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Specificity of some ganglion stimulants

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Summary

1. The specificity of several ganglion stimulants has been tested on the isolated guinea-pig ileum by measuring the dose ratios produced by concentrations of hexamethonium.

2. Most ganglion stimulants are also active at postganglionic receptors, some as blocking agents (for example, lobeline and dimethylphenylpiperazinium), others as agonists (for example, *o*-aminophenethyltrimethylammonium and, to a lesser extent, nicotine). The most specific ganglion stimulant, with the least activity at postganglionic receptors, was *p*-aminophenethyltrimethylammonium.

3. The affinity constants of lobeline and dimethylphenylpiperazinium for the muscarine sensitive receptors in the guinea-pig ileum are $1{\cdot}05 \times 10^6$ and $3{\cdot}71 \times 10^4$, respectively.

4. The antagonism of *p*-aminophenethyltrimethylammonium by hexamethonium gave results consistent with competition up to dose ratios of about 20. Such results could also be obtained if the antagonism were non-competitive, however, provided large responses could be obtained with less than about 5% of the receptors in the ganglia activated. The affinity constant of hexamethonium is about $2{\cdot}6 \times 10^5$.

5. It is suggested that the affinity of hexamethonium can largely be ascribed to hydrophobic bonding.

Introduction

Ever since Langley (1890) used nicotine to locate autonomic ganglia, pharmacologists have used ganglion stimulants and ganglion blocking agents as tools in research. Dimethylphenylpiperazinium (DMPP) (Chen, Portman & Wickel, 1951), for example, has been extensively used as a ganglion stimulant. The usefulness of such tools, however, depends upon their specificity for receptors in ganglia. Although ganglionic receptors are clearly different in structure from the muscarine sensitive postganglionic parasympathetic receptors on smooth muscle and organs, they must have some features in common, because both are activated by acetylcholine, even though higher concentrations are required to stimulate the receptors in ganglia than those on smooth muscle or organs. Substances which stimulate ganglia may therefore be expected to have some activity also at muscarine sensitive receptors and it is important to know the difference between the concentrations which affect the two types of receptor.

A rough idea of the relative specificity of ganglion stimulants for the receptors in ganglia can be obtained by comparing their activities on a tissue containing both ganglia and postganglionic receptors, such as intestine, in the presence and in the

absence of a ganglion blocking agent. We have attempted to obtain a more precise idea of their specificity by measuring the antagonism produced by several concentrations of the ganglion blocking agent, using hexamethonium in concentrations of 5×10^{-6} to 8×10^{-5}M. If hexamethonium and the ganglion stimulants are competing for the same receptors, the dose ratio produced by a particular concentration of hexamethonium should be the same, whatever the agonist used. The graph of (dose ratio -1) against the concentration should be a straight line with a slope equal to the affinity constant. If the agonist also stimulates the muscarine sensitive receptors, this will become apparent when hexamethonium is present and higher concentrations of agonist are being used ; the antagonism will no longer appear competitive and the dose ratio will be less than expected. If the ganglion stimulant has a blocking action at the muscarine sensitive receptors, the dose ratio will be more than would be expected. These experiments will also test whether or not the blocking action of hexamethonium is consistent with competitive antagonism.

The agonists used were (−)-nicotine, dimethylphenylpiperazinium, (−)-cytisine, (−)-lobeline, choline phenylether, *o*-, *m*-, and *p*-aminophenethyltrimethylammonium and *m*-hydroxyphenylpropyltrimethylammonium. Some of the latter were extremely active on the nicotine sensitive receptors of the frog rectus muscle (Barlow & Thompson, 1969) and we were interested to know how active and how specific they were at nicotine sensitive receptors in ganglia.

