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Anticholinergic Compounds as Adjuncts to Atropine in Preventing Lethality by Sarin in the Rabbit

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Studies of a wide variety of anticholinergic compounds as prophylactic agents in experimental poisoning by isopropyl methylphosphonofluoridate (Sarin) in the rat, rabbit, dog and monkey have led to certain general conclusions about the molecular configuration necessary for maximal activity in this sort of test.[1] A smaller number of anticholinergic compounds has been tested for activity as adjuncts to atropine in prevention of fatal poisoning by Sarin in the rabbit. The purpose of this paper is to present the available information and to examine the possibility of reaching some general conclusion about the molecular configuration requisite for adjunctive effectiveness. It has been pointed out already[2] that adjuncts must exert some action not possessed by atropine itself; this may mean that in turn they must possess some structural feature not present in atropine.

Method

Prophylactic efficacy was assessed by injecting the test preparation intravenously into one marginal ear vein of a rabbit 2 min before the injection of a test dose of Sarin into the marginal ear vein of the other ear. Groups of 6 rabbits were used for each experimental condition. Doses of Sarin starting at 30 μg/kg and increasing by increments of 30 μg/kg were given until a dose killing at least 3 rabbits in a group of 6 had been administered. The criteria for concluding that a given substance is adjunctive to atropine were that a mixture of the drug and 2 mg/kg of atropine sulphate either

prevented death in 5 of 6 rabbits given 30 μg/kg of Sarin or prevented death in at least 1 of 6 rabbits given 60 μg/kg of Sarin. In this series of experiments, the LD_{50} dose for Sarin alone was $15 \pm 1 \cdot 2$ μg/kg; that for Sarin given after intravenous injection of 2 mg/kg of atropine sulphate was $17 \pm 5 \cdot 6$ μg/kg and that after 5 mg/kg of atropine sulphate was $19 \pm 2 \cdot 2$ μg/kg.

Results

The results are expressed qualitatively in Tables I through III where inactive and active compounds of somewhat related structures are shown side by side. The most active adjuncts in each Table are marked by asterisks. When the formulae of the 9 outstanding compounds from the Tables are examined for similarity, the only clearly evident relationships are that (1) either tertiary or quaternary amines may be effective, and (2) that in 8 of the 9 most active compounds there is a –C–O–C–C– linkage between the nitrogen atom and the bulk of the molecule. These two characteristics obviously do not aid greatly in the design of new and possibly more effective adjuncts to atropine in the prevention of poisoning by anticholinesterase compounds.

Discussion

A further look at the compounds in Table I discloses that there is a not entirely consistent tendency for the compounds having a free amino group to be more active than those having the nitrogen atom enclosed in a ring. In Table II, however, this tendency seems to be reversed. Perhaps it would be reasonable to say on the basis of these two tables that whenever the nitrogen atom is a component of a ring, the compound has a higher probability of being an adjunct to atropine in the prevention of death from an injection of Sarin when the nitrogen atom is quaternized. Table III suggests that this rule does not extend to those amines in which the bulk of the molecule is phenothiazine. Table III also makes the fact apparent that the partial analogues of the phenothiazine-based compounds, such as the two derivatives of xanthene and one of acridine in this Table, have no significant adjunctive activity with atropine despite the comparatively high activity

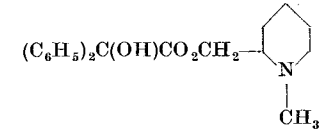
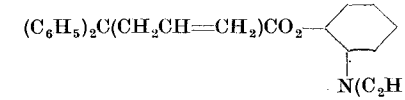
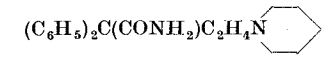
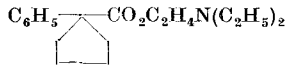
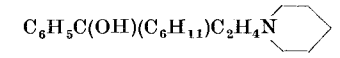
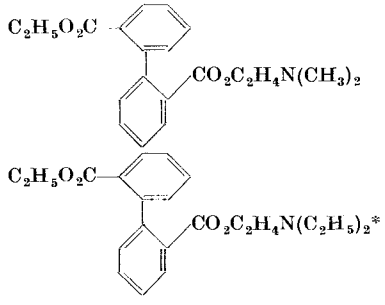
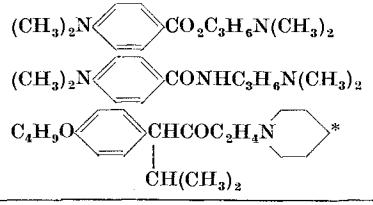
Active	Inactive
$(C_6H_5)_2C(OH)CO_2C_2H_4N(C_2H_5)_2$*	
$(C_6H_5)_2CHCO_2C_2H_4N(C_2H_5)_2$	
$(C_6H_5)_2C(CH_2CH{=}CH_2)CO_2C_2H_4N(C_2H_5)_2$	
$(C_6H_5)_2C(CH_2CH{=}CH_2)CO_2CH(CH_3)CH_2N(CH_3)_2$	$(C_6H_5)_2C[CH_2C(CH_3){=}CH_2]CO_2C_2H_4N(C_2H_5)_2$
	
