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THE STEREOSPECIFICITY OF NICOTINE

BY

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It is of great interest to know the relative activity of the two isomers of nicotine because this should indicate the number of groups in the molecule which interact with the receptor. If one isomer is more active than the other, at least three groups must be important; if the isomers are equiactive, it is probable that only two groups are important, because if more than two groups were involved they would all have to interact with groups in the receptor which lay along a straight line.

Natural nicotine is laevorotatory (sodium D line). Racemic nicotine is tedious to prepare, either by racemizing natural nicotine (Pictet & Rotschy, 1900) or by synthetic methods (for example, Craig, 1933a); (+)-nicotine is even more tedious to prepare because it involves the resolution of the racemic mixture (Pictet & Rotschy, 1904) or a process starting from an optically pure intermediate, such as that used by Hicks & Sinclair (1947), who methylated pure (+)-nornicotine extracted from *Duboisia hopwoodii*. Probably because of the difficulty of obtaining (±)- and (+)-nicotine, relatively little is known about their pharmacological properties.

Mayor (quoted by Pictet & Rotschy, 1904) found that in guinea-pigs and rabbits (−)-nicotine appeared to be about twice as toxic as (+)-nicotine and to produce effects which were qualitatively quite different. An injection of (−)-nicotine produced marked stimulation, a shriek from the animal (suggesting intense pain) and convulsions; (+)-nicotine did not produce a shriek and caused twitching of the skin, followed by paralysis,rather than convulsions. Although other workers (for example, Craig, 1933b; Macht & Davis, 1934) have found that (−)-nicotine was more toxic than (±)-nicotine in a variety of species, including goldfish, tadpoles and aphides, the results vary greatly from one set of experiments to another. Hicks & Sinclair (1947), for example, failed to find any significant difference between the toxicities of (−)- and (+)-nicotine in rats and guinea-pigs.

The first direct comparison of the activities of the isomers, other than their toxicities, was made by Hicks, Mackay & Sinclair (1947), who found that (+)-nicotine was much less active than (−)-nicotine in raising the blood-pressure of cats and rabbits and in stimulating and then blocking the perfused superior cervical ganglion of the cat. Unfortunately there were not sufficient results for a quantitative comparison of activity to be made. Domino (1964) found that (+)-nicotine was less active than (−)-nicotine in raising the blood-pressure of dogs anaesthetized with pentobarbitone sodium; his sample of (+)-nicotine was estimated to be 87% optically pure and he found that 4.2-times as much of this material

was needed to produce the same effect as that of the pure (−)-isomer. Domino also investigated the effects of the isomers on behavioural responses and on the ability to cause convulsions in rats.

In general, the lack of detailed quantitative information about the relative activities of the two isomers of nicotine at sites in the peripheral nervous system was considered to justify preparing some pure (+)-nicotine and comparing its activity with that of (−)-nicotine on a variety of preparations.

METHODS

Drugs

(−)-Nicotine was tested as the di-(+)-hydrogentartrate dihydrate (B.D.H.). This material melted at 89 to 90° C and had $\alpha_D^{17} = +25.4°$ (for the anhydrous salt); Pictet & Rotschy (1904) recorded a melting point 88 to 89° C, and $\alpha_D^{27} = +26.6°$.

(+)-Nicotine was tested as the di-(−)-hydrogentartrate dihydrate, obtained by racemizing natural nicotine, as described by Pictet & Rotschy (1900) but with 40-g quantities in a stainless steel autoclave instead of 10-g quantities in a sealed glass tube, and resolving the racemate as described by Pictet & Rotschy (1904). The material was recrystallized until the melting point was 89 to 90° C and α_D^{17} was −25.5° (for the anhydrous salt). The purest sample obtained by Pictet & Rotschy (1904) had $\alpha_D^{15} = -25.6°$; if, however, it is assumed that the rotation should be the same as that of the diastereoisomer, (−)-nicotine di-(+)-hydrogentartrate dihydrate, but of the opposite sign, the material used in this work should be 97.8% optically pure. Free (+)-nicotine base, which we obtained from specimens of the di-(−)-hydrogentartrate dihydrate having α_D^{17} between −25.0 and −25.4°, had $\alpha_D^{17} = +167.6°$. Pictet & Rotschy (1904) found $\alpha_D^{20} = +163.2°$ for their purest sample of this isomer, and values of −166.4 and −168.0° for various samples of the other (natural) isomer.