Methods

The guinea-pig ileum was set up in Tyrode's solution at 37° C. To avoid desensitization by the ganglion stimulants, the interval between doses was 5 min (compared with 90 s for an agonist acting at muscarine sensitive receptors). The stimulant was in contact with the tissue for 30 seconds. Automated apparatus was used and the dose ratios were measured as described previously (Abramson, Barlow, Mustafa & Stephenson, 1969 ; Edinburgh Staff, 1970), with two dose levels of agonist tested before and after the addition of hexamethonium. Responses were obtained in the absence of hexamethonium, in the presence of two different concentrations of hexamethonium, and then again in the absence of hexamethonium. In some experiments it was possible subsequently to test a third concentration of hexamethonium. In many experiments the responses obtained in the second period in which hexamethonium was absent were similar to those obtained initially. In this situation the dose ratios were calculated using only the initial set of responses in the absence of hexamethonium. In some experiments, however, the preparation became more sensitive with time and the responses obtained in the second period in which hexamethonium was absent were bigger than those obtained initially. In this situation the dose ratio for the first concentration of hexamethonium was calculated using the first set of responses in the absence of hexamethonium ; that for the second concentration of hexamethonium was calculated using the responses obtained in the second period in which hexamethonium was absent. The responses in this latter period were always used for the calculation of the dose ratio for any third concentration of hexamethonium.

Some of the compounds were blocking postganglionic acetylcholine receptors and their affinity constants for these were measured with carbachol as agonist in the presence of hexamethonium (3×10^{-4}M), by the method of Abramson, Barlow, Mustafa & Stephenson (1969).

Drugs

The following drugs were obtained commercially: (−)-nicotine hydrogentartrate (B.D.H.), dimethylphenylpiperazinium iodide (Aldrich), (−)-lobeline sulphate (Sigma), and (−)-cytisine (Fluka). The analyses of the other compounds used are given by Barlow & Thompson (1969).

Results

The dose ratios obtained with the different concentrations of hexamethonium and the various agonists are shown in Table 1. The values obtained with a particular concentration of hexamethonium depended on the agonist used. The differences

TABLE 1. *Dose ratios produced by concentrations of hexamethonium with different agonists*

Agonist:	Dose ratio produced by hexamethonium 0·5	1·0	2·0	4·0	$8·0 \times 10^{-5}$M	Agonist conc.
(—)-Nicotine		3·4 ± 0·6 (5)	5·4 ± 0·3 (5)	9·0 ± 0·3 (4)	9·8 ± 0·8 (4)	$2·5 \times 10^{-6}$M
Dimethylphenylpiperazinium	3·4 ± 0·1 (5)		12·2 ± 0·4 (4)		121*	$2·5 \times 10^{-6}$M
(—)-Cytisine		3·1 ± 0·5 (3)	4·7 ± 1·2 (5)	8·0 ± 1·0 (4)	12·2 ± 1·2 (4)	2×10^{-5}M
(—)-Lobeline	3·7	14·6	20·5			$2·5 \times 10^{-7}$M
Choline phenyl ether		3·7 ± 0·5 (2)	6·0 ± 0·4 (2)	7·4 ± 1·0 (4)	13·1 ± 2·4 (2)	2×10^{-6}M
o-Aminophenethyltrimethylammonium		2·7	2·3 ± 0·1 (2)	2·8 ± 0·5 (2)	2·9	8×10^{-6}M
m-Aminophenethyltrimethylammonium			1·2 ± 0·0 (2)	2·0	2·8 ± 0·5 (2)	4×10^{-6}M
p-Aminophenethyltrimethylammonium	2·3	3·9 ± 0·0 (2)	7·2 ± 1·1 (3)	14·7 ± 0·7 (3)	21·0 ± 1·7 (4)	4×10^{-6}M
m-Hydroxyphenylpropyltrimethylammonium	2·3 ± 0·2 (2)	3·0 ± 0·2 (2)	6·4 ± 0·1 (2)	10·3 ± 0·6 (4)	15·9 ± 3·5 (2)	2×10^{-6}M
Calculated values	2·3	3·6	6·2	11·4	21·8	

The value expected, if the antagonism is competitive and the affinity constant of hexamethonium is $2·6 \times 10^5$, is shown for comparison at the bottom of the table. The relative activities of the agonists are indicated by the final column, which shows the concentration which usually produced small responses in the absence of hexamethonium; twice this concentration usually produced large responses, the two sets of responses forming the first part of the measurement of the dose ratio. The concentration of hexamethonium used in the experiments with dimethylphenylpiperazinium marked with an asterisk was 10^{-4}M, not 8×10^{-5}M, and the dose ratio consistent with competition is 27.

were small with low concentrations; with 10^{-5}M hexamethonium the mean dose ratios lay between 2·7 and 3·9, except when lobeline was used as the agonist, where the dose ratio was 14·6. The concentration of hexamethonium which produced a dose ratio of 2 appeared to be around $0{\cdot}5 \times 10^{-5}$M and its affinity constant therefore around 2×10^5. When the graph of log (hexamethonium concentration) was plotted against log (dose ratio − one) the results obtained with nicotine as agonist indicated that log K was 5·44 and those with *p*-aminophenethyltrimethylammonium indicated that log K was 5·40 (Fig. 1). The mean of these two values corresponds to K= $2{\cdot}6 \times 10^5$. From this the dose ratios for the various concentrations of hexamethonium were calculated, assuming the antagonism to be competitive, and these are included in Table 1 for comparison with the experimental values.

