Dual mechanism of the antidotal action of atropine-like drugs in poisoning by organophosphorus anticholinesterases

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Atropine has long been used in the treatment (Wills, 1964) of poisoning by anticholinesterases on the simple basis that it blocks the effects of excess of acetylcholine at muscarinic receptors in the central nervous system (cns) and in the periphery (pns). The potential usage of synthetic organophosphorus compounds as chemical warfare agents (Heath, 1961) and the increasing use of organophosphorus compounds as insecticides (Eto, 1974), make poisoning by anticholinesterases a serious problem (Namba, 1971) and many attempts have been made (Coleman, Little & Bannard, 1962, 1963; Wills, 1964; Madill, Stewart & Savoie, 1967; Brimblecombe, Green & others, 1970) to improve therapeutic procedures, most of which involve atropine–oxime mixtures, by replacing atropine by other antimuscarinic drugs. In these investigations no clear relation was demonstrated between the protection afforded by atropine-like drugs against poisoning by organophosphorus anticholinesterases and their antimuscarinic activity in the pns and cns. Meaningful discussion as to whether or not there is a relation between antimuscarinic activity and protective ability, however, has been restricted by a lack of information about the relative importance in therapy of the effects of antimuscarinic drugs in the cns and pns and also by a lack of information about the relative antimuscarinic activities of such drugs in the cns and pns. Recent results (Brimblecombe, Green & others, 1971; Inch, Green & Thompson, 1973; Burgen, Hiley & Young, 1974) clearly demonstrate that antimuscarinic drugs act by a similar mechanism in both the cns and pns and that antimuscarinic activities may be determined separately in the pns and cns. This has prompted a new comparison of the antimuscarinic and therapeutic effects of atropine-like drugs. We now report results which show that, only for protection against low doses of organophosphorus anticholinesterases, does any relation exist between the antimuscarinic and therapeutic activities of atropine-like drugs. Further, we show that some atropine-like drugs have anticonvulsant action which may contribute to their ability to counteract the effects of poisoning by high doses of anticholinesterases.

The central and peripheral antimuscarinic activities of atropine and 5 related drugs (see Table 1) in mice, together with a measure of their abilities, when used with an oxime, to protect rats against poisoning by isopropyl methylphosphonofluoridate (Sarin) are given in Table 1. (Rats are more suitable than mice for measuring the protection afforded by atropine-like drugs in combination with oximes since greater degrees of protection in rats are possible allowing differences in therapeutic combinations to be more apparent. Mice, rather than rats, were used for quantitative measurements of antimuscarinic activity using oxotremorine since quantitative measurements of tremors in rats is difficult. The use of rats in one test and mice in the other is unlikely to lead to serious error since it has been shown that the muscarinic receptors in each species are similar (Burgen & others, 1974). Relations between protection and the administered dose of the atropine-like drugs are summarized in Fig. 1. All the drugs were superior to atropine in protecting against Sarin poisoning, with II (G3063) and III (PMCG) being the most effective. Although *R*(—)V was a much more potent antimuscarinic drug than its enantiomer, *S*(+)V, in both the pns and cns, there was no difference in the protection that the enantiomers afforded against poisoning by Sarin, except at lower doses of Sarin (e.g. up to *ca* 5 LD50's) when for all six atropine-like drugs there does appear to be a relation between central antimuscarinic activity and protection.

Differences between the antimuscarinic and therapeutic actions of atropine-like drugs is further exemplified by a comparison of the time course of the antimuscarinic and therapeutic actions of the enantiomers of V. The highly stereoselective differences in the antimuscarinic time—activity profiles of *R*(—)V and

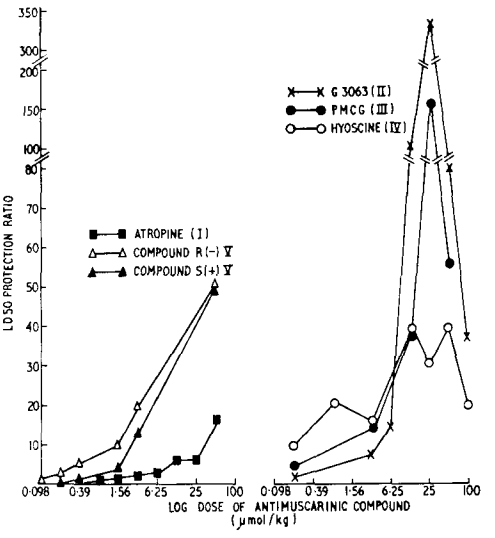

FIG. 1. Protection afforded in rats by various atropine-like compounds, in combination with 140 μmol kg^{-1} P2S, given i.m. 15 min before poisoning with s.c. Sarin.

* Correspondence.

