67668 was shown to possess hallucinogenic properties and to be less potent than animal tests had predicted (Gillett, Hedges, Metcalf, Richens & Royds, 1972; Wray & Cowan, 1973) so that further development of the compound was abandoned.

We now present results obtained with RX 72601, *cis*-2-(3-hydroxy-phenyl)-1-pyrrolidino cyclohexane methobromide, a quaternary derivative of the parent compound. It was predicted that such chemical modification of the original molecule would result in increased anticholinesterase activity together with reduced CNS activity whilst retaining the parent compound's specificity for

nicotinic sites. We have studied the ability of RX 72601 to reverse competitive neuromuscular blockade in normal volunteers. In addition, the effects of the compound at muscarinic sites have been assessed.

Methods

Four healthy male volunteers, from whom informed consent had been obtained, aged between 21 and 24 years, were used for the study.

Biochemistry

Blood samples (10 ml) were withdrawn from an indwelling cannula and placed in heparinized bottles (Evans Medical Ltd) in order to determine the degree of acetylcholinesterase inhibition achieved in whole blood by the anticholinesterase

drug. Enzyme activity was determined as described by Smith (1974) and the activity was expressed as percentage of control values. The blood samples were stored on dry ice until assayed, because previous animal experiments had shown that, in the case of neostigmine, blood samples recovered their acetylcholinesterase activity on standing at 4°C due to decarbamylation of the inhibited enzyme.

Measurement of muscle twitches

Subjects reclined on a couch during the experiments. One leg was lifted on to a rigid frame so that the knee was flexed to about 45°. The foot was strapped to a foot plate to which a Grass FT 10C strain gauge transducer was attached. Nerve stimulation was by pad electrodes strapped over the lateral popliteal nerve at the head of the fibula. Supramaximal square wave stimuli at a frequency of 0.16 Hz and 1 msec duration were applied to the nerve with a Disa Ministim stimulator. Twitches of the anterior compartment muscles were recorded isometrically and displayed on a Devices M2 recorder. Muscle stimulation began prior to tubocurarine administration (see below), and was continued until at least 15 min after administration of the anticholinesterase. Results were expressed as a percentage of control values.

Measurement of grip strength

Voluntary grip strength was measured with a transducer comprising a spring cylinder enclosing a light-emitting diode, a butterfly shutter and a photocell. The output of this device was recorded on a Smith's Servoscribe recorder. Two measurements were taken, grip strength before exercise and secondly grip strength after exercise (maximum grip for 1 s every 2 s over a period of 60 seconds). The latter was taken as an index of fatigue. Results were expressed as a percentage of control values.

Measurement of intestinal activity

Intestinal activity was estimated by three different methods:

(a) Recordings of intestinal activity were made with a pressure sensitive radio pill (Rigel Research Ltd) which was swallowed 2-3 h before the experiment began. No food was taken by the volunteers between swallowing the pill and the completion of an experiment. The frequency-modulated signal from the pill was detected by an aerial positioned over the abdomen and recorded via a radio-receiver (Rigel Research Ltd) and a Servoscribe recorder.

Absolute measurements of pressure were made in cm water by calibrating the output of each pill against a simple water manometer before an experiment began.

(b) Simultaneous measurements of gastrointestinal activity were made by one of us (G.B.G.) by auscultation of the abdomen. The effects produced by the drugs were quantified by adopting a simple scoring system in which 1 = diminished activity, 2 = normal activity and 3 = increased activity.

(c) The subjects were asked to volunteer comments on the effects which they attributed to the drugs administered (the subjects were students who had received instruction in pharmacology).

Blood pressure and pulse rate

These were measured with a sphygmomanometer and by palpation of the radial pulse by one of us (G.B.G.) who was unaware which treatment had been administered.

Drug administration

All drugs were administered intravenously via an indwelling cannula. Tubocurarine was injected slowly (1 mg/min) until the twitches evoked in the anterior compartment muscles decreased to approximately 55% of their control amplitude. The total dose required to produce this effect varied between 9 mg and 17.5 mg on different occasions. Three minutes were then allowed to elapse before the anticholinesterase was administered. This interval allowed the maximum tubocurarine effect to develop which usually resulted in an approximately 50% decrease in twitch amplitude. In the main study each of the four subjects received tubocurarine on three separate occasions and the anti-curare effect of three treatments was compared:

(a) atropine 1.2 mg followed by neostigmine 2.5 mg,

(b) 0.9% w/v NaCl (saline) followed by RX 72601 10 µg/kg,

(c) saline followed by saline.

An interval of 1 min was allowed between administration of the two drugs comprising each treatment. The treatments were administered in a randomized order and neither the subjects nor the clinician responsible for the assessments was aware of treatment given on any occasion.

Drugs used

The drugs used were atropine sulphate and (+)-tubocurarine chloride (Burroughs Wellcome &

Fig. 1 The inhibition of acetylcholinesterase in blood produced by RX 72601 (●), neostigmine plus atropine (■), or saline (▲) when measured after tubocurarine (TC) administration. Results are mean values obtained from four subjects.

