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The effects of anti-Parkinson drugs on cortical neurones

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Summary

1. The effects of a potential anti-Parkinson drug, benapryzine, have been compared with those of benzhexol, atropine and procaine on the excitatory responses induced by acetylcholine and L-glutamate on feline cortical neurones using the microiontophoretic technique.

2. All the drugs tested reduced the excitatory responses evoked by acetylcholine and L-glutamate. However, benapryzine, benzhexol and procaine more effectively reduced the excitatory responses to L-glutamate than those to acetylcholine whereas atropine was more effective against acetylcholine-induced excitation.

3. In the presence of procaine the amplitude of the extracellular spikes was decreased. This effect was also observed during applications of benapryzine and benzhexol.

4. Tests on the isolated frog sciatic nerve indicated that benapryzine and benzhexol had local anaesthetic actions respectively greater than and equivalent to those of procaine.

5. It was concluded that the effects of benapryzine and benzhexol on cortical neurones were probably related to their local anaesthetic properties. The possibility that a local anaesthetic action may account for the effects of these drugs and of many other commonly used anti-Parkinson drugs in Parkinson's disease is discussed.

Introduction

Benapryzine (*N*-n-propyl-*N*-ethylaminoethyl benzilate hydrochloride) is a potential anti-Parkinson drug presently undergoing clinical trials. It is of particular interest since though it reduces oxotremorine-induced tremor it possesses little peripheral anti-cholinergic action in contrast to many of the commonly used anti-Parkinson drugs (Hughes & Spicer, 1969 ; Brown, Hughes & Mehta, 1969 ; Leslie & Conway, 1970). However, the beneficial effects in Parkinson's disease of many of the anti-Parkinson drugs are often considered to be due to their central anticholinergic actions (Klawans, 1968). Thus the effects of benapryzine have been compared with those of the anti-Parkinson drugs, benzhexol and atropine on the excitatory responses to acetylcholine and L-glutamate on feline cerebral cortical neurones using the microiontophoretic technique.

The effects of procaine have also been examined and compared with those of benapryzine on cortical neurones since observations made during the early part of this investigation indicated that benapryzine might have local anaesthetic proper-

ties. In addition, the local anaesthetic potencies of the drugs investigated were determined on the isolated frog sciatic nerve preparation.

Though many of the anti-Parkinson drugs are thought to act in the striatum (Duvoisin, 1967) the present study was carried out in the cerebral cortex because available evidence suggests that the pharmacology of neurones in the cortex is similar to that in the striatum and the former is more easily accessible.

A brief report of some of these results has already appeared (Clarke, Davies & Straughan, 1972).

Methods

Experiments were performed on 38 cats of either sex, weighing 2·5–3·5 kg. Anaesthesia was induced by N_2O-halothane and was maintained with 60% nitrous oxide in oxygen and 1% halothane. The animals' EEG, ECG, respiratory rate, rectal temperature (maintained at 38° C with the aid of a heating blanket) and femoral blood pressure were monitored routinely throughout the experiment.

The anterior suprasylvian gyrus was exposed and cortical pulsations reduced by means of a transparent plastic pressor plate (Krnjević & Phillis, 1963a). Extracellular action potentials were recorded through the centre barrel (containing 2 M NaCl) of 7 barrel micropipettes with overall tip diameters of 4–9 μm. The spikes were amplified, displayed on an oscilloscope and recorded on magnetic tape. The number of spikes in a 2, 5 or 10 s epoch were electronically counted and displayed on a pen recorder trace. A conventional analogue rate meter was also used for measuring the frequency of firing.

The following drugs were used in aqueous solution in the micropipettes: acetylcholine chloride 500 mM, L-glutamate 200 mM (adjusted to pH 8 with NaOH), benapryzine hydrochloride (BRL-1288) 20 mM, benzhexol hydrochloride 20 mM, atropine sulphate 20 mM and procaine hydrochloride 20 mM. Acetylcholine, L-glutamate and benapryzine were used routinely in three barrels of the micropipettes, a fourth barrel containing either benzhexol, atropine or procaine. The remaining two drug barrels contained 2 M NaCl and were used for passing neutralizing currents so that the net current flow at the microelectrode tip was kept at zero during ejection and retention of drug ions.

