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Neuromuscular blocking agents. Replacement of quaternary ammonium groups in bis-onium compounds by amidinium, guanidinium, thiouronium, sulphonium and sulphoxonium groups

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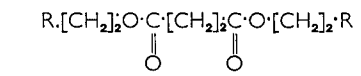
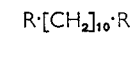
The quaternary ammonium groups of suxamethonium have been replaced by amidinium, guanidinium and thiouronium groups. The guanidine compound exhibited competitive neuromuscular activity of a low order of potency; the other compounds were ineffective. Replacement of the quaternary ammonium groups in decamethonium by ethylmethylsulphonium or dimethylsulphoxonium groups gave less potent, depolarizing agents.

The primary chemical requirement for a neuromuscular blocking agent, according to Stenlake (1963), is a strongly basic centre capable of permanent existence as a positively charged ion. While in most active compounds this basic centre is a quaternary ammonium group, blocking properties have been demonstrated for other onium compounds such as sulphonium, phosphonium, arsonium and stibonium compounds, certain tertiary amines, and guanidine derivatives.

As part of a program aimed at the development of a short-acting, competitive, blocking drug, we have investigated the effect on the nature and duration of activity of suxamethonium (Ia) and decamethonium (IIa) brought about by replacement of the quaternary ammonium groups by other strongly basic groups.



(I)



(II)

a: R = $\overset{+}{N}Me_3, X^-$

b: R = $-C(:NH)NH_2, HCl$

c: R = $-NH\cdot C(:NH)NH_2, HCl$

d: R = $-S\cdot C(:NH)NH_2, HBr$

e: R = $-S\cdot C(:NMe)NMe_2, HBr$

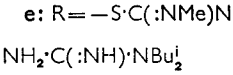
f: R = $-S(:O)Me$

g: R = $-S(:O)Et$

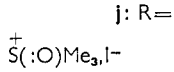
h: R = $-\overset{+}{S}(:O)Me_2, Ts^-$

i: R = $-\overset{+}{S}Me_2, I^-$

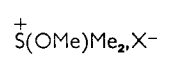
j: R = $-\overset{+}{S}MeEt, I^-$



(III)



(IV)



(V)

COMPOUNDS STUDIED

Amidine, guanidine and isothiourea derivatives

Although salts of the di-amidine analogue IIb of decamethonium are well known, none appears to have been tested for neuromuscular blocking activity. The di-guanidine analogue IIc, however, has been shown to produce relaxation in the frog

sciatic-sartorius muscle preparation which was antagonized by potassium chloride and neostigmine (Ozawa, Fukuda & Goto, 1962, Ozawa, Gomi & Watanabe, 1964) Certain *NN*-disubstituted guanidines have been found to cause flaccid paralysis in fowls, cats, mice and rabbits which was not antagonized by cholinesterase inhibitors (Barzaghi, Mantegazza & Riva, 1965). One of the most active compounds was *NN*-di-isobutylguanidine (III), which was more active than gallamine triethiodide in mice, but less active in the rabbit.

The di-isothiourea analogues IId and IIe of decamethonium produced about 1/30 the activity of tubocurarine in the rabbit head-drop test and 1/30 and 1/100 the activity of tubocurarine respectively in the frog rectus abdominis muscle preparation (Cheymol, Chabrier & others, 1953).

Preparation of the di-amidine (Ib), di-guanidine (Ic) and di-isothiourea (Id) analogues of suxamethonium was therefore undertaken.

Sulphonium and sulphoxonium salts

The di-sulphonium compound IIi has been found to be approximately one-third as active as gallamine triethiodide in the rabbit head-drop test for neuromuscular blocking activity (Walker, 1950).

Major & Hess (1958) demonstrated in dogs that trimethylsulphoxonium iodide (IV) showed some of the muscarinic and nicotinic properties of acetylcholine, but the compound was only 1/1000 to 1/10,000 as active as acetylcholine in lowering the blood pressure and its nicotinic activity was less than that of acetylcholine.

The possibility that analogous di-sulphoxonium salts such as IIh might exhibit short-acting neuromuscular blocking activity was suggested by our colleague Dr. R. Slack. That salt was therefore examined and the intermediate compound used in its preparation was also used to prepare a higher homologue (IIj) of the di-sulphonium salt (IIi).