Table 1. *Protection, anticonvulsant and antimuscarinic activity of atropine-like drugs.*

Drug	Protection against Sarin[a] poisoning		Antimuscarinic activity[b] (ED50 μ mol kg^{-1}, i.p.)		Anticonvulsant activity[c] (ED50 μmol kg^{-1}, i.m.)		
	MED (μmol kg^{-1} i.m.)	LD50 P/R (24 h mortality)	Central Antagonism of tremors	Peripheral OTM-induced-salivation	Maximal electro-shock	Nicotine	Leptazol
I	50	16 (11–23)	16·2 (10·0–26·6)	0·44 (0·3–0·66)	>800	(d)	>800
II	25	337 (130–873)	4·7 (1·9–11·3)	5·5 (3·4–8·6)	7·3 (2·2–14·1)	14·5 (7·8–29·4)	>400
III	25	158 (59–427)	3·5 (2·2–5·7)	1·7 (0·4–7·1)	4·3 (2·7–14·9)	27·7 (17·1–45·0)	>400
IV	25	44 (23–85)	1·1 (0·6–2·5)	0·05 (0·02–0·08)	19·2 (9·3–34·8)	(d)	>400
R(−)V	50	51 (26–172)	0·56 (0·3–1·3)	0·18 (0·04–0·31)	10·8 (4·8–21·8)	17·2 (iv.) (10·6–29·5)	>100
S(+)V	50	50 (23–190)	9·42 (5·2–16·6)	7·75 (3·8–10·2)	14·6 (5·7–50·0)	12·7 (iv.) (7·7–21·4)	>100

95% confidence limits in brackets. OTM = oxotremorine. MED = max. effective dose. P/R = protection ratio.

Compounds: I, atropine sulphate; II, G3063 (*N*-methyl piperidin-4-yl-phenylcyclopentane-carboxylate hydrochloride), (Coleman & others, 1962, 1963); III, PMCG [(*N*-ethyl pyrollidine-2)methyl-2-cyclopentyl-2-hydroxy-2-phenylacetate hydrochloride], (Brimblecombe & others, 1970); IV, hyoscine hydrobromide; *R*(−)V, *N*-methyl-piperidin-4-yl(*R*-)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (Brimblecombe & others, 1971); *S*(+)V, *N*-methyl-piperidin-4-yl(*S*+)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (Brimblecombe & others, 1971).

(a) Rats given atropine-like drugs and 140 μmol kg^{-1} P2S (*N*-methylpyridinium-2-aldoxime methane sulphonate) intramuscularly (i.m.) 15 min before subcutaneous (s.c.) injection of Sarin (Brimblecombe & others, 1970). LD50 protection ratio = LD50 of Sarin in treated animals/LD50 of Sarin in untreated animals.

(b) Previously published results (Brimblecombe & others, 1970, 1971; Inch & others, 1973). ED50 values obtained in mice when oxotremorine (0·49 μmol kg^{-1}) was given intravenously (i.v.) 15 min before intraperitoneal (i.p.) injection of atropine-like compound.

(c) Rats given atropine-like drug i.m. 15 min before testing for antagonism of convulsions produced by: (i) electric shock (100 mA strength, 0·2 s duration, frequency of 100 Hz with 1 ms pulse width) applied across the ears. (ii) i.v. injection of 2 μmol kg^{-1} nicotine hydrogen tartrate. (iii) i.m. injection of 506 μ mol kg^{-1} leptazol.

(d) An estimate of the ED50, with 95% confidence limits, was not calculable due to an insignificant probit slope. The dose to produce antagonism of convulsions in 50% of animals was *ca* 12·5 μmol kg^{-1} but a dose of 200 μmol kg^{-1} antagonized convulsions in <80% animals.

S(+)V in mydriasis experiments (Brimblecombe & others, 1971), on salivation and tremors (Inch & others, 1973) and on elevation of eeg arousal threshold in cats (Green, 1973) are most conveniently illustrated by the mydriasis results in Fig. 2. The *R*(—)V, in addition to being more potent, has a far more protracted effect. For comparison, the therapeutic time—activity profiles in Fig. 3A show that at equal doses the enantiomers of V, show no overall stereoselectivity and have similar time—activity profiles which do not relate to the antimuscarinic action except perhaps after 240 min. After this time *R*(—)V still protects against 5 LD50's of Sarin, whereas *S*(+)V does not, the differences being statistically significant ($P < 0.05$). Also the protection afforded by 0·25 μmol kg^{-1} of *R*(—)V was more protracted than that afforded by 10 μmol kg^{-1} of *S*(+)V. These results again indicate that only for protection against low doses of Sarin is there a relation between protective action and antimuscarinic action.