Hicks *et al.* (1947) recorded $\alpha_D = +168°$ (temperature not specified) for their sample of (+)-nicotine.

The ultraviolet absorption spectra of the two diastereoisomeric tartrates in water were found to be identical, λ_{max} 258 mμ (log ε molar 3.49), with plateaux at 254 mμ (log ε molar 3.45) and 264 mμ (log ε molar 3.35). These spectra were regularly made use of to check the composition of aqueous stock solutions of both isomers.

Preparations

Striated muscle. The chick biventer cervicis preparation was set up, exactly as described by Ginsborg & Warriner (1960), in Krebs-Henseleit solution (Krebs & Henseleit, 1932) at 37° C bubbled with 95% oxygen and 5% carbon dioxide. It was not stimulated electrically; only the responses of the slow fibres were recorded. The volume of the bath was approximately 25 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act for 10 min and then washed out. The interval between doses was 25 min. The relative ability of the two isomers to cause contracture was estimated by a four-point assay in which as much of a 4×4 Latin square was used as was convenient (in most of the experiments it was possible to complete all sixteen doses).

A few experiments were performed on the frog rectus abdominis muscle preparation (Langley, 1907, 1913) in Clark's modification of Ringer solution (Clark, 1913) at room temperature and bubbled with air. The volume of the bath was approximately 10 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act for 2 min and then washed out. In these experiments it was found that the tissue was desensitized for a considerable time after the addition of the drug, particularly after doses of (+)-nicotine which caused any appreciable contracture. It was necessary to allow from 20 to 30 min interval between doses of (−)-nicotine but, with doses of (+)-nicotine which produced comparable contractures of similar size, the tissue had to be left for a much longer period before its sensitivity was fully returned. In the circumstances an accurate assay of the activity of the two isomers was not feasible.

The rat diaphragm preparation was set up exactly as described by Bülbring (1946), in Tyrode solution at 37° C bubbled with 95% oxygen and 5% carbon dioxide. The phrenic nerve was stimulated with rectangular-wave shocks, which produced maximal twitches, of 0.75 msec duration at 5 shocks/min. The volume of the

bath was approximately 25 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act for 7 min and then washed out. The interval between doses was 15 min. The relative blocking activity of the two isomers was measured by a four-point assay in which as much of a 4×4 Latin square was used as was possible (it was seldom possible to complete all sixteen doses).

The anterior tibialis muscle preparation of the cat (anaesthetized with chloralose) was set up as described by Brown (1938). The peroneal nerve was stimulated with rectangular-wave pulses of short duration, which produced maximal twitches at a rate of 12 shocks/min, and drugs were injected, in a volume not exceeding 0.2 ml., retrogradely into the anterior tibial artery and washed in with 0.9% saline. The relative blocking activity of the two isomers was estimated by comparing the doses which produced roughly similar effects.

Ganglionic preparations. The superior cervical ganglion preparation of the cat (anaesthetized with chloralose) was set up as described by Paton & Perry (1953). The preganglionic sympathetic nerve was stimulated with rectangular-wave pulses of short duration, at a rate of 10 shocks/sec, producing a maximal contracture of the membrane. Injections were made retrogradely into the lingual artery and washed in with 0.9% saline. Both isomers themselves stimulated the ganglion but this effect was rapidly followed by a block in transmission. The relative blocking activity of the two isomers was estimated by comparing the doses which produced roughly similar effects.

The relative ability of the two isomers to raise arterial blood pressure was studied in rats and kittens anaesthetized with urethane. Blood pressure was recorded from a carotid artery and the drugs were injected into a femoral artery, in a volume not exceeding 0.2 ml., and washed in with 0.9% saline.

A few experiments were performed on the guinea-pig isolated ileum preparation set up in Tyrode solution at 37° C and bubbled with air. The volume of the organ-bath was approximately 25 ml. and the drugs were added by pipette in a volume not exceeding 0.4 ml., allowed to act for 1 min, and then washed out. On this preparation it was found that doses of the (+)-isomer which caused any appreciable contracture produced appreciable desensitization of the tissue; a maximal response of the tissue could not, in fact, be obtained with this compound. By a suitable choice of doses and by allowing sufficient time (usually about 8 min) for the full recovery of the tissue it was possible to perform a four-point assay of the relative activity of the two isomers, but it was clear that the relative activity could not really be expressed as a number. All the responses were abolished by the addition of hexamethonium to the bath (in a concentration approximately 6×10^{-5} M).

