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A COMPARISON OF CINOBUFOTENINE (THE QUATERNARY DERIVATIVE OF 5-HT) AND SOME RELATED COMPOUNDS WITH CORYNEINE (THE QUATERNARY DERIVATIVE OF DOPAMINE) ON THE FROG RECTUS, GUINEA-PIG ILEUM AND RAT FUNDUS STRIP PREPARATIONS

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1 Coryneine is 2.7 times as active as cinobufotenine on the frog rectus but on the guinea-pig ileum cinobufotenine is 1.5 times as active as coryneine. Cinobufotenine is a potent stimulant of parasympathetic ganglia and its effects are competitively antagonized by hexamethonium.

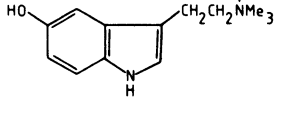
2 The effects of pH on activity relative to a standard whose ionisation is constant ($Me_4\overset{+}{N}$ or the trimethylammonium analogue of tryptamine) are consistent with the phenate form being weaker than the phenolic form but the changes are smaller than with coryneine because cinobufotenine is a weaker acid.

3 The hydroxyl group makes a large contribution to activity. Cinobufotenine is 9 times as active as the analogue without a hydroxyl on the frog rectus and 12 times as active as it on the ileum. The 5-methoxy analogue is an antagonist on the frog rectus and a very weak partial agonist on the ileum.

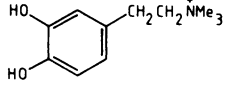
4 Cinobufotenine and the quaternary derivative of tryptamine have less than one-thousandth of the activity of 5-hydroxytryptamine on the rat fundus strip.

Introduction

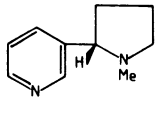
The quaternary trimethylammonium derivative of 5-hydroxytryptamine (5-HT), cinobufotenine (I), was found by Gaddum, Hameed, Hathway & Stephens (1955) to be only very feebly active at 5-HT receptors but they noted that it caused a contraction of the guinea-pig ileum similar to that produced by nicotine. In this it apparently resembles the quaternary trimethylammonium derivative of dopamine, coryneine (II), which has powerful nicotine-like properties (Barlow, Bowman, Ison & McQueen, 1974) though it has little activity at dopamine receptors (Ginsborg, House & Turnbull, 1976). Neither compound bears a particularly obvious resemblance to nicotine (III), and it is remarkable that the quaternary derivatives of amines with such different biological properties should both activate nicotine-sensitive acetylcholine receptors in parasympathetic ganglia in the ileum.



I Cinobufotenine



II Coryneine



III (-)-S-nicotine

This paper describes an attempt to obtain quantitative information about the relative activities of the two compounds and includes observations with the analogue in which the 5-hydroxy group of cinobufotenine is methylated and the analogue in which it is replaced by hydrogen (the trimethylammonium derivative of tryptamine). The effect of ionization of the phenolic group on the activity of cinobufotenine has also been studied, as in previous work with coryneine, by observing the effects of pH on its activity relative to a standard, tetramethylammonium or the quaternary derivative of tryptamine, whose ionization is unaffected by the changes in pH.

Methods

Preparations

The rectus abdominis muscle from *Rana temporaria* was set up as described by Edinburgh Staff (1974) at

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room temperature (16 to 18°C) with the contractions recorded isotonically. Direct comparisons of cinobufotenine with coryneine were made in aerated frog-Ringer solution (Edinburgh Staff, 1974) but in the experiments to determine the effects of pH on activity, the two preparations obtained from each frog were set up in aerated frog-Ringer solutions containing (mM): NaCl 115, KCl 2, $CaCl_2$ 1.8, tris(hydroxymethyl)-aminomethane 10, glucose, 11 and either 9 mM HCl or with this omitted. At room temperature these solutions had a pH of 7.8 and 9.8 respectively and experiments were done in parallel with one preparation at each pH.

Drug solutions were made up in a 10 ml stoppered measure, poured onto the preparation (the bath was first emptied) and allowed to act for 5 min; there was an interval of 16 min between doses. In each comparison two concentrations of the test drug and of the standard were found which produced small and large responses. The four concentrations were given in such a way that alternate small and large responses were obtained, to avoid extreme changes in sensitivity associated with two small or two large doses given in sequence. Because of the length of time necessary, each concentration of each compound was usually tested only twice, as in similar experiments with tetramethylammonium and coryneine (Barlow, 1976). The results were used to calculate concentrations producing matching responses and expressed as the equipotent molar ratio. The trimethylammonium derivative of 5-methoxytryptamine was found to be an antagonist and when present in a concentration of 10^{-4} M produced a dose-ratio of around 2 with tetramethylammonium as the agonist.

