THE GANGLIONIC BLOCKING ACTIVITY OF A SERIES OF TERTIARY SULPHONIUM QUATERNARY AMMONIUM SALTS

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A number of polymethylene bis-tertiary sulphonium quaternary ammonium salts have been examined for their ganglionic blocking activity on the superior cervical ganglion of the cat. The effect of substituting different alkyl groups on the sulphur and nitrogen atoms and varying the length of the polymethylene chain has been investigated. Maximum activity was found in the tetramethylene di-ethyl sulphonium tri-ethyl ammonium salt, which was about three times as active as hexamethonium. With tri-, penta- and hexamethylene salts maximum activity occurs when there are a total of 13 to 15 carbon atoms in the molecule. It is concluded that the substitution of sulphur for nitrogen in the bis-quaternary ammonium salts does not necessarily lead to a reduction in relative potency. The importance of the groupings on the "onium" centres in producing ganglionic block is stressed.

The effect of substituting sulphur for nitrogen in polymethylene bis-quaternary ammonium salts has been investigated by several workers. Walker[1] has shown that replacing one nitrogen with sulphur in decamethonium led to a decrease in neuromuscular blocking activity. Muir and Lewis[2] confirmed this and demonstrated that the type of neuromuscular block produced by analogous sulphur-nitrogen compounds is unaffected. Barlow and Vane[3] synthesised a number of bis-alkyl tertiary sulphonium analogues of hexamethonium which proved to have less ganglionic blocking activity than their corresponding bis-quaternary ammonium salts. Wien[4] reported the replacement of nitrogen by sulphur in hexamethonium and its homologues to reduce activity. From these investigations it would appear that the replacement of nitrogen by sulphur in polymethylene bis-alkylammonium salts causes a decrease in relative activity.

We have investigated the ganglionic blocking activity of a series of straight chain polymethylene tertiary sulphonium quaternary ammonium salts synthesised by Doyle and Stove[5]. Among the compounds tested a number have been shown to possess ganglionic blocking activity in excess of hexamethonium.

Methods

The ganglionic blocking activity of compounds was assessed on the cat nictitating membrane preparation. The method was slightly modified from that described by Barlow and Vane[3]. Cats were anaesthetised with ether followed by intravenous chloralose-urethane mixture (0·7 per cent chloralose, 2·8 per cent urethane, 8·0 ml./kg.). A square wave stimulus of 1·0 millisecond duration and frequency of 14 shocks per second was

applied for 15 seconds every 3 minutes to the preganglionic fibres of the superior cervical ganglion. The voltage was adjusted to give just maximal contraction of the membrane. The nictitating membrane completely relaxed after each period of stimulation. The compounds were administered intravenously, into the femoral vein, 30 seconds before stimulation. The dose of hexamethonium bromide to give approximately 50 per cent inhibition of contraction (0·5 to 1·0 mg./kg.) was

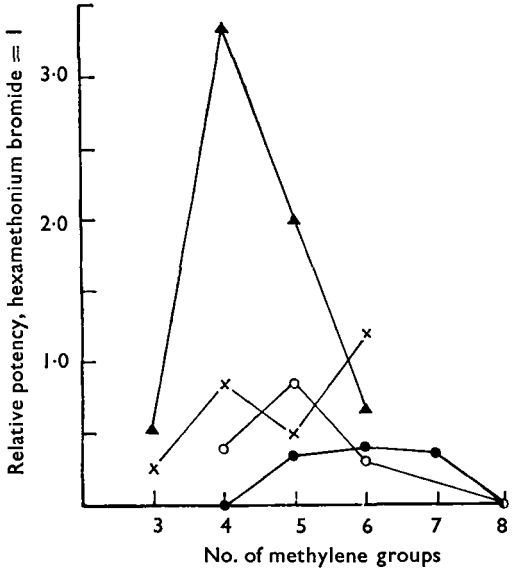

FIG. 1. Relationship between sympathetic ganglion blocking activity and chain length in a series of polymethylene bis-tertiary sulphonium quaternary ammonium salts.

