Journal of Medicinal and Pharmaceutical Chemistry

VOL. 1, No. 5 (1959)

Comparative Ganglion-blocking Potencies of the Geometric Isomers of Two Cyclic 1,2-Amino Alcohols*

FRANK G. STANDAERT,† SEYMOUR L. FRIESS and ROGER O. DOTY

Naval Medical Research Institute, Bethesda, Maryland, U.S.A.

Introduction

A recent communication from this laboratory[1] described the effects of the two geometric isomers of 2-hydroxy*cyclo*pentyltrimethylammonium iodide and 2-hydroxy*cyclo*hexyltrimethylammonium iodide (Fig. 1) on the neuromuscular junction of the cat. In the course of the experiments leading to that publication it was noted that, in addition to the action on the neuromuscular apparatus, the intravenous administration of these compounds resulted in paralysis of the nictitating membranes. In fact, it appeared that when two of the isomers were administered in rapid succession the relaxation of the membrane was greater than when either one was given alone. This was in marked contrast to the effects on the skeletal muscle system where the two isomers are antagonistic.

This communication reports the results of an assay of the comparative potencies of the isomers individually and in mixture. The impression of increased activity when the isomers are mixed has been confirmed and found to be a true synergism of action.

* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

† Present address: Department of Pharmacology, Cornell University Medical College, New York, New York.

Methods

Adult cats were anaesthetized with Dial-Urethane (Ciba) 0·6 ml/kg i.p. After the trachea had been cannulated the carotid artery was ligated just distal to the origin of the occipital pharyngeal trunk. The cervical sympathetic trunk was isolated and sectioned centrally. The distal stump was placed over platinum electrodes for stimulation which, in each case, consisted of square waves of 0·1 msec duration delivered from a Grass stimulator at a

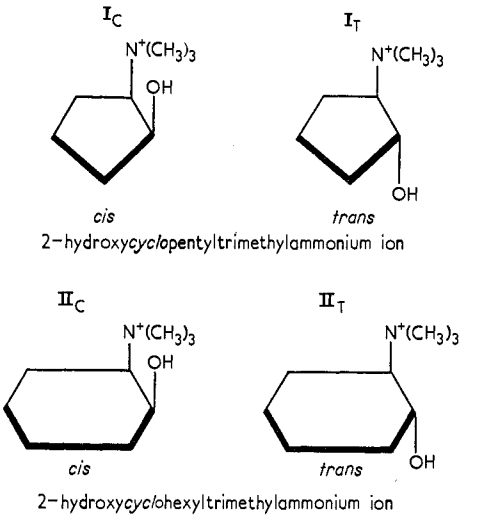

Fig. 1. Structures of amino alcohols

rate of 25 per sec for a period of 15 sec. The voltage was adjusted for each animal to produce a maximum contraction of the nictitating membrane. Contractions were recorded on a kymograph by means of a light isotonic lever. Drugs, dissolved in 0·2 ml of isotonic saline, were injected rapidly into the common carotid artery just proximal to the ligature. The nerve was stimulated 15 sec after the injection. Control stimulation was done 5 min before and after each injection.

At least two, and usually three, drugs were compared for blocking potency on each animal. In advance of each experiment, the compounds to be assayed and the concentrations of each which were anticipated to provide a dose-response curve were tabulated.

The sequence in which the various combinations of drug and concentration would be given was then selected at random. Duplicate administrations of several concentrations were included to test reproducibility. Although there was some potency variation between animals, results were consistent for any given animal.

For each drug, several experiments were done in which the post-ganglionic fibres were stimulated 15 sec after the administration of a concentration of the agent which produced a 100 per cent block of preganglionic stimulation. In no case could block of the post-ganglionic stimulation be demonstrated.