Acetylcholinesterase. Purified acetylcholinesterase from ox red cells was obtained from Nutritional Biochemicals Corporation. The effects of the compounds on the hydrolysis of acetylcholine (1.1×10^{-3} M) by this enzyme were studied by manometric methods as described by Barlow & Zoller (1964). The two concentrations of each isomer tested were 10^{-3} M and 5×10^{-4} M and the concentration producing 50% inhibition of the hydrolysis was calculated. In each experiment both isomers were tested simultaneously, and an equipotent molar ratio was calculated by comparing the concentrations which should cause 50% inhibition.

RESULTS

The results are summarized in Table 1 which shows the equipotent molar ratios for (+)-nicotine relative to (−)-nicotine (that is the number of molecules of the former required to produce the same effect as one molecule of the latter; if the number is greater than one it indicates that the (+)-isomer is less active than the (−)-isomer). For reasons already discussed in Methods, the results for the experiments on the frog rectus and guinea-pig ileum preparations do not have any quantitative significance and merely indicate that the (+)-isomer is appreciably less active than the (−)-isomer on the frog rectus and distinctly less active on the guinea-pig ileum preparation.

The result for the inhibition of the hydrolysis of acetylcholine by the acetylcholinesterase of ox red cells shown in Table 1 is the mean of the equipotent molar ratios obtained in each comparison. The mean values of the actual concentrations causing 50% inhibition were 1.05×10^{-3} M for the (−)-isomer and 8.0×10^{-4} M for the (+)-isomer.

TABLE 1

EQUIPOTENT MOLAR RATIOS FOR (+)-NICOTINE RELATIVE TO (−)-NICOTINE

Mean values are shown with standard errors and the numbers of results in parentheses. The results marked with an asterisk must be taken only to indicate the order of activity, because the tissues are easily desensitized

Preparation	Equipotent molar ratios
Chick biventer (contracture)	4·60±0·07 (6)
Frog rectus (contracture)	c. 10 (3)*
Rat diaphragm (block)	0·96±0·08 (4)
Cat tibialis (block)	3·1 ±0·5 (4)
Cat superior cervical ganglion (block)	13·7 ±1·2 (3)
Guinea-pig ileum (contracture)	42 ±2 (4)*
Rat blood pressure (rise)	14·0 ±1·5 (4)
Kitten blood pressure (rise)	6·1 ±0·2 (3)
Ox red cell acetylcholinesterase (inhibition)	0·75±0·10 (6)

The results with the chick biventer agree reasonably with what might be expected from the comparison of the effects on this preparation of (−)-nicotine and (±)-nicotine (obtained by catalytic reduction of nicotyrine; Barlow, 1964). In so far as it is possible to judge from the results of Hicks *et al.* (1947), the results obtained here on the superior cervical ganglion of the cat and on blood pressure appear to be reasonable; they are also consistent with the findings of Domino (1964).

DISCUSSION

The differences between the activity of the two isomers appear to depend greatly on the site at which they are tested. The greatest difference was found on the guinea-pig ileum at which the isomers were acting on parasympathetic ganglia (as the contractions were antagonized by hexamethonium), but there was also considerable stereospecificity on the cat superior cervical ganglion and in the experiments on blood pressure. At the receptors in the slow fibres the differences were less marked, though it was quite clear that the (−)-isomer was more active, but the blocking activity of the compounds at the neuromuscular junction was only slightly stereospecific. At the active centres of the acetylcholinesterase of ox red cells the stereospecificity was even reversed; (+)-nicotine was consistently a more active inhibitor than (−)-nicotine, though neither compound was particularly effective.

From these results it is possible to understand why there has been some confusion about the relative toxicities of the two isomers. Mayor's original description (Pictet & Rotschy, 1904) indicates that in the species he studied (−)-nicotine acted in a different way from (+)-nicotine, producing considerable central stimulation accompanied by respiratory paralysis, whereas (+)-nicotine was probably toxic because it paralysed breathing by desensitizing the muscle endplates of the diaphragm. How far the toxic action of (−)-nicotine was central and how far it was peripheral (and similar to that of the (+)-isomer) is not clear, but the fact that the drug can act both centrally and peripherally may well explain why in some species it is much more active than the (+)-isomer, whereas in others it is only equiactive. Presumably in the former it is toxic by some action such as its central one, whereas in the latter it is toxic by its peripheral one.