General formula: $R_1R_2S^+(CH_2)_nN^+R_3R_4R_5$

	R_1	R_2	R_3	R_4	R_5
●—●	CH_3	CH_3	CH_3	CH_3	CH_3
○—○	CH_3	C_2H_5	CH_3	CH_3	C_2H_5
×—×	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5
▲—▲	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5

determined. Three doses of hexamethonium were then bracketed with two doses of the test compound and from the log-dose response lines obtained the relative potency of the compound was calculated.

The acute intravenous toxicities were determined in male mice weighing 18 to 22 g. and expressed as the LD50 in mg./kg.

RESULTS

Figure 1 illustrates the alteration in ganglionic blocking activity as a result of increasing the length of the polymethylene chain and substituting successively ethyl groups for methyl groups on the sulphur and nitrogen atoms. Compounds with methyl groups only on the sulphur and nitrogen have demonstrable ganglionic blocking activity, but this is always less

than hexamethonium. The pentamethylene, hexamethylene and heptamethylene compounds are equipotent, and have a relative ganglionic blocking activity of about 0·4 times hexamethonium. The tetramethylene and octamethylene compounds are inactive. When an ethyl group is substituted for a methyl group on both the sulphur and nitrogen atoms the relative activity within the series increases; the tetramethylene derivative has an activity of 0·4 times hexamethonium, while maximum activity occurs with the pentamethylene derivative which has a potency of 0·85

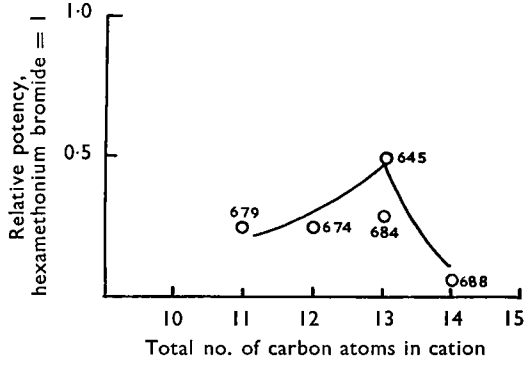

FIG. 2. Relationship between sympathetic ganglion blocking activity and total number of carbon atoms in the cation of a series of trimethylene bis-tertiary sulphonium quaternary ammonium salts.

General formula: $R_1R_2S^+(CH_2)_3N^+R_3R_4R_5$

Code No.	R_1	R_2	R_3	R_4	R_5
679	C_2H_5	CH_3	C_2H_5	C_2H_5	CH_3
645	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5
674	CH_3	CH_3	C_3H_7n	C_3H_7n	CH_3
684	CH_3	C_2H_5	C_3H_7n	C_3H_7n	CH_3
688	CH_3	C_3H_7n	C_3H_7n	C_3H_7n	CH_3

times hexamethonium. As the chain length is increased activity falls away rapidly and no significant ganglionic blocking activity is detectable in the octamethylene salt.

When a further methyl group is replaced by an ethyl group on the nitrogen atom, maximum activity occurs with the hexamethylene compound—the ethylmethylsulphonium di-ethylmethylammonium salt. This compound was slightly more active than hexamethonium. The higher members of this series, however, have not been investigated and therefore a complete picture of the variation in activity within the series has not been obtained.

Replacement of all the methyl groups by ethyl groups markedly alters the activity. There is a sharp increase in activity between the compounds having 3 or 4 carbon atoms in the chain. While the former is only 0·5 times as active as hexamethonium the latter is 3·3 times as active. The pentamethylene derivative is less active than the tetramethylene derivative, but is still twice as active as hexamethonium. The hexamethylene derivative has a relative potency of only 0·6.