The preparation of the compounds has been described previously.[2, 3] In brief, identical procedures were employed in the synthesis of the *cis* and *trans* isomers in either cyclic series, starting with each respective 2-chlorocyclanone. First, the chloroketone was aminated with dimethylamine in aqueous alcoholic medium, and the resulting dimethylamino ketone purified by distillation under reduced pressure, followed by reduction with hydrogen and a platinum catalyst in alcoholic suspension. The tertiary amino alcohol was then fractionally distilled for separation of the *cis* and *trans* isomers, in a procedure made remarkably facile by virtue of the large differences in boiling points previously noted.[2, 3] Then, each fractionated tertiary isomer was methylated with a fivefold excess of methyl iodide in absolute ether solution, and the precipitated quaternary iodides were re-crystallized repeatedly from either methanol–ether or acetone–ether mixtures. All were used as the iodides. As an additional control, solutions of sodium iodide in concentrations comparable to those used in the assay of the amino alcohols were administered. They had no apparent effect upon the preparations or upon ganglionic transmission.

Results

None of these compounds showed any evidence of stimulating the ganglion. The blocking potency is presented graphically in Fig. 2 (*cyclo*pentanols) and Fig. 3 (*cyclo*hexanols). The concentrations of the drugs are plotted on a logarithmic scale on the abscissa and the amount of block, i.e. decrease in contraction height after drug as percentage of control, on the ordinate. In both figures, COMB represents the results obtained employing an

equimolar mixture of the two isomers. In this case the concentrations indicated represent the total amount of material present. For example, the 10^{-1} M combination contained 5×10^{-2} moles of the *cis* isomer and 5×10^{-2} moles of the *trans* isomer per litre.

In the illustrations the points represent the means of the observations. For statistical analysis, the raw data were subjected to an arcsin transformation and then pooled to obtain greater reliability. Regression coefficients were calculated and the lines

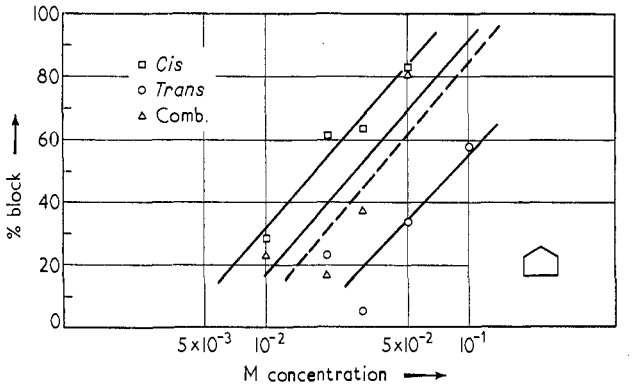

Fig. 2. Relationship between blockade of the superior cervical ganglion of the cat and the concentration of *cis* and *trans* 2-hydroxy*cyclo*pentyltrimethylammonium ions and an equimolar mixture (COMB) of the two isomers. The dotted line represents action calculated to result if the *trans* isomer in the mixture were inert

tested statistically following the methods of Bliss.[4] The lines shown in the illustrations were calculated on the basis of the regression coefficients. The three lines in each illustration have the same slope ($0 \cdot 10 < p < 0 \cdot 25$).

The results depicted in Fig. 2 show a potency relationship between the *cis* and *trans* isomers of 2-hydroxy*cyclo*pentyltrimethylammonium ion very similar to that previously noted for neuromuscular preparations,[1] i.e. the *cis* isomer is approximately 10 times more potent than the *trans* isomer ($p < 0 \cdot 001$). The curve representing the results obtained using a mixture of the isomers does not differ significantly from either of the other two curves ($0 \cdot 10 < p < 0 \cdot 25$ *versus* the *cis* and $0 \cdot 05 < p < 0 \cdot 10$ *versus*

the *trans*), but it is very suggestive of a phenomenon which is much more clearly seen with the ammonio*cyclo*hexanol derivatives.