CHEMISTRY

Condensation of 2-amidinoethanol or 2-guanidinoethanol with succinyl chloride at 130° gave respectively di-2-amidinoethyl succinate dihydrochloride (Ib) or di-2-guanidinoethyl succinate dihydrochloride (Ic) in low yield. Di-2-amidinothioethyl succinate dihydrobromide (Id) was obtained in moderate yield from di-2-bromoethyl succinate and thiourea in boiling ethanol. For all three compounds the structures assigned were supported by the presence of ester and amidine bands in the infrared spectra.

Decamethylenebis(ethylmethylsulphonium iodide) (IIj) was obtained directly from 1,10-di(methylthio)decane and ethyl iodide at room temperature. Attempts to ethylate 1,10-di(ethylthio)decane with ethyl iodide to give a higher homologue were not successful.

Kuhn (1957) and Kuhn & Trischmann (1958) found that dimethyl sulphoxide and methyl iodide react to give the stable sulphoxonium salt IV. However, Smith & Winstein (1958) showed that the action of dimethyl sulphoxide on reactive halides or esters can give rise to two different types of derivative, the *S*-alkyl compound such as IV or the *O*-alkyl compound such as V. The unstable *O*-alkyl derivatives tend to isomerize in solution to the stable *S*-alkyl adducts, which are the normal reaction products from a sulphoxide with methyl iodide or an alkyl toluenesulphonate (cf. also Natus & Goethals, 1965).

1,10-Di(methylsulphinyl)decane (IIf) has been described by Jerchel, Dippelhofer & Renner (1954), who obtained it by oxidation of 1,10-di(methylthio)decane with hydrogen peroxide in acetic acid. It was conveniently prepared under conditions where sulphone formation is negligible by periodate oxidation of the sulphide, the same method also being employed for the preparation of the corresponding ethyl compound IIg.

Methylation of 1,10-di(methylsulphinyl)decane was achieved in low yield by prolonged heating at 120° with methyl toluene-*p*-sulphonate to give the di-sulphoxonium tosylate IIh. Attempted ethylation of either IIf or its ethyl homologue IIg with ethyl toluene-*p*-sulphonate or ethyl 2,4-dinitrobenzenesulphonate was not successful.

PHARMACOLOGICAL METHODS

Cats were anaesthetized with ether followed by chloralose (80–100 mg/kg) intravenously. The tibialis muscle of one leg was prepared for recording responses to supramaximal stimulation of the sciatic nerve as described by Bamford, Biggs & others (1967). Drugs were injected via a polythene cannula inserted into a femoral vein. Dose response lines were constructed for each compound where possible. Mechanism of action was investigated (i) by attempting to reverse neuromuscular blockade with edrophonium (0·5 mg/kg) intravenously and (ii) by intravenous injection of each compound into day-old chicks.

The intravenous LD50 of each compound was determined in mice, and mortalities were observed for 24 h.

The drugs used were: α-chloralose (Koch–Light Labs.), decamethonium iodide, edrophonium chloride ("Tensilon", Roche Products Ltd.), gallamine triethiodide ("Flaxedil", May & Baker Ltd.) and suxamethonium bromide ("Brevidil M", May & Baker Ltd.). All doses refer to the appropriate salt of each compound.

Table 1. *Analogues of suxamethonium*

$R{\cdot}[CH_2]_2{\cdot}O{\cdot}CO{\cdot}[CH_2]_2{\cdot}CO{\cdot}O{\cdot}]CH_2]_2{\cdot}R$

Compound	R	LD50 in mice mg/kg intravenously	Blocking dose on cat sciatic nerve-tibialis muscle preparation ED50 mg/kg intravenously	Duration of action of ED50 in min	Mechanism of action
Ia Suxamethonium)	$-\overset{+}{N}Me_3$, Br^-	0·71	0·032	4·5	Depolarizing
Ib	$-C(:NH){\cdot}NH_2,HCl$	*ca* 100	>40·0	—	—
Ic	$-NH{\cdot}C(:NH){\cdot}NH_2,HCl$	*ca* 75	10% at 30·0	—	Competitive
Id	$-S{\cdot}C(:NH){\cdot}NH_2,HBr$	*ca* 100	>40·0	—	—
Gallamine triethiodide	—	48	0·68	18	Competitive

RESULTS AND DISCUSSION

Di-amidine, -guanidine and -isothiourea analogues of suxamethonium (Table 1)

Only the di-guanidine analogue Ic showed any neuromuscular blocking activity at the dose levels used, but it was much less active than either gallamine triethiodide or suxamethonium.

