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THE STEREOSPECIFICITY OF ACETYL-α-METHYLCHOLINE

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Acetyl-α-methylcholine has been known to have " nicotinic " activity similar in strength to that of acetylcholine ever since the work of Simonart (1932). By contrast it has little " muscarinic " activity, in respect of which acetyl-β-methylcholine was found to have a potency similar to that of acetylcholine.

Acetyl-β-methylcholine is highly stereospecific. Molitor (quoted by Major & Bonnett, 1935) found that the (+)-isomer was more active than the racemic mixture on the blood pressure and the intestine (animal not stated), whereas the (−)-isomer had only one-hundredth the activity of the racemate on the blood pressure and was " considerably less " active than the racemate on the intestine. Ellenbroek & Van Rossum (1960) and Beckett, Harper, Clitherow & Lesser (1961) have since confirmed the very high relative activity of the (+)-isomer. Ellenbroek & Van Rossum (1960) and Beckett, Clitherow & Harper (1960) have also shown that the (+)-isomer has the L-configuration (as was expected from the stereospecificity and stereochemistry of muscarine).

The (+)- and (−)-forms of acetyl-α-methylcholine have recently been prepared by Beckett *et al.* (1960) and Beckett, Harper & Clitherow (1963), who have also established the absolute configurations of these compounds. Although the (+)-isomer, which has the D-configuration, is much less active than acetylcholine on the guinea-pig ileum and cat blood pressure preparations, it is eight times as active as its enantiomorph, L-(−)-acetyl-α-methylcholine, on the former and six times as active on the latter (Beckett *et al.*, 1961).

It is of interest to know whether or not a similar degree of stereospecificity can be demonstrated at the receptors involved in " nicotinic " activity.

METHODS

The isomers used had $\alpha_D^{20.7}+8.61°$ and $\alpha_D^{27}-9.07°$, and were in the form of the iodide salts. They were compared in 2+2 assays of Latin square design, using not less than sixteen doses, on the following preparations:

Isolated rectus abdominis muscle of the frog. The muscle was mounted in aerated Ringer solution in a 10-ml. organ-bath at room temperature. The drugs were applied at 4- to 7-min intervals, left in contact with the tissue for 45 to 90 sec, and then washed out. Contractures were recorded with a frontal-writing lever on a smoked drum.

Chick innervated biventer cervicis muscle. Chicks, weighing 70 to 120 g, were anaesthetized with pentobarbitone sodium (0.1 ml./100 g of body weight) injected into a gastrocnemius muscle. The preparation

was isolated (Ginsborg & Warriner, 1960), set up in a 10-ml. organ-bath of Krebs solution gassed with a mixture of 95% oxygen and 5% carbon dioxide, and stimulated with maximal shocks at 10-sec intervals. The drugs were given at 7- to 10-min intervals and left in contact with the preparation for 3 min, after which the fluid was replaced and the stimulator switched off for 2 min. Contracture of the lightly stretched muscle was recorded on a smoked drum with a Starling heart-lever. In some experiments the preparation was kept in contact with 10^{-7} physostigmine sulphate for 30 min and the ratio of potency was redetermined in the new conditions.

Blood pressure of the atropinized cat. Cats of either sex, weighing 1.5 to 3.5 kg, were anaesthetized with chloralose (80 mg/kg) and the blood pressure was recorded from a carotid artery. Atropine, in doses of 1 mg/kg, was injected into a femoral vein every hour. The drugs, which now caused a rise in blood pressure, were administered intravenously at 15-min intervals.

Blood pressure of the atropinized rat. Rats, weighing 250 to 400 g, were anaesthetized with urethane (1 g/kg) given intraperitoneally. Blood pressure was recorded from a carotid artery. Drugs were injected into a femoral vein, in volumes not exceeding 0.4 ml. Atropine (5 mg/kg) was found to abolish depressor responses to the choline esters used, which thereafter caused a rise in blood pressure.

Superior cervical ganglion of the cat. Cats of either sex and weighing 1.5 to 3 kg were anaesthetized with chloralose (80 mg/kg). The blood pressure was recorded continuously from the left femoral artery, and a simultaneous recording was made also of the tension in the right nictitating membrane. The right lingual artery was cannulated retrogradely with fine polyethylene tubing, and injections were made into the cannula after a clamp had been placed on the common carotid artery immediately cephalad to the lingual artery, to ensure that the greater part of each injected dose was carried by the blood stream to the ganglion. The immediate effect of the drugs on the blood pressure was to cause a rise, which appeared to be independent of dose, followed by a fall which was maximal in the doses used. The drugs caused a swift contraction, which varied with dose, of the nictitating membrane, followed by a comparably rapid recovery, which responses were recorded by a frontal-writing lever on a smoked drum. All doses used in the comparisons of potency were given in a volume of 0.1 ml.