TABLE I

SYMPATHETIC GANGLION BLOCKING ACTIVITY AND INTRAVENOUS TOXICITIES OF A SERIES OF $R_1R_2.S^+(CH_2)_4N^+R_3R_4R_5$ SALTS

Code number	Substituent groups					Toxicity in mice. LD50 mg./kg. I.V.	Ganglionic block hexamethonium bromide = 1·0
	R_1	R_2	R_3	R_4	R_5		
BRL 443	CH_3	CH_3	CH_3	CH_3	CH_3	194	<0·02
534	CH_3	C_2H_5	CH_3	CH_3	CH_3	170	0·2
519	CH_3	C_2H_5	CH_3	CH_3	C_2H_5	119	0·4
524	CH_3	CH_3	CH_3	C_2H_5	C_2H_5	103	0·4
644	C_2H_5	C_2H_5	CH_3	CH_3	C_2H_5	99	0·9
525	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5	54	0·9
653	CH_3	C_2H_5	C_2H_5	C_2H_5	C_2H_5	25	1·3
611	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5	45	3·3
530	CH_3	CH_3	CH_3	C_3H_7n	C_3H_7n	75	0·6
683	CH_3	CH_3	CH_3	C_3H_7i	C_3H_7i	26	1·8
678	CH_3	C_2H_5	CH_3	C_3H_7i	C_3H_7i	26	2·5
654	CH_3	C_3H_7i	CH_3	C_3H_7i	C_2H_7i	20	0·4
531	CH_3	C_3H_7n	CH_3	C_3H_7n	C_3H_7n	40	0·2
652	CH_3	C_2H_5	C_2H_5	C_3H_7n	C_3H_7n	50	1·4
667	C_2H_5	C_3H_7n	C_2H_5	C_2H_5	C_3H_7n	31	0·4
589	CH_3	CH_3	CH_3	C_4H_9n	C_4H_9n	41	0·5

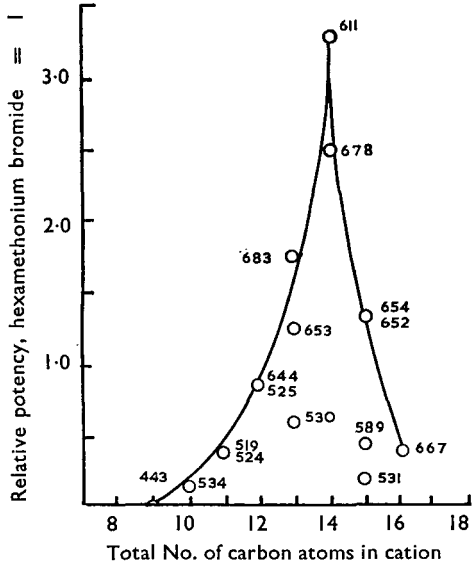

FIG. 3. Relationship between sympathetic ganglion blocking activity and total number of carbon atoms in the cation of a series of tetramethylene bis-tertiary sulphonium quaternary ammonium salts.

Numbers refer to structures shown in Table I.

The effect on the ganglionic blocking activity of substituting in turn methyl groups by larger groups in the tri-, tetra-, penta- and hexamethylene series was investigated further. The variation in activity of the compounds in the trimethylene series is illustrated in Figure 2. Maximum ganglionic blocking activity occurs when only ethyl groups are present on the sulphur and nitrogen atoms. When there are more than a total of 13 carbon atoms in the molecule activity is virtually abolished.

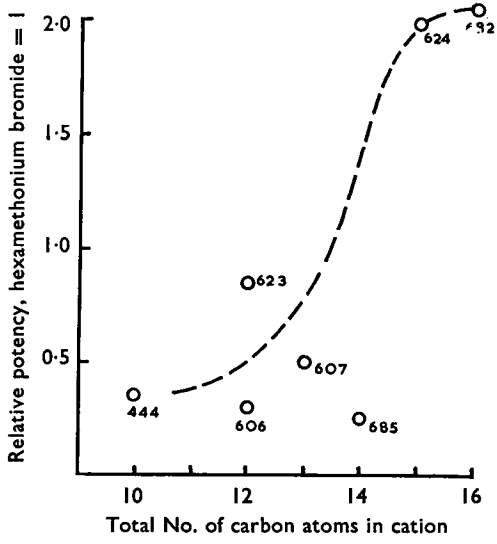

FIG. 4. Relationship between sympathetic ganglion blocking activity and tota number of carbon atoms in the cation of a series of pentamethylene bis-tertiary sulphonium quaternary ammonium salts.