	$(C_6H_5)_2CHNHCONHO_2CCH_3$
	
	
	$C_6H_5CO_2CH(CH_3)CH_2NHC_6H_{11}$ $C_6H_5CH_2O_2CN(C_6H_5)C_2H_4N(CH_3)_2$
	

Table II. Derivatives of quaternary amino alcohols as adjuncts to atropine in the prevention of lethal poisoning by Sarin in the rabbit

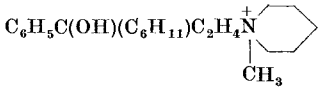
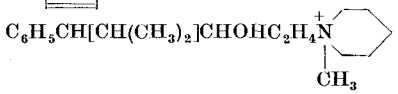
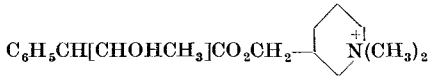
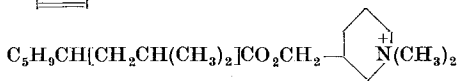
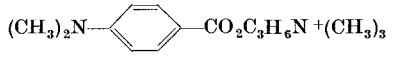
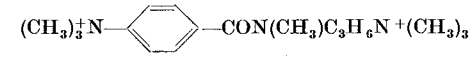
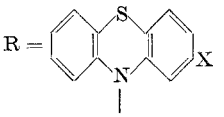
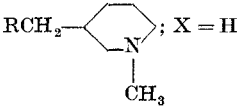
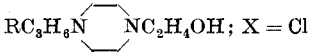
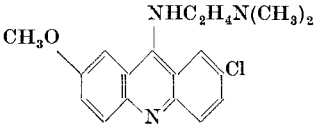
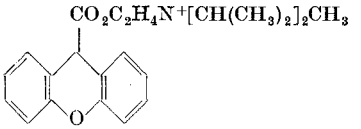
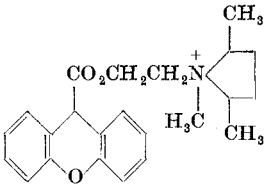
Active	Inactive
$(C_6H_5)_2CHCO_2C_2H_4N^+(C_2H_5)_2CH_3$*	
$C_6H_5C(OH)(C_6H_{11})CO_2C_2H_4N^+(CH_3)_3$*	
$C_6H_5C(OH)(C_6H_{11})CO_2C_2H_4N^+(C_2H_5)_2CH_3$	
$C_6H_5CH(C_6H_{11})CO_2C_2H_4N^+(C_2H_5)_2CH_3$	
	
$(C_6H_5)_2NCON(CH_3)C_2H_4N^+(C_2H_5)_2CH_3$	
$C_6H_5CH{=}C(C_6H_5)CONHC_2H_4N^+(C_2H_5)_3$	$C_6H_5CH{=}C(OC_6H_5)CONHC_2H_4N^+(C_2H_5)_2CH_3$
	
	
	$C_6H_5CH[CH(OH)CH_3]CO_2C_2H_4N^+(C_2H_5)_2CH_3$
$C_5H_9C(OH)CO_2C_2H_4N^+(C_2H_5)_3$	
	
	
	
$(C_4H_9)_2NCO_2CH(CH_3)CH_2N^+(CH_3)_2C_2H_5$	$(CH_3)_2NCO_2CH(CH_3)CH_2N^+(CH_3)_2C_2H_5$
$(C_4H_9)_2NCONHC_2H_4N^+(CH_3)_2C_2H_5$	
$(C_4H_9)_2NCSOC_2H_4N^+(CH_3)_5$*	$(C_2H_5)_2NCSOC_2H_4N(CH_3)_3$
N-Isopropylatropinium bromide	
N-Benzylatropinium chloride	