The guinea-pig isolated ileum preparation was set up as described by Edinburgh Staff (1974) at 37°C with the contractions recorded isotonically. Direct comparisons of cinobufotenine with coryneine were made in aerated Tyrode solution (Edinburgh Staff, 1974) but in the experiments done to determine the effects of pH on activity, preparations were set up in an aerated physiological saline (Burgen, 1965) containing (mM): NaCl 118, KCl 4.5, $MgCl_2$ 0.25, $CaCl_2$ 0.5, glucose 5, glycine 50 and either NaOH 25 mM or with this omitted. These solutions had a pH of 9.3 and 7.6, respectively. As with the frog rectus, experiments were done in parallel with one preparation at each pH.

Drugs were added by pipette and allowed to act for 90 s; there was an interval of 3 min between doses. Activity relative to the standards, tetramethylammonium or the quaternary derivative of tryptamine, was estimated in 4 × 4 assays of latin square design and the results expressed as the equipotent molar ratio. The trimethylammonium derivative of 5-methoxytryptamine was found to be an antagonist and its affinity constant was estimated in experiments with automated apparatus (Abramson, Barlow, Mustafa & Stephenson, 1969) with carbachol as agonist and also with the more specific ganglion stimulant, *m*-hydroxyphenylpropyltrimethylammonium (Barlow & Franks, 1971), as agonist. The ganglionic nature of the stimulation produced by cinobufotenine and the trimethylammonium derivative of tryptamine was tested by measuring the dose-ratios produced by hexamethonium in similar experiments.

The rat fundus strip preparation (Vane, 1957) was set up exactly as described by Edinburgh Staff (1974) in Krebs solution aerated with 95% O_2 and 5% CO_2. Contractions were recorded isotonically.

Compounds

Carbachol, hexamethonium bromide and 5-hydroxytryptamine oxalate were purchased from Sigma; at the very low concentrations of 5-HT which are needed on the rat fundus the oxalate anion is without effect. For analytical details of cinobufotenine bromide and the other related quaternary ammonium bromides see Barlow & Burston (1980). Solutions of cinobufotenine are more stable than those of coryneine. Both were made up freshly for each experiment though at pH < 7.6 these are stable for many hours. In tests at more alkaline pH the solutions were applied immediately after dilution of a stock solution (in water) with the alkaline physiological saline.

Results and Discussion

In the direct comparisons on the frog rectus the equi-

Table 1 Antagonism of cinobufotenine by hexamethonium

	Hexamethonium	
Agonist	2×10^{-5} M	4×10^{-5} M
5-HTQ	6.37 ± 0.44 (6)	14.96 ± 1.26 (3)
TQ	6.98 ± 0.48 (6)	
Coryneine		12.59 ± 1.21 (4)
Theoretical	*6.2*	*11.4*

The numbers show the dose-ratios (mean ± s.e. and number of estimates) produced by hexamethonium on the guinea-pig ileum (37°C). The numbers in italics indicate values calculated by Barlow & Franks (1971) from the estimated value 2.6×10^5 for the affinity constant of hexamethonium.
5-HTQ indicates cinobufotenine; TQ indicates the trimethylammonium analogue of tryptamine.

potent molar ratio for cinobufotenine relative to coryneine was 2.66 ± 0.126 (s.e., 6 estimates) but on the guinea-pig ileum cinobufotenine was the more active compound and the equipotent molar ratio was 0.654 ± 0.013 (s.e., 6 estimates). It is therefore a highly potent ganglion stimulant which may be relatively selective for parasympathetic ganglia. Dr D.S. McQueen (Department of Pharmacology, University of Edinburgh) kindly tested it for pressor activity in pithed rats by methods already described (Barlow *et al.*, 1974) and found the equipotent molar ratio for cinobufotenine relative to coryneine was 3.5 (mean of 3 experiments); the ratio for the trimethylammonium analogue of tryptamine relative to coryneine was 9.9 (mean of 3 experiments).

The experiments on the rat fundus confirmed the earlier reports of the low activity of cinobufotenine (Gaddum *et al.*, 1955; Barlow & Khan, 1959); the equipotent molar ratio relative to 5-HT was 1290 ± 294 (s.e., 6 estimates); the result for the trimethylammonium analogue of tryptamine was 2970 ± 462 (5) and for the 5-methoxy quaternary compound it was 7610 ± 1340 (10).