Fig. 3 shows that the *cis* and *trans* isomers of 2-hydroxy*cyclo*hexyltrimethylammonium ion are equipotent on this preparation ($0 \cdot 10 < p < 0 \cdot 25$). This is in contrast to the results previously reported for the neuromuscular junction preparation where the *cis* isomer is much more active than is the *trans* isomer. Of greater interest are the results obtained with the mixture of the two iso-

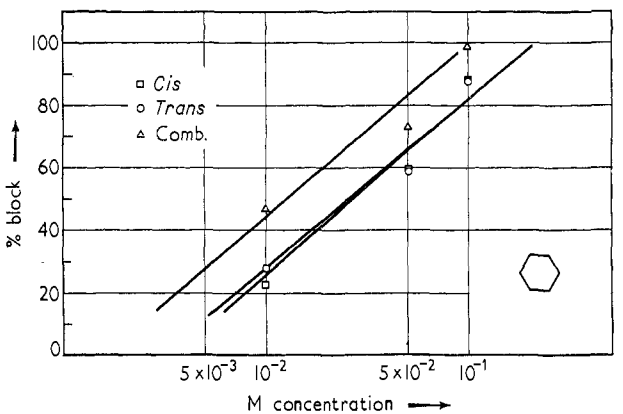

Fig. 3. Relationship between blockade of the superior cervical ganglion of the cat and the concentration of *cis* and *trans* 2-hydroxy*cyclo*hexyltrimethylammonium ions and an equimolar mixture (COMB) of the two isomers

mers. The illustration shows quite clearly that, when the two isomers are present at the same time, they are about twice as active as equimolar amounts of either one of the isomers.

For example, from the illustration it can be estimated that production of a 50 per cent block of transmission requires a $2 \cdot 8 \times 10^{-2}$ M solution of the *cis* or of the *trans* isomer, but only a $1 \cdot 3 \times 10^{-2}$ M solution of the mixture, a solution which, in fact, is only $0 \cdot 65 \times 10^{-2}$ M in each isomer. This difference, which is statistically significant ($p < 0 \cdot 01$), extends over the entire concentration range as shown. At any point along the lines it appears as if the two components of the mixture were acting independently and that the blockades were summating. Thus, the injection of 2 μmoles ($0 \cdot 2$ ml of a 1×10^{-2} M solution) of either isomer produces

about a 27 per cent block, but if the same amount of each compound is included in one solution, yielding a 2×10^{-2} M solution of the mixture, an injection of 0·2 ml produces a block of about 60 per cent.

A similar summation cannot be as clearly seen in Fig. 2 but there is a strong suggestion that it is present. This becomes more apparent when the line representing the mixture is compared to the dotted line, which represents a line calculated to result if the *trans* isomer in the mixture were completely inert. It can then be noted that the potency of the mixture is greater than that of its *cis* component alone and by an amount which closely approximates the individual action of the *trans* isomer in the solution.

Discussion

In a recent comprehensive review of the subject of potentiation and synergism Veldstra[5] classed as synergistic those compounds of which 'the combination effects a certain response with a smaller number of molecules than that required for the most active compound separately'. The results indicate that these amino alcohol derivatives meet this requirement in their action on the superior cervical ganglion. In this regard, the two isomers of 2-hydroxy*cyclo*hexyltrimethylammonium ion appear to represent a rare example of two closely related compounds which independently have identical actions on a biological system and, in addition, are synergistic with each other on that system.

The structural and chemical features of these compounds, with particular regard to the effects these may have upon reactions with a receptor surface, have been discussed previously.[1] In brief, the two isomers have structural formulae which differ only in the spatial orientation of the ring substituents. This distinction confers upon them chemical and physical properties which, although qualitatively similar, are in many respects quantitatively different. Therefore, in spite of their apparent similarity, it is best to consider the two isomers as separate entities which are much more closely related to each other than are the compounds in most series of analogues.

We do not at present have any direct evidence for the mechanisms of synergism, but of the several possible some appear to be

more probable than others. For one, interaction between the two compounds to produce a more active product is very unlikely, since, *in vitro*, at least, there is no such reaction; nor is there any appreciable isomerization. On the contrary, the compounds are very stable and inert.