This observation suggests that potency in this series is related to basic strength. The following values of pK_a have been reported (Perrin, 1965): guanidine, 13·6;

N-methylguanidine, 13·4; amidinomethane (acetamidine), 12·1, 12·4; *S*-methylisothiourea, 9·78, 9·81. Although a strong base, the diguanidine analogue Ic would be weaker than suxamethonium, which as a quaternary ammonium salt must necessarily be completely ionized (Albert, 1968).

Table 2. *Analogues of decamethonium*

$R\cdot[CH_2]_{10}\cdot R$

Compound	R	LD50 in mice mg/kg intravenously	Blocking dose on cat sciatic nerve-tibialis muscle preparation ED50 mg/kg intravenously	Duration of action of ED50 in min	Mechanism of action
IIa (Decamethonium)	$-\overset{+}{N}Me_3,I^-$		0·008	18	Depolarizing
IIf	-S(:O)Me	37·5	> 7·0	—	—
IIg	-S(:O)·Et	>30	>10·0	—	—
IIh	$-\overset{+}{S}(:O)\cdot Me_2,Ts^-$*	*ca* 10	1·1	12	Depolarizing
IIj	$-\overset{+}{S}(Me)\cdot Et,I^-$	12·8	0·55	16	Depolarizing

* Ts = toluene-*p*-sulphonate.

Sulphonium and sulphoxonium analogues of decamethonium (Table 2)

The uncharged sulphoxides IIf and IIg were devoid of activity at the dose levels used, but both the disulphoxonium salt IIh and disulphonium salt IIj showed activity on the cat nerve-muscle preparation. Both compounds were much less effective than decamethonium, but had potencies of the order of that of gallamine triethiodide. Both compounds had a depolarizing mechanism of action. A typical experiment with compound IIj is shown in Fig. 1.

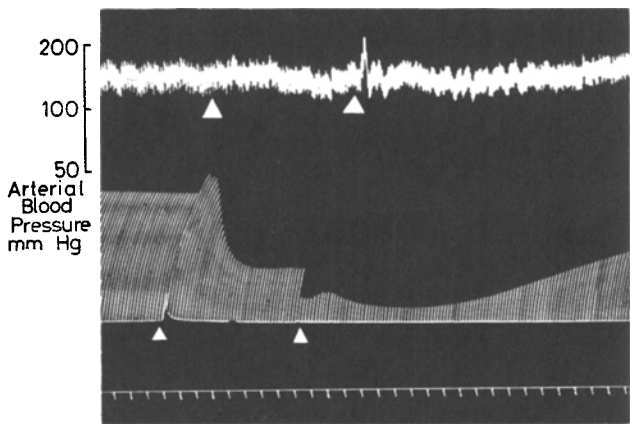


FIG. 1. Cat sciatic nerve–tibialis muscle preparation. At the first mark compound IIj (0·375 mg/kg, intravenously) was injected. This caused twitch potentiation followed by a neuromuscular blockade which was potentiated by injection of edrophonium (0·5 mg/kg, intravenously) at the second mark. Time scale in min.

It was noteworthy that the di(ethylmethylsulphonium) salt IIj was approximately equipotent with gallamine triethiodide in the cat (see Table 1), for in the rabbit the di(dimethylsulphonium) salt IIi had only approximately one-third the activity of gallamine triethiodide (Walker, 1950). Replacement of one methyl group by an ethyl group therefore seems to have increased neuromuscular blocking activity whereas in decamethonium itself this change decreases potency (Barlow, 1964).

EXPERIMENTAL

Di-2-amidinothioethyl succinate dihydrobromide. A mixture of di-2-bromoethyl succinate (Fusco, Palazzo & others, 1949) (8·3 g, 0·025 mol) and thiourea (3·8 g, 0·05 mol) was heated under reflux for 5 h in ethanol (50 ml). Ethyl acetate was added to produce incipient turbidity, and the mixture was then chilled. The separated material (6·1 g, m.p. 164–167°) was crystallized from ethanol-ether to give the *di-isothiourea* derivative (5·3 g, 44%) as colourless needles, m.p. 166–167° (Found: N, 11·6; Br, 32·7. $C_{10}H_{18}N_4O_4S_2$,2HBr requires N, 11·6; Br, 33·0%).

