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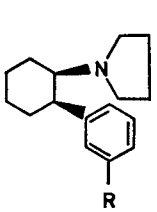
COMMUNICATIONS

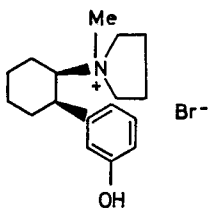
New inhibitor of acetylcholinesterase with preferential activity at nicotinic sites

In earlier work on substituted cyclohexylamines, some members of the *cis*-2-arylcyclohexylpyrrolidine series were found to possess anticholinesterase properties; one compound in particular, RX 67668 (I), aroused interest because it appeared to exert its action selectively at nicotinic sites (Doxey, Metcalf & others, 1972). Development of RX 67668 halted when tests in man showed the compound produced hallucinations and that it was less potent than animal tests had predicted (Gillett, Hedges & others, 1972).

By converting the tertiary amino group of (I) into a quaternary salt it was hoped to retain selectivity for nicotinic sites, to increase anticholinesterase activity and to reduce cns side-effects. In the *m*-hydroxy analogue, *cis*-2-(3-hydroxyphenyl)-1-pyrrolidinocyclohexane methobromide, RX 72601 (II), it is believed that these hopes have been realized. This compound is only in the preliminary stages of evaluation in man, but we report here brief details of the chemical, biochemical and pharmacological properties that led to its selection for further development.



I RX 67668 R=H
III R=OH



II RX 72601

RX 72601 was prepared by quaternization of the amine (III) obtained from 2-(3-methoxyphenyl-)-cyclohexanone (Kametani, Noguili & others, 1971) by reaction with pyrrolidine and formic acid, followed by demethylation with 48% hydrobromic acid. RX 72601 crystallized from ethyl acetate as a white amorphous solid with a melting point of 192–195°. It is freely soluble in water to yield stable aqueous solutions.

Anticholinesterase activity was measured *in vitro* by the method of Michel (1949) using acetylcholine as substrate, washed human erythrocytes as the source of acetylcholinesterase (acetylcholine acetyl hydrolase, EC 3.1.1.7) and human plasma as the source of cholinesterase (butyrylcholinesterase, acylcholine acylhydrolase, EC 3.1.1.8).

Table 1. *Concentrations of anticholinesterases necessary to produce 50% inhibition of enzymes.*

Drug	Molar concentration necessary to produce 50% inhibition of: Acetylcholinesterase	Butyrylcholinesterase
RX 72601	2×10^{-8}	2×10^{-7}
RX 67668	5×10^{-6}	$5{\cdot}3 \times 10^{-6}$
Neostigmine	5×10^{-7}	$1{\cdot}5 \times 10^{-7}$
Edrophonium	5×10^{-5}	5×10^{-4}

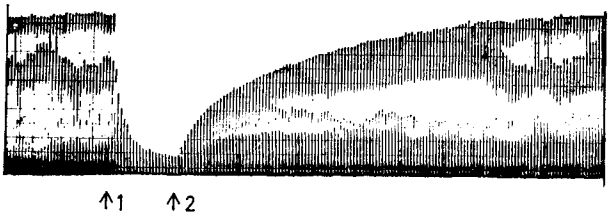


FIG. 1. Reversal of tubocurarine induced muscle blockade in the cat anterior tibialis preparation by RX 72601. 1. tubocurarine chloride 200 μg kg^{-1}. 2. RX 72601 10 μg kg^{-1}.

Acetylcholine was added immediately after mixing the inhibitor and the enzyme. Concentrations of anticholinesterases necessary to produce 50% inhibition of the enzymes were determined (Table 1).

RX 67668 and RX 72601 were added to diluted human erythrocytes in concentrations which produced 50% inhibition of the enzyme. On dilution, enzyme activity was immediately restored. Thus the compounds resemble the reversible anticholinesterases (e.g. edrophonium) rather than the oxydiaphoric anticholinesterases (e.g. neostigmine) in their mode of action. Despite this, the duration of action *in vivo* for RX 72601 was comparable to that of neostigmine.

Inhibitors of acetylcholinesterase are used therapeutically in cases where there is an apparent deficiency of acetylcholine released from cholinergic nerve endings (e.g. myasthenia gravis) or where a competitive acetylcholine antagonist has been used (e.g. reversal of competitive muscle relaxants). Pharmacological experiments using either the rat or cat anterior tibialis preparations demonstrated that RX 72601 (3–30 μg kg^{-1}, i.v.) was able to reverse the competitive muscle blockade induced by tubocurarine (Fig. 1). Animals treated with RX 72601, unlike those treated with other anticholinesterases, exhibited only minimal signs of concurrent muscarinic stimulation (e.g. lowering of blood pressure, increased gut motility, bradycardia, salivation). Rats and dogs dosed daily for one month with RX 72601 at doses up to 100 μg kg^{-1}, i.v. showed no toxic effects.

The preceding results have been confirmed in volunteers where RX 72601 proved to be an effective antagonist of tubocurarine at a dose (10 μg kg^{-1}) which produced only minimal concurrent muscarinic stimulation.

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