With many agonists there is a reasonable agreement between calculated and experimental figures with the lower concentrations of hexamethonium but with higher concentrations there were often big differences. This might be due to actions of the agonists at postganglionic receptors. With agonists which have some

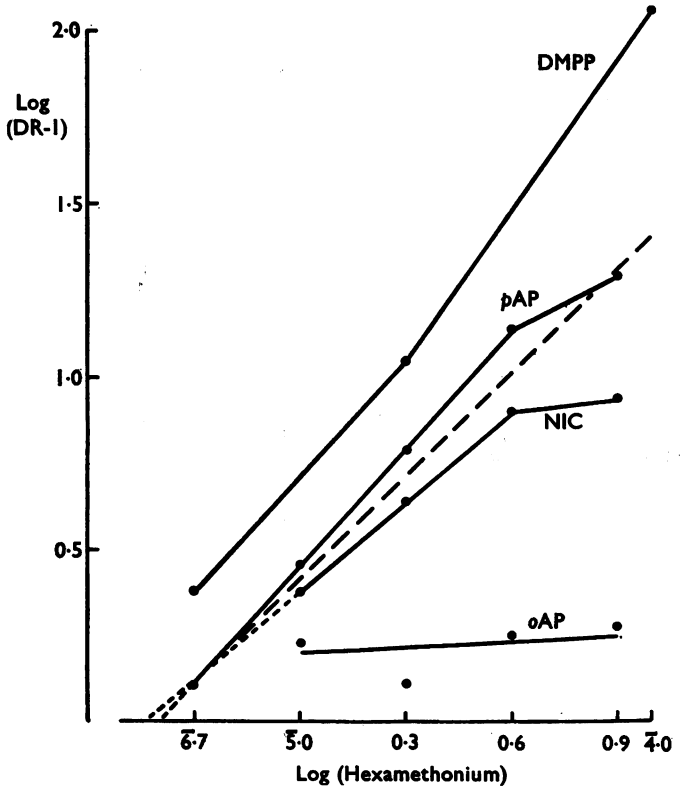

FIG. 1. Values of log (dose ratio −1) are plotted against log (hexamethonium concentration). Results are shown for nicotine (NIC), *p*-aminophenethyltrimethylammonium (*p*AP), *o*-aminophenethyltrimethylammonium (*o*AP), and dimethylphenylpiperazinium (DMPP). The dashed line (– – –) has a slope of unity and indicates the expected values if the agonist and hexamethonium are competitive and the value of log K for hexamethonium is 5·42. This is the mean of the values obtained by extrapolating the results obtained with nicotine and with *p*-aminophenethyltrimethylammonium (5·44 and 5·40, respectively). Note that with dimethylphenylpiperazinium the dose ratios are higher than is consistent with competition, indicating its atropine-like properties. With *o*-aminophenethyltrimethylammonium the dose ratios are always much lower than is consistent with competition, indicating its muscarine-like properties. The results with nicotine indicate that it too has some muscarine-like activity in high concentrations.

muscarine-like activity, the dose ratios produced by hexamethonium will be less than expected, whereas with those which have some atropine-like activity they will be more than expected.

It appeared that dimethylphenylpiperazinium and, to an even greater extent, lobeline, were blocking postganglionic acetylcholine receptors and their affinity constants for these receptors were therefore measured. With carbachol as agonist and in the presence of hexamethonium (3×10^{-4}M) the mean values of log K ($\pm$the standard error) were 4·575 ($\pm$0·022, eight estimates) for dimethylphenylpiperazinium, tested in concentrations producing dose ratios up to 10, and 6·022 ($\pm$0·051, six estimates) for lobeline, tested in concentrations producing dose ratios up to 30.