General formula: $R_1R_2S^+(CH_2)_5N^+R_3R_4R_5$

Code No.	R_1	R_2	R_3	R_4	R_5
444	CH_3	CH_3	CH_3	CH_3	CH_3
623	CH_3	C_2H_5	CH_3	CH_3	C_2H_5
606	CH_3	CH_3	CH_3	C_2H_5	C_2H_5
607	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5
624	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5
685	CH_3	CH_3	CH_3	C_3H_7n	C_3H_7n
682	CH_3	C_2H_5	C_2H_5	C_3H_7n	C_3H_7n

In the tetramethylene series a different picture evolves. The variations in the groupings and their relative ganglionic blocking activities obtained are given in Table I and are illustrated graphically in Figure 3. Maximum activity in this series again occurs when only ethyl groups are present on the sulphur and nitrogen atoms, but in this instance the compound is about 3 times as active as hexamethonium. Larger or smaller groups than ethyl on the sulphur and nitrogen atoms leads to diminished activity. There is also an interesting relationship between the compounds possessing *n*-propyl and isopropyl groups. A comparison between the dimethyl sulphonium di-isopropyl methyl ammonium salt (B.R.L. 683) and the

corresponding *n*-propyl isomer (B.R.L. 530) and a comparison between the methyl isopropyl sulphonium methyl di-isopropyl ammonium salt (B.R.L. 654) and the *n*-propyl isomer (B.R.L. 531) shows that the *n*-propyl compound is in both instances much less active than the corresponding isopropyl isomer.

The compounds prepared with the pentamethylene chain form a rather incomplete picture. The activities are expressed graphically in Figure 4. Maximum activity again occurs when only ethyl groups are present on the sulphur and nitrogen, but the ethylmethylsulphonium di-*n*-propyl ethyl

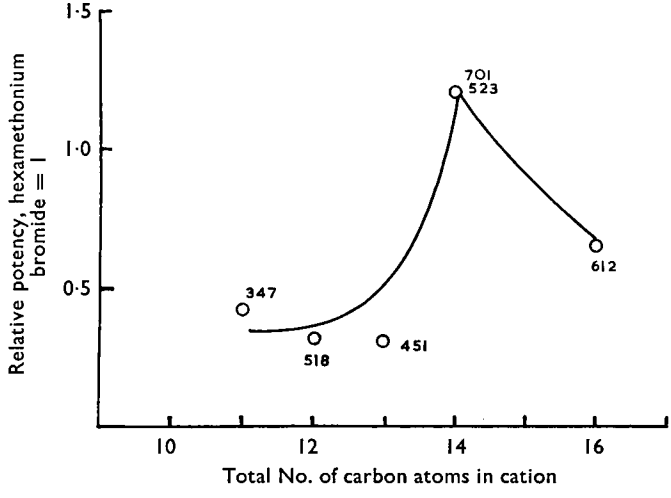

FIG. 5. Relationship between sympathetic ganglion blocking activity and the total number of carbon atoms in the cation of a series of hexamethylene bis-tertiary sulphonium quaternary ammonium salts. General formula: $R_1R_2S^+(CH_2)_6N^+R_3R_4R_5$.

Code No.	R_1	R_2	R_3	R_4	R_5
347	CH_3	CH_3	CH_3	CH_3	CH_3
518	CH_3	C_2H_5	CH_3	CH_3	CH_3
451	CH_3	C_2H_5	CH_3	CH_3	C_2H_5
701	C_2H_5	C_2H_5	CH_3	CH_3	C_2H_5
523	CH_3	C_2H_5	CH_3	C_2H_5	C_2H_5
612	C_2H_5	C_2H_5	C_2H_5	C_2H_5	C_2H_5

ammonium derivative is of the same order of activity. Compounds with larger groups than ethyl on the sulphur were not prepared, and hence it has been impossible to determine at which stage maximum activity does occur.