From the close structural relationship between the two isomers it might be anticipated that they are reacting at the same site. Synergism, under these circumstances, is very difficult to picture. Rather, it would be expected that if they were competing for the same receptor groups they would either inhibit each other, or, at best, produce a simple addition of action, equal to, but not surpassing, that of an equimolar concentration of one administered alone.

Synergism could occur if the receptor surface were so modified by the presence of one that it is more than normally susceptible or reactive to the second. This, for instance, is the mechanism proposed for the synergism between physostigime and decamethonium[6] and between epinephrine and acetylcholine.[7] These, however, unlike the present case, represent synergism between compounds of widely different structures and primary actions.

Alternatively, the surface might be so modified by one that it becomes less sensitive to that species, but retains its original sensitivity to the second. This would account for the apparent mathematical addition of the independent effects of the compounds. The reduction in sensitivity, in all probability, does occur. Clark[8] in his discussion of dose-response curves pointed out that they are in general either hyperbolic or exponential in nature although portions of them may approximate to a straight line, or be made to approximate to a straight line, by plotting the logarithm of the dose. This was originally attributed to the occupation of the receptors by the drug, thereby decreasing the possibility of further drug receptor encounters, but Stephenson[9] has pointed out the probability that only a small proportion of the receptors are occupied by an active drug. It is conceivable that the unreacted receptors would be available for reaction with a second compound. At present, however, it is difficult to see why such secondary reactions should not be equivalent to additional primary reactions.

It is more probable that the synergism is occurring because the

two isomers are reacting with different structures within the ganglion. Although it has long been considered that blocking agents acted by impeding the access of acetylcholine to receptors, recent evidence[10,11] has called attention to the fact that there is an important action, both for stimulating and for blocking agents, on the pre-synaptic nerve terminals. More recently it has been shown[12] that acetylcholine and hexamethonium have dual sites of action within the superior cervical ganglion in that they affect both the pre-synaptic and the post-synaptic fibres. Hexamethonium, in addition, seems to have a differential action; that is, it acts on both sides of the synapse but not with equal potency. In the present case, it is quite possible that by acting differentially on pre- and post-synaptic sites the two isomers could produce independent partial blockades which summate to produce the measured response.

Summary. The *cis* and *trans* isomers of 2-hydroxy*cyclo*pentyltrimethylammonium and 2-hydroxy*cyclo*hexyltrimethylammonium ions were assayed for potency in blocking transmission at the superior cervical ganglion of the cat. The ammonio*cyclo*hexanol isomers were particularly interesting in that in addition to having blocking actions which are qualitatively and quantitatively identical they are synergistic with each other on this preparation.

(*Received* 22 *August*, 1959)

References

[1] Standaert, F. G. and Friess, S. L. *J. Pharmacol.*, (1960) in press
[2] Baldridge, H. D., McCarville, W. J. and Friess, S. L. *J. Amer. chem. Soc.*, **77**, 739 (1955)
[3] Friess, S. L. and Baldridge, H. D. *J. Amer. chem. Soc.*, **78**, 2482 (1956)
[4] Bliss, C. I. *The Statistics of Bioassay.* (1952). New York; Academic Press
[5] Veldstra, H. *Pharmacol. Rev.*, **8**, 339 (1956)
[6] Zaimis, E. J. *J. Physiol.*, **112**, 176 (1951)
[7] Burn, J. H. *Phys. Rev.*, **25**, 377 (1945)
[8] Clark, A. J. General Pharmacology, in Heffter's *Handbuch der Experimentellen Pharmakologie, Ergänzungswerk*, Vol. 4. (1937). Berlin; Springer
[9] Stephenson, R. P. *Brit. J. Pharmacol.*, **11**, 379 (1956)
[10] Riker, W. F., Roberts, J., Standaert, F. G. and Hiroshi Fujimori. *J. Pharmacol.*, **121**, 3 (1957)
[11] Riker, W. F., Werner, G., Roberts, J. and Kuperman, A. *J. Pharmacol.*, **125**, 150 (1959)
[12] Riker, W. K. and Szreniawski, Z. *J. Pharmacol.*, **126**, 233 (1959)