In contrast, many of the other substances tested as ganglion stimulants appeared to stimulate postganglionic receptors. The *o*- and *m*-aminophenethyl compounds were extreme examples. With these it was not possible to obtain dose ratios greater than about 3 with hexamethonium, indicating that the concentrations which stimulate postganglionic receptors are only about three times those which stimulate ganglia in the preparation. Even with nicotine there appeared to be a limit of about 10 to the dose ratio which could be obtained with hexamethonium, suggesting that the concentrations of nicotine which stimulate the postganglionic muscarine-sensitive receptors are about ten times those which stimulate the ganglia. The compound which appears to be most specific in stimulating ganglia is *p*-aminophenethyltrimethylammonium, with which it was possible to obtain results which were reasonably consistent with competition up to dose ratios of about 20.

Table 1 also shows the concentrations of the agonists which were used to produce effects in the absence of hexamethonium. This gives a rough idea of their relative potency and shows that the most active compounds are not necessarily the most specific for the receptors in ganglia.

Discussion

The results suggest that a specific ganglion stimulant is difficult to find. The reputed specificity of dimethylphenylpiperazinium probably arises from its atropine-like properties, which were observed by Bennett & Whitney (1966). Significant atropine-like effects were produced in our experiments by concentrations which were as little as two or three times those which stimulated ganglia. The value of the log affinity constant for the postganglionic acetylcholine receptors, 4·575, is comparable with the value, 4·533, for phenylacetylcholine (Abramson, Barlow, Mustafa & Stephenson, 1969); both compounds contain a single benzene ring. The much higher affinity of lobeline for these receptors (log K=6·022) is consistent with its bigger size; it contains two benzene rings. The atropine-like properties of lobeline are particularly unfortunate because it is by far the most active ganglion stimulant of the compounds tested, in that it produced responses with the most dilute solutions. The most specific of the ganglion stimulants we have studied appears to be *p*-aminophenethyltrimethylammonium, but this is slightly less active than nicotine. It remains to be seen whether it is possible to alter the structure in such a way as to increase ganglion stimulant potency without producing either muscarine-like or atropine-like activity.

The results do not provide absolutely convincing evidence that the blocking action of hexamethonium is competitive. Competitive dose ratios of up to 20 would be

obtained with a noncompetitive antagonist provided that responses could be obtained with only 5% of the receptors available to the agonist. The preparation is not a good choice for testing the competitive nature of the antagonism, however, because it is unlikely that any ganglion stimulant will be totally devoid of activity at the postganglionic muscarine sensitive receptors when the dose ratio is as high as 100. Tests to check competitive antagonism should be made on sympathetic ganglia or on parasympathetic ganglia which can be separated from postganglionic receptors. An additional practical difficulty is the need to use high concentrations of both antagonist and agonist to obtain high dose ratios because neither is particularly strong (compared, for instance, with some agonists and antagonists at muscarine sensitive receptors). It is possible to test for competition by other methods (Abramson, Barlow, Mustafa & Stephenson, 1969) but these require that a known competitive antagonist is available and the evidence that any other ganglion blocking agent is competitive is no better than that for hexamethonium.

The value of the affinity constant of hexamethonium for the receptors in ganglia, $2{\cdot}6 \times 10^5$, corresponds to a free energy of adsorption of 7·7 kcal (32·2 kJ)/mol. It seems possible that this is largely ascribable to hydrophobic bonding. There are six methylene and six methyl groups in the ion and, if these contribute the amount suggested by Tanford (1962), (0·75 kcal (3·1 kJ)/mol)/methylene group, the total would be 9·0 kcal (37·6 kJ)/mol. The affinity of pentyltrimethylammonium for the muscarine sensitive receptor (log K, 3·73; Abramson, Barlow, Mustafa & Stephenson, 1969) corresponds to a free energy of adsorption of 5·3 kcal (22·2 kJ)/mol. This ion has four methylene and four methyl groups and the free energy of adsorption is almost exactly two-thirds of that for hexamethonium at the ganglionic receptor ($7{\cdot}7 \times \frac{2}{3} = 5{\cdot}1$). It seems unlikely, therefore, that the second onium group contributes significantly to binding, and it would be interesting to test 7,7-dimethyl-*n*-octyltrimethylammonium.

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