RESULTS

The results are summarized in Table 1. They are expressed as equipotent molar ratios for the (+)-isomer relative to the (−)-isomer, that is the number of molecules of the former

TABLE 1

EQUIPOTENT MOLAR RATIOS FOR (+)-ACETYL-α-METHYLCHOLINE RELATIVE TO (−)-ACETYL-α-METHYLCHOLINE

* Sixteen-dose Latin square not completed

Preparation	Equipotent molar ratios: Mean	Equipotent molar ratios: Fiducial limits $P=0{\cdot}05$
Chick biventer (contracture)	0·38	0·31–0·45
	0·42	0·38–0·47
	0·47	*
	0·61	*
Frog rectus abdominis (contracture)	0·57	0·52–0·64
	0·52	0·42–0·65
	0·70	0·63–0·79
Cat superior cervical ganglion (contraction of nictitating membrane)	1·26	1·06–1·49
	1·31	1·06–1·63
	2·37	1·52–3·8
Atropinized cat blood pressure (rise)	2·01	1·61–2·51
	2·23	1·95–2·56
	2·14	*
Atropinized rat blood pressure (rise)	0·75	0·71–0·78
	0·76	0·68–0·84
Chick biventer (contracture in presence of physostigmine, 10^{-7})	0·29	0·23–0·38
	0·25	0·22–0·28

which should produce the same effect as one molecule of the latter. They indicate that the potency of the (+)-isomer ranges from half to twice that of the (−)-compound, except in the experiments on the chick biventer cervicis preparation in the presence of physostigmine, when it is about three times as active, in the presence of a hundredfold increase in sensitivity to both.

DISCUSSION

All the preparations may be supposed to be affected in a similar manner by the drugs used, in that the response measured is produced by a stimulation of the smooth or striated muscle or nerve cells involved. It cannot be claimed, however—as the term " nicotinic " may suggest—that a common receptor system is implicated in all instances; and, indeed, while superficially it might appear that the two atropinized preparations come closest in this respect, the results obtained from them are much further apart than those of the two striated muscle preparations. What is generally accepted is that, in some way, each of the test tissues used presents a measure of nicotinic activity.

While the differences in equipotent molar ratio are small, the calculated fiducial limits indicate that they are real enough. On the other hand, the ratios of potency lend little support to the notion that the isomers, and hence the receptor systems on which they act, exhibit any important degree of stereospecificity, which would require at a minimum that one isomer was usually more potent than the other. This situation contrasts with that on the muscarinic side.

The results of the experiments with the chick biventer preparation in the presence of physostigmine are the opposite of what might have been expected. Child (1955) stated that the variability of the response of the semispinalis cervicis preparation to acetylcholine was " presumably " due to differing amounts of cholinesterases present in the muscle. These cholinesterases were subsequently found to be mainly acetylcholinesterase (Blaber & Cuthbert, 1962), which was inhibited to a similar extent to mammalian acetylcholinesterase by the selective monoquaternary salt [*m*-(*N*-*p*-chlorophenyl-*N*-methylcarbamoyloxy)-phenyl] trimethylammonium bromide (Ro 2-1250). Of the two enantiomorphs of acetyl-α-methylcholine the (−)-isomer is more readily broken down by the acetylcholinesterase of ox red cells (Beckett *et al.*, 1963). Yet in the presence of physostigmine, which had by itself no effect on the resting length of the muscle of the biventer, the relative potency of the (−)-isomer was reduced. It is possible to speculate on the effects which differing affinities and concentrations might have; but the simplest explanation appears to be that the cholinesterases present in the tissue are unlikely to be implicated in the result.

" Nicotinic " actions are well known to cover a very wide range of disparate effects. Even when nicotine itself is used a wide range of equipotent molar ratios result (Barlow & Hamilton, 1965) when the optical enantiomorphs likely to reveal stereospecificity are used. It may therefore be that the failure to repeat for nicotine and acetyl-α-methylcholine the success in prediction achieved for muscarine and acetyl-β-methylcholine is the result of the fact that, while nicotinic receptors—in the sense of a site of action sensitive to nicotine—may exist, other drugs which cause " nicotinic " effects act through a different system or systems. If this were true it would follow that the term " nicotinic " has outlived its usefulness.

SUMMARY

1. The potencies of the (+)- and (−)-forms of acetyl-α-methylcholine have been compared with a view to demonstrating the stereospecificity of the nicotinic receptor.

2. The preparations used were the rectus abdominis muscle of the frog, the innervated biventer cervicis muscle of the chick, the blood pressure of the atropinized cat and rat, and the superior cervical ganglion of the cat.

3. The relative potencies were determined on the basis of 2+2 dose assays of Latin square design, using not less than sixteen doses.

4. The equipotent molar ratios for (+)-relative to (−)-acetyl-α-methylcholine range from about 0.40 to 2.12. The possibility that the relatively high potency of the (+)-isomer might be due to tissue cholinesterases that preferentially inactivated the (−)-isomer was tested in the biventer cervicis preparation and found to be unlikely.

5. The results reflect relatively small differences of potency of the two isomers and no consistent degree of stereospecificity. The results also suggest that tissue cholinesterases are unlikely to affect the relative potency in the biventer cervicis preparation.

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