In the tests on the ileum, therefore, the effects of cinobufotenine are likely to be largely due to actions at acetylcholine receptors. These actions were competitively blocked by hexamethonium. Table 1 shows the dose-ratios produced by concentrations of hexamethonium in experiments on the ileum with cinobufotenine, the trimethylammonium analogue of tryptamine and coryneine as agonists. From the affinity constant of hexamethonium for the receptors in ganglia in the ileum (Barlow & Franks, 1971) it is possible to calculate the dose-ratios to be expected for any agonist and these are close to the experimental values. This technique has already been used to test the site of action of coryneine (Barlow *et al.*, 1974) which was also included in the present experiments.

The relative activities of cinobufotenine and coryneine will depend on the degree of ionisation of the phenolic group and should alter with pH. The phenolic form of coryneine is much more active than the ionised phenate (Barlow, 1976) but cinobufotenine is a weaker acid. In 0.1 M NaCl the pK_a of coryneine is 8.9 at 25°C and 8.7 at 37°C; the corresponding values for cinobufotenine are 10.4 and 10.3 (Barlow & Burston, 1980). The changes in pH which are possible, therefore, only alter the ionisation of cinobufotenine slightly. On the frog rectus at 16 to 18°C the change from pH 7.8 to 9.8, which should reduce the proportion of the phenolic form from 99.8% to 79.9% changed the equipotent molar ratio relative to $Me_4\overset{+}{N}$ from 0.175 ± 0.015 (s.e. 5 estimates) to 0.180 ± 0.007 (5). If the phenate form were completely inactive the ratio at the more alkaline pH should be 0.218. In similar experiments with the trimethylammonium derivative of tryptamine the equipotent molar ratio relative to $Me_4\overset{+}{N}$ was 1.682 ± 0.070 (5) at pH 7.8 and 1.654 ± 0.057 (4) at pH 9.8.

On the guinea-pig ileum at 37°C the change from pH 7.6 to 9.3 reduced the proportion of phenolic cinobufotenine from 99.8% to 90.3% and the equipotent molar ratio relative to the trimethylammonium derivative of tryptamine changed from 0.082 ± 0.004 (6) to 0.089 ± 0.014 (7). If the phenate form were completely inactive the ratio at the more alkaline pH should be 0.091. Similar experiments were made with $Me_4\overset{+}{N}$ as standard and the ratio for cinobufotenine changed from 0.056 ± 0.003 (6) to 0.119 ± 0.008 (7). This excessive change occurs because $Me_4\overset{+}{N}$ acts primarily at postganglionic (muscarine-sensitive) acetylcholine receptors which appear to be more sensitive to the effects of alkali than the ganglionic (nicotine-sensitive) receptors. The ganglionic nature of the effects of the trimethylammonium analogue of tryptamine were checked by testing with hexamethonium (Table 1).

The effects of pH are consistent therefore with the phenate form of cinobufotenine being less active than the phenolic form. The hydroxyl group makes a large contribution to activity. On the frog rectus, cinobufotenine is 1.68/0.18 = 9 times as active as the trimethylammonium analogue of tryptamine: on the guinea-pig ileum it is 1/0.08 = 12 times as active as that compound. The importance of the hydroxyl group for efficacy at these nicotine-sensitive receptors is shown by the finding that the 5-methoxy analogue is an antagonist on the frog rectus with log affinity constant = 3.95 ± 0.06 (4). On the guinea-pig ileum it is a very weak partial agonist. Its affinity for the postganglionic acetylcholine receptors was estimated in experiments with carbachol as agonist and log affinity constant = 4.34 ± 0.05 (6). It had higher affinity for the ganglionic receptors; with the ganglion stimulant *m*-hydroxyphenylpropyltrimethylammonium as agonist the mean estimate of log affinity was 4.63 ± 0.10 (6).

This work establishes the high ganglion-stimulant activity of cinobufotenine though the extent of its possible selectivity for parasympathetic ganglia requires further study. The high ganglion-stimulant activity of compounds with such diverse structures as nicotine, coryneine and cinobufotenine has some similarities with the situation observed by Crum Brown & Fraser (1869), who found that the quaternary metho salts of alkaloids with widely diverse biological properties were all neuromuscular blocking agents. However, there is the difference that in the present situation, methylation has produced agonists rather than antagonists. This is particularly remarkable in view of the large size of cinobufotenine. Its apparent molal volume is bigger than that of coryneine by more than 20 cm^3/mole and about 40 cm^3/mole bigger than nicotine (Barlow, unpublished). Increased

size is usually associated with increased affinity and the emergence of antagonist activity, as can be seen, for instance, in homologous *n*-alkyltrimethylammonium salts (Stephenson, 1956).

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