Although the way in which the stereospecificity of nicotine varies from site to site suggests that the receptors at these sites are slightly different in structure, it cannot be taken to indicate that, relative to the (−)-isomer, the (+)-isomer does not fit some receptors (for

example those in the ganglia) as well as it does others. At receptors where nicotine is acting as an agonist the difference could well be one of efficacy rather than one of affinity. Although it is not possible to make a definite decision about this, it is striking that even on preparations, such as the guinea-pig ileum, where (+)-nicotine is a much weaker agonist than is (−)-nicotine, it is still reasonably potent in producing desensitization, consequently it must have considerable affinity for the desensitized receptors (even though this may not apply to normal receptors).

From what is known about the stereospecificity and absolute configuration of muscarine it has been possible successfully to predict the absolute configuration of the more active isomer of acetyl-β-methylcholine at postganglionic acetylcholine receptors (Barlow, 1960; Ellenbroek & Van Rossum, 1960; Beckett, Clitherow & Harper, 1960). From the absolute configuration of nicotine (Hudson & Neuberger, 1950) and from what is now known about the stereospecificity of nicotine at certain receptors, it might likewise be possible to predict the stereospecificity of acetyl-α-methylcholine, whose absolute configuration has been worked out by Beckett *et al.* (1960). Natural (−)-nicotine is related to the natural form of the aminoacid proline and hence has the *S*-configuration (Fig. 1). By analogy the active

Fig. 1. The absolute configurations of natural (−)-nicotine (left, as ion) and (−)-*S*-α-methyl-*O*-acetylcholine (right).

form of acetyl-α-methylcholine should have the same arrangement at the carbon atom next to the onium group, that is it should have the *S*-configuration, which is known to be that of the (−)-isomer. The work of Lesser (1965), however, indicates that acetyl-α-methylcholine is not markedly stereospecific, even at sites, such as ganglia, where nicotine is distinctly stereospecific; moreover (−)-acetyl-α-methylcholine is at many sites less active than the (+)-isomer. It seems, therefore, that it is unjustifiable to compare nicotine and acetyl-α-methylcholine as has been shown in Fig. 1, and one possible explanation for this could be that the onium group in nicotine and the methylene group to which it is attached are part of a planar five-membered ring, whereas in acetyl-α-methylcholine (and also in acetyl-β-methylcholine and muscarine) they are part of a flexible aliphatic chain (though there may be some restriction on the rotation possible because of the bulk of the substituents attached to this chain). In the circumstances the substituents on the onium group in nicotine may be presented to the receptor in quite a different alignment from what they are in the other compounds. Some justification for this idea is provided by the fact that the relationships between activity and the composition of the onium group are different in nicotine from what they are in the other compounds; nicotine monomethiodide, for instance, is not appreciably more active than nicotine (Barlow & Dobson, 1955) whereas acetylcholine is much more active than (2-acetoxyethyl)dimethylamine, and the other quaternary compounds are more active than the corresponding tertiary bases.

SUMMARY

1. This paper describes a quantitative comparison of the activities of the (−)- and (+)-isomers of nicotine at sites in the peripheral nervous system.

2. Samples of the two isomers of high optical purity were tested for ability to cause contracture of the frog rectus abdominis and chick biventer muscle preparations, to block transmission on the rat diaphragm and cat tibialis anterior muscle preparations, to raise the blood pressure in rats and kittens, to block transmission in the cat superior cervical ganglion, to cause a contraction of the guinea-pig isolated ileum (which was antagonized by hexamethonium), and to inhibit the hydrolysis of acetylcholine by the acetylcholinesterase of ox red cells.

3. The difference between the activities of the two isomers varied considerably on the different preparations. Stereospecificity appeared to be greatest at receptors in ganglia, particularly at the parasympathetic ganglia in the guinea-pig ileum. It was less marked at receptors in slow fibres and less still for blocking activity at the neuromuscular junction. On preparations where stimulant activity was being compared, particularly on the guinea-pig ileum and frog rectus, doses of the (+)-isomer which were needed to cause stimulation tended to produce marked subsequent desensitization. In the experiments with acetylcholinesterase the stereospecificity appeared to be reversed, the (+)-isomer being slightly more potent than the natural (−)-isomer, but both compounds were only active in high concentrations (around 10^{-3} M).

4. From the known absolute configuration of (−)-nicotine it is suggested that at the receptors where there is marked stereospecificity to nicotine there might also be stereospecificity to acetyl-α-methylcholine and that the (−)-*S*-form of this compound may be more active than the (+)-*R*-isomer.

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