Co.), neostigmine methylsulphate (Roche Products Ltd) and RX 72601 (Reckitt & Colman Ltd).

The results were analysed using the Student's *t* test.

Results

Pilot studies

Two such studies were undertaken prior to the main experiment to identify a dose of RX 72601 suitable for the main study.

In the initial study two subjects were used. Tubocurarine was not administered. The first subject received RX 72601 in doses which increased logarithmically from 0.3 to 10 $\mu\text{g/kg}$ (a cumulative dose of 14.3 μg administered over 2 hours). At this latter dose the subject reported transient signs of cholinergic stimulation and subsequent biochemical estimations showed that inhibition of acetylcholinesterase increased from 20% at 0.3 $\mu\text{g/kg}$ to 90% at 10 $\mu\text{g/kg}$. The result from a second subject treated with a single dose of 10 $\mu\text{g/kg}$ RX 72601 confirmed that such a dose was well tolerated and produced 94% inhibition of acetylcholinesterase.

In the second pilot study it was ascertained that

Fig. 2 The effect of RX 72601 (●), neostigmine plus atropine (■), or saline (▲) on muscle twitch measured after tubocurarine (TC). Results are mean values obtained from four subjects. * Result significantly greater than other two results, $P < 0.05$.

RX 72601 produced a significant anti-curare effect. Tubocurarine was infused to produce a 50% decrease in twitch height (see Methods). After 10 $\mu\text{g/kg}$ RX 72601 twitch height was restored to control values after 10 min; this compared with 30 min in subjects treated with saline in a previous study (Gillett *et al.*, 1972).

Main study

On the basis of these results a detailed comparison was undertaken of the anti-curare properties of RX 72601, and of neostigmine, the anticholinesterase currently used by most anaesthetists. Atropine was given before neostigmine (see Methods) to concur with current anaesthetic practice but not before RX 72601 or saline.

Enzyme inhibition. Both RX 72601 and neostigmine produced marked inhibition of whole blood acetylcholinesterase which was maximal 5 min after drug administration (Figure 1). There was no significant difference between the degree of inhibition produced by the two drugs. Saline produced no inhibition of acetylcholinesterase.

Fig. 3 The effect of RX 72601 (●), neostigmine plus atropine (■), or saline (▲) on grip strength (a) before exercise, and (b) after exercise, both measured subsequent to tubocurarine (TC) administration. Results are mean values obtained from four subjects. * and ** Result significantly greater than other two results at $P < 0.05$ and $P < 0.01$ respectively.

Muscle twitch. Subjects treated with either RX 72601 or neostigmine made a more rapid recovery than those treated with saline (Figure 2). The pattern of recovery seen with RX 72601, however, differed from that seen with neostigmine. The latter drug showed its maximum recovery at 5 min and no further improvement was evident. In contrast, RX 72601 showed continuous improvement during the 15 min after drug administration.

Grip strength. The results are shown in Figure 3. Several points were noted:

(a) The dose of tubocurarine necessary to decrease twitch height by 50% reduced grip strength before exercise to 20-30% of control values and almost completely abolished grip strength after exercise (2-6%).

(b) Grip strength before exercise recovered more quickly than grip strength after exercise.

(c) Recovery of grip strength before exercise was maximal with neostigmine at 5 min, whereas for RX 72601 recovery was more gradual.

(d) After exercise recovery was more prompt

when the subjects were treated with neostigmine plus atropine than when they received either RX 72601 or saline, although at 30 min after drug administration there was no difference between the two anticholinesterases.

Intestinal activity. Intestinal activity was recorded as a basal pressure (50-125 cm H₂O) punctuated by a series of spikes each of which represented a sudden change in intraluminal pressure. Assessment of drug effects was made by two methods; by counting the frequency (spikes/min) of spikes during the course of the experiment and secondly by measuring the spike amplitude. The frequency results are presented in Table 1. Administration of neostigmine produced a transient rise in spike count during the 10 min following injection but after that values returned to control values. In contrast, RX 72601 produced an increased spike count which was greatest in the period 10-20 min after injection but was still apparent 20-30 min after injection. This difference, however, was probably exaggerated by the consistently higher spike counts recorded at all

stages of these experiments with RX 72601. When amplitude of the pressure spikes was assessed RX 72601 was observed to produce an increase in three of the four experiments whereas neostigmine produced an increase in only one experiment and saline did not produce an increase in spike amplitude.

The results obtained by auscultation of the abdomen (Table 2) were in general agreement with those obtained using the radio pill and showed neostigmine to exert its maximum intestinal effect 5 min after drug administration whereas the maximum effect obtained with RX 72601 occurred 15 min after injection. The comments related by the subjects described the intestinal effects of the anticholinesterases as mild. The effect was usually compared to a feeling of hunger, and may in fact have been related to the subjects' lack of food. Only on two occasions during the 12 experiments were stomach pains experienced. On both of these occasions the subjects had received neostigmine plus atropine.