The above results are consistent with the notion that in addition to their antimuscarinic properties, atropine-like drugs provide protection against poisoning by Sarin by at least one additional mechanism. Observations that convulsant seizures caused by anticholinesterases are reversed by anticonvulsant drugs (Rump, Grudzinska & Edelwejn, 1973) prompted a survey of the anticonvulsant activities of the drugs listed in Table 1. The test procedures used for assessing anticonvulsant activity in rats were antagonism of convulsive activity produced by maximal electroshock (MES) and blockade of convulsions produced by nicotine and leptazol (pentylenetetrazol). The results are included in the Table.

None of the atropine-like drugs blocked the convulsions produced by leptazol. In the MES test atropine did not possess anticonvulsant activity whereas the other compounds that were superior to atropine in the protection experiments were all active. All the compounds were effective in blocking convulsions produced by nicotine. However neither for the MES test nor for the nicotine test was it possible to relate anticonvulsant activity quantitatively to protection.

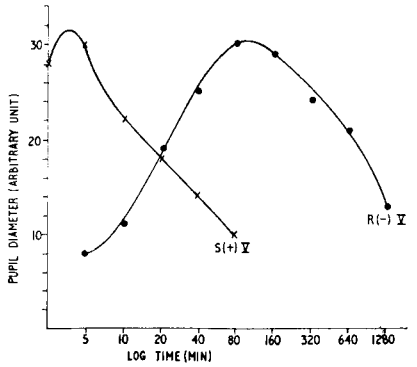

FIG. 2. Potency and duration of mydriatic action of compounds *R*(−)V (0·1 μmol kg^{-1}) and *S*(+)V (2 μmol kg^{-1}) in mice.

It was possible to relate time—activity profiles for protection and anticonvulsant time—activity profiles (Fig. 3). The anticonvulsant time—activity profiles (Fig. 3B) for *R*(—)V and *S*(+)V in the MES and nicotine tests showed no stereoselectivity and above the 5 LD50 protection level were similar to their therapeutic time—activity profiles. The dose of 0·25 μmol kg^{-1} *R*(—)V, which had insignificant anticonvulsant but significant antimuscarinic activity, protected at, but not above, the 5 LD50 level.

The above results are consistent with the hypothesis that the protection afforded by atropine-like drugs against low dosages of organophosphorus anticholinesterases is related to their antimuscarinic activity whereas the anticonvulsant properties of atropine-like drugs become important for protection against higher dosages of anticholinesterases. Thus in man, where only lower doses of antimuscarinic drugs are usually administered, it will be the antimuscarinic properties that are most important. At present there is no direct evidence to show that the convulsive mechanisms which are operative in the anticonvulsant tests on which this hypothesis is partly based are similar to the convulsive mechanisms which result from poisoning by anti-

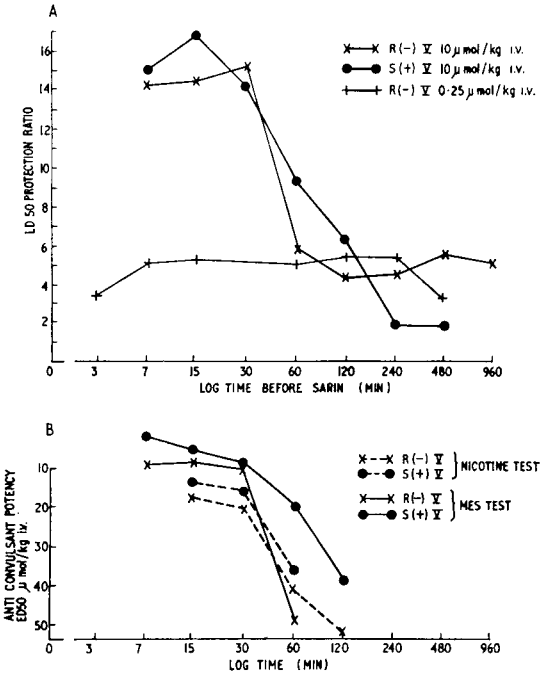

FIG. 3. Protection afforded by, and anticonvulsant activity of compounds *R*(−)V and *S*(+)V with time in rats. In all experiments, *R*(−)V and *S*(+)V were given by the i.v. route and in the protection experiments the animals were given 140 μmol kg^{-1}, i.m. P2S 15 min before poisoning with s.c. Sarin.

cholinesterases, (indeed the nicotinic actions of acetylcholine may be important) and it is fully appreciated that such direct evidence would strengthen this hypothesis. However, additional evidence has recently been provided (Lipp, 1972; Johnson & Wilcox 1975) by results which show that diazepam, a powerful anticonvulsant in the three tests described here (Swinyard & Castellion, 1966), when used in conjunction with atropine—oxime mixtures, affords considerable protection against anticholinesterases.

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