The ganglionic blocking activity in the hexamethylene series is illustrated in Figure 5. Maximum activity occurs with the diethyl sulphonium dimethylethyl ammonium salt, and the ethylmethyl sulphonium diethylmethyl ammonium salt. Both these compounds are slightly more active than hexamethonium. The diethyl sulphonium triethyl ammonium salt is about 0·6 times as active as hexamethonium. Compounds possessing larger groups on the sulphur and nitrogen were prepared, but none of these showed any significant ganglionic blocking activity.

Discussion

To achieve the maximum ganglionic blocking activity 4 carbon atoms are required in the carbon chain separating the sulphur and nitrogen atoms and the presence of only ethyl groups on the nitrogen and sulphur atoms is necessary. Barlow and Vane[3] similarly found that maximum activity in the hexamethylene bis-alkyl sulphonium series occurred when only ethyl groups were attached to the sulphur atoms. In the compounds with 3 carbon atoms in the chain the activity is not marked, but when the chain length is increased to 5 carbon atoms compounds with activity of twice that of hexamethonium have been obtained. Increasing the length of the chain to 6 carbon atoms likewise results in compounds with slightly greater activity than hexamethonium, but activity in this series follows a somewhat different pattern than seen in the preceding series. Relative activity would appear to follow more closely the change in activity seen with the bis-nitrogen compounds when the methyl groups are substituted in turn by ethyl groups as shown by Wien, Mason, Edge and Langston[6]. These workers found that the penta- and hexamethylene bis-dimethylethyl quaternary ammonium salts were the most active of the compounds tested, and of the 4 carbon chain compounds the bis-diethylmethyl ammonium salt was the most active. In the sulphur-nitrogen series the most active hexamethylene derivatives are the diethyl sulphonium dimethylethyl ammonium salt and the ethylmethylsulphonium diethylmethyl ammonium salts. Both are equiactive.

Wien and his colleagues also showed that the salt of hexamethylene bis-triethylammonium was practically devoid of ganglionic blocking action, but had a significant neuromuscular paralysing action. No such alteration in effect has been demonstrated in the sulphur-nitrogen series even with the substitution of larger groups. The shorter chain sulphur-nitrogen compounds would seem to be remarkably free from neuromuscular blocking action.

The modifications to structure of mono-quaternary and bis-quaternary ammonium salts which have shown ganglionic blocking activity has been investigated by several workers. Burn and Dale[7] found that substitution of the methyl groups by ethyl groups in tetramethylammonium abolished nicotinic stimulatory action on the blood pressure while maintaining the nicotine paralysing action. Subsequently Paton and Zaimis[8] demonstrated in a series of polymethylene bis-trimethylammonium salts the importance of the distance between the two onium centres. They showed that maximum activity occurred when the polymethylene chain contained 5 or 6 carbon atoms, the hexamethylene derivative being 20 times as active as tetraethylammonium. Wien and others[6] extended this work and investigated the effect of substitution of methyl by ethyl on the nitrogen atoms. They showed that optimal activity depended on both chain length and the nature of the substituents on the nitrogen atoms. The results of the present investigation emphasise the importance of the groupings on the onium centres and also stresses the fact that 5 to 6 carbon atoms separating the onium centres are not necessary for optimal activity. Nevertheless, depending on the type of onium centre present, an optimum

number of carbon atoms would appear to be necessary. In tertiary sulphonium quaternary ammonium compounds optimal activity occurs when there are only 4 carbon atoms in the polymethylene chain. However, to ensure an adequate fit on a receptor site, and so inducing ganglionic block, both the total length and general shape of the molecule appear to be of importance. That the volumes and effective radii of the groupings on the onium centre play a part in determining activity is evident from the difference in activity in the isomeric compounds containing *n*-propyl and *iso*propyl groups. The end groupings probably regulate the approach of the molecule to the receptor site and maximum ganglionic blocking activity is attained when there is correct electrolytic balance between drug and receptor site.

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After Mr. Brown presented the paper there was a DISCUSSION. The following point was made.

It was suggested that the higher charge density round the N atom due to its small atomic radius, is the reason for the physiological activity being more affected by change in substitution on this atom than on the S atom.