Blood pressure and pulse rate. None of the three treatments had any significant effect on blood pressure or pulse rate (Figure 4). In the case of neostigmine plus atropine, pulse rate was raised

during the period following atropine but prior to neostigmine.

Discussion

In the present study RX 72601 has been compared with neostigmine, a conventional anticholinesterase established in medical practice, and the results show that the doses chosen (0.66-0.83 mg RX 72601; 2.5 mg neostigmine) produced similar inhibition of acetylcholinesterase in whole blood, so that using this criterion the new compound appears 3-4 times more potent than neostigmine *in vivo*. Both RX 72601 and neostigmine completely reversed the neuromuscular block induced by tubocurarine although the time course of the reversal was different for the two drugs. Both methods used to assess neuromuscular function, voluntary grip strength and indirectly evoked muscle twitches, showed that neostigmine produced a maximum anti-curare effect about 5 min after injection and that there was no further improvement after this time. In contrast, with RX 72601, the anti-curare effect was slower in onset but showed continuous improvement over 30 minutes. This was especially noticeable when grip

Table 1 Intestinal activity assessed by counts of pressure spikes recorded by the radio pill in four healthy male volunteers. Results are given as the mean count/min $\pm$ s.e. mean.

Drug	Control		Post drug		
	Pre-TC	Post-TC	0-10 min	10-20 min	20-30 min
Saline	0.44 $\pm$ 0.29	0.33 $\pm$ 0.19	0.55 $\pm$ 0.23	0.78 $\pm$ 0.30	0.68 $\pm$ 0.23
Neostigmine + atropine	0.54 $\pm$ 0.07	0.38 $\pm$ 0.11	0.85 $\pm$ 0.36	0.58 $\pm$ 0.25	0.30 $\pm$ 0.11
RX 72601	0.92 $\pm$ 0.29	0.81 $\pm$ 0.34	1.0 $\pm$ 0.65	1.58 $\pm$ 0.45	1.48 $\pm$ 0.30*

* Significant difference from corresponding spike count after neostigmine ($P < 0.02$).
TC, tubocurarine.

Table 2 Intestinal activity assessed by auscultation of bowel sounds in four healthy male volunteers. Each value is the mean score on a rating scale in which: 1 = diminished activity; 2 = normal activity; 3 = enhanced activity.

Drug	Mean bowel sound rating				
	Control		Post drug		
	Pre-TC	Post-TC	5 min	15 min	30 min
Saline	2.25	1.75	2.25	2.00	2.00
Neostigmine + atropine	2.00	1.50	2.75	2.50	2.00
RX 72601	2.00	1.00	2.75	3.00	2.75

TC, tubocurarine.

Fig. 4 The effect of RX 72601 (●), neostigmine plus atropine (■), or saline (▲) on pulse rate subsequent to tubocurarine (TC) administration. Results are mean values obtained from four subjects. The large increase seen with neostigmine plus atropine treatment is attributable to atropine before neostigmine is administered.

References

- DOXEY, J.C., METCALF, G., SMITH, M.H. & WHITTLE, B.A. (1972). Some pharmacological properties of RX 67668, a new anticholinesterase. *Br. J. Pharmac.*, **46**, 568P-569P.
- GILLET, G.B., HEDGES, A., METCALF, G., RICHENS, A. & ROYDS, R.B. (1972). Reversal of competitive neuromuscular blockade by RX 67668 in normal volunteers. *Br. J. Pharmac.*, **46**, 581P-582P.
- SMITH, M.H. (1974). Determination of blood acetyl-

strength after exercise was measured, which was probably the most searching test of neuromuscular function undertaken. The reason for the difference in time course of the effects of the two anticholinesterases is not clear. At the end of the trial all the subjects stated that they felt strongest at the end of the test in which they had received RX 72601, despite their failure to identify correctly the order of treatments.

Although 10 $\mu\text{g/kg}$ (0.66-0.83 mg total dose) RX 72601 proved to be an effective anti-curare dose, it provided only minimal stimulation at muscarinic sites as evidenced by the limited effect on pulse rate and intestinal activity. The effects obtained with RX 72601 at these sites were comparable with the other two treatments.

We feel that RX 72601 could be safely used to reverse the effects of competitive neuromuscular blocking agents without the need for pre-medication with a post-ganglionic parasympathetic blocking drug such as atropine. Unlike its parent compound, RX 72601 did not produce adverse effects on the central nervous system. Together these results suggest that the compound may find a useful place in clinical practice.

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- cholinesterase inhibition caused by reversible inhibitors *in vivo*. *Biochemical Medicine*, in press.
- WRAY, S.R. & COWAN, A. (1973). Correlation between animals and clinical findings with a psychotomimetic anticholinesterase. *Neuropharmacology*, **12**, 397P-400P.

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