Table III. Derivatives of phenothiazine and analogous heterocycles as adjuncts to atropine in preventing lethal poisoning by Sarin in the rabbit



Active	Inactive
$RCH_2CH(CH_3)N(CH_3)_2$; X = H	$RCH_2CH(CH_3)N^+(CH_3)_2C_2H_4OH$; X = H
$RC_2H_4N(C_2H_5)_2$; X = H	
	
$RC_3H_6N(CH_3)_2$; X = Cl*	
	
$RCO_2C_2H_4N(C_2H_5)_2$; X = H*	
	
	
	

of most of the phenothiazine derivatives. The fact that the two xanthene derivatives are both quaternary amines may contribute to this finding, but the inactivity of the acridine derivative should not depend upon this particular factor. Provisionally, at least, we conclude that such heterocyclic radicals as xanthene and

acridine may not be used to replace phenothiazine in anticholinergic amines and still leave intact an adjunctive effect when the resultant amines are given along with atropine to animals before they are poisoned by injection of Sarin.

To return to Table I again: it is evident by inspection of the two lists of compounds that differences in the bulk of the molecule cannot explain the difference in properties. Furthermore, the amine portions of the molecule in the right-hand and the left-hand portions of Table I are identical in many cases, so that variation in this part of the structure can hardly be the factor determining whether the compound does or does not possess adjunctive potency. This leaves, then, the central portion of the molecule as the apparently critical site. But here again there are no obvious differences. One is thus forced to the conclusion that activity or inactivity is determined by a complex of factors. For example, from the first pair of compounds in Table I one could say that inactivity may depend upon the inclusion of the nitrogen atom in a ring structure. In Table II, however, the twelfth compound on the 'active' side possesses exactly the same structure for the amino alcohol portion, but a different one for the bulk of the molecule, as the first inactive compound of Table I. One must conclude, therefore, that the inactivity conferred by enclosing the nitrogen atom within a ring structure in the first case is modified or antagonized by the change in structure of the acidic portion of the molecule in the second case.

What has been said above leads to the general conclusion that each family of compounds (that is, compounds either with identical acidic, or bulk, and connecting portions but with various amino alcohol moieties or with identical amino alcohol and connecting portions but with differing bulk groups) must be considered as a rule to itself, so that no general rules for making new structures capable of being adjuncts to atropine and still lying within the general field of anticholinergic compounds seem possible.

Conclusions

1. Adjuncts to atropine in preventing lethal poisoning by Sarin in the rabbit have been found among ester-linked, amide-linked and carbon-linked derivatives of tertiary and quaternary amino alcohols.

2. Among the 51 compounds so studied, no general rule for adjunctive structures is apparent. Each family of related compounds seems to follow a rule of its own.

3. There is a tendency for ester-linked compounds to be more actively adjunctive than those with other types of linkages.

4. Xanthene and acridine heterocycles, although analogues of the phenothiazine heterocycle, probably can not substitute for the latter in effective adjuncts to atropine.

Summary. Fifty-one compounds have been tested for activity as adjuncts to atropine in the prevention of fatal poisoning by Sarin in the rabbit. The existence of adjunctive action was assumed if a mixture of any drug and 2 mg/kg of atropine sulphate prevented death in five of six rabbits receiving 30 μg/kg of Sarin, or one of six rabbits given 60 μg/kg of Sarin. In this series of experiments, all rabbits given 30 μg/kg of Sarin without anticholinergic prophylaxis died; the 2 mg/kg dose of atropine sulphate saved a mean of 2 rabbits out of the group of 6 from death after 30 μg/kg of Sarin, but saved none of the group after 60 μg/kg of Sarin. The 5 mg/kg dose of atropine sulphate also saved 2 rabbits in the group of 6 from death after 30 μg/kg of Sarin and saved an occasional animal from death after 60 μg/kg. The drugs which were tested fall into three groups: derivatives of tertiary and quaternary amino alcohols and of phenothiazine or similar heterocycles. An examination of the most active compounds yields some indication that a –C–O–C–C– linkage between the nitrogen atom and the bulk portion of the molecule confers adjunctive power. No general rule for designing new anticholinergic molecules capable of being adjuncts to atropine in the prevention of death from intoxication by organophosphorus anticholinesterases seems possible.

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