Di-2-guanidinoethyl succinate dihydrochloride. 2-Guanidinoethanol hydrochloride (Beatty & Magrath, 1960) (1·4 g, 0·01 mol) was mixed with succinyl chloride (2 ml, 0·005 mol) whilst cooling in ice, and the mixture was then heated in an oil bath at 130° until a clear viscous solution formed. This was shaken with a mixture of acetone and ether, and the solvents were decanted from a pasty residue, which became solid on treatment with ethanol, and was filtered off and washed with ether. Recrystallization of the product (0·61 g, m.p. 167–170°) from ethanol-ether gave the *di-guanidine hydrochloride* (0·48 g, 14%) as a colourless powder, m.p. 168–170° (Found: N, 22·9; Cl, 19·8. $C_{10}H_{20}N_6O_4$,2HCl requires N, 23·3; Cl, 19·6%).

Similarly 2-amidinoethanol (Price & Zomlefer, 1949) (4·15 g, 0·033 mol) and succinyl chloride (2·6 g) gave *di-2-amidinoethyl succinate dihydrochloride* (0·65 g, 12%) as a colourless powder, m.p. 153–154° (Found: C, 36·5; H, 6·4; Cl, 21·6. $C_{10}H_{18}N_4O_4$,2HCl requires C, 36·3; H, 6·1; Cl, 21·4%).

Decamethylenebis(ethylmethylsulphonium iodide). A mixture of 1,10-di(methylthio)decane (Walker, 1950) (0·234 g, 0·001 mol) and ethyl iodide (3 ml) was allowed to stand at room temperature for 3 days. Anhydrous ether was added, and the oil thus formed slowly solidified. Crystallization of the solid from acetone gave the *salt* (0·33 g, 60%) as colourless needles, m.p. 110–111° (Found: S, 11·5; I, 46·1. $C_{16}H_{36}I_2S_2$ requires S, 11·7; I, 46·5%).

1,10-Di(ethylthio)decane, prepared (61%) in an impure state by the method of Walker (1950), had b.p. 195–197°/15 mm. (Found: C, 64·9; H, 11·8; S, 23·6. Calc. for $C_{14}H_{30}S_2$: C, 64·1; H, 11·4; S, 24·4%), and was oxidized directly (see below).

1,10-*Di(methylsulphinyl)decane.* The procedure of Leonard & Johnson (1962) was employed. 1,10-Di(methylthio)decane (2·34 g, 0·01 mol) in methanol (100 ml) was added with stirring to sodium metaperiodate (42 ml of a solution made by dissolving 5·35 g in 50 ml water) (0·021 mol), and the mixture stirred vigorously at 0° for 12 h. The solution was filtered, and the residue remaining after removal of the methanol was extracted thrice with 50 ml portions of boiling chloroform. Removal of the chloroform gave a colourless solid (1·9 g, m.p. 108–110°), which was recrystallized from benzene to give the sulphoxide (1·6 g, 60%) as colourless leaflets, m.p. 110–112° (Found: C, 53·9; H, 9·6; S, 24·2. Calc. for $C_{12}H_{26}O_2S_2$: C, 54·1; H, 9·8; S, 24·1%). Jerchel & others (1954) give m.p. 119–120°.

Similarly 1,10-di(ethylthio)decane (2·62 g, 0·01 mol) gave 1,10-*di(ethylsulphinyl)decane* (2·3 g, 78%) as colourless needles from benzene, m.p. 108–109° (Found: C, 57·4; H, 10·3; S, 21·9. $C_{14}H_{30}O_2S_2$ requires C, 57·1; H, 10·3; S, 21·7%).

Decamethylene bis(dimethylsulphoxonium toluene-p-sulphonate). A mixture of 1,10-di(methylsulphinyl)decane (1·33 g, 0·005 mol) and methyl toluene-*p*-sulphonate (1·86 g, 0·01 mol) was heated in an oil bath at 120° for 96 h. The reaction mixture was triturated with acetone and filtered. The brown solid residue (0·09 g, m.p.

235–238°) was crystallized from ethanol-ether (charcoal) to give the salt (0·07 g, 2·2%) as a colourless powder, m.p. 238–240° (Found: C, 52·8; H, 7·2; S, 20·2 $C_{28}H_{46}O_8S_4$ requires C, 52·6; H, 7·3; S, 20·1%).

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