In order to obtain meaningful data from these experiments the following procedures were adhered to throughout the investigations:

1. Neurones were excited by regular applications of acetylcholine and/or L-glutamate with currents sufficient to produce a plateau response before the application was terminated. The iontophoretic currents and durations of application required to obtain these responses usually fell within the range of 25–50 nA and 15–45 s respectively for both acetylcholine and L-glutamate.

2. The test drugs were applied 15 s before and concurrently with the excitants since this regimen gave the most consistent and reproducible results. In particular this short application of test drugs favoured rapid recovery of the control response which is often difficult to achieve with potent drugs such as atropine.

3. Depression of firing caused by the test drugs was measured by expressing the total spike count during the application of the excitant in the presence of the drug as a percentage of the total spike count during the previous application of the excitant alone.

4. Since there were often considerable variations in the control responses only test drug depressions exceeding 20% of the control excitant response were accepted as being meaningful.

5. As the ejection characteristics of different microelectrodes vary (Cole & Halpern, 1969; Bradley & Candy, 1970; Hoffer, Neff & Siggins, 1971), comparisons of the effects of the test drugs on the excitatory responses to acetylcholine and L-glutamate were made either on (a) the same neurone, or (b) on individual cholinoceptive and non-cholinoceptive neurones using the same microelectrodes. An identical procedure was adopted when comparing benapryzine with the other test drugs.

6. All the neurones encountered in this study were tested for current sensitivity by passing positive and negative currents through one of the NaCl barrels. If the firing rate of a neurone was appreciably affected by current it was abandoned.

Local anaesthesia of nerve

The local anaesthetic properties of benapryzine, benzhexol, procaine and atropine were compared on the isolated desheathed frog sciatic nerve preparation at room temperature (Ritchie, Ritchie & Greengard, 1965). The drugs were dissolved in frog Ringer and adjusted to pH 7·2. Desheathed nerves were mounted in a Perspex bath so that the central portion was immersed in 0·1 ml of the drug solution. One end of the nerve was kept in moist air and stimulated with rectangular pulses. Nerve action potentials were recorded from the other end, displayed on an oscilloscope and averaged using a Biomac 1000 computer. The strength of the test drugs was adjusted to equieffective concentrations causing a 50–60% reduction in the height of the action potential within 10 minutes. At this time the drug solution was replaced by frog Ringer and recovery time was noted.

Results

Most of the cholinoceptive neurones encountered during this investigation were spontaneously firing cells occurring at depths of 750–1,500 μm. Extracellular recordings were made from 96 of these neurones, the majority of which were sensitive to currents of 25–50 nA acetylcholine and exhibited a characteristic delay in both the onset and cessation of excitation in response to iontophoretic application of acetylcholine. No attempt was made to identify these neurones but in the light of previous reports they were probably Betz cells (Krnjević & Phillis, 1963b; Crawford & Curtis, 1966). L-Glutamate excited most of the neurones found between depths of 150–1,800 μm and the effects of drugs on over 200 of these were studied. Currents of 25–50 nA L-glutamate were usually sufficient to cause a substantial increase in the firing rate of these neurones. However, relatively few (20%) of the cholinoceptive neurones could be consistently excited with L-glutamate.

Benapryzine

Short applications of 25–50 nA benapryzine for 30–60 s reduced the acetylcholine-evoked excitation of 42 out of 47 neurones and the L-glutamate-evoked excitation of 95 out of 97 neurones. With respect to time course and potency the effects of benapryzine on the response to acetylcholine or L-glutamate were similar whether compared on neurones sensitive to both acetylcholine and L-glutamate or on

separate populations of cells responsive to each excitant alone. On terminating the application of benapryzine the excitatory response to acetylcholine recovered rapidly within about 1·5 min, in contrast full recovery of L-glutamate induced excitation was often slow usually taking about 2–4 min (Fig. 1). This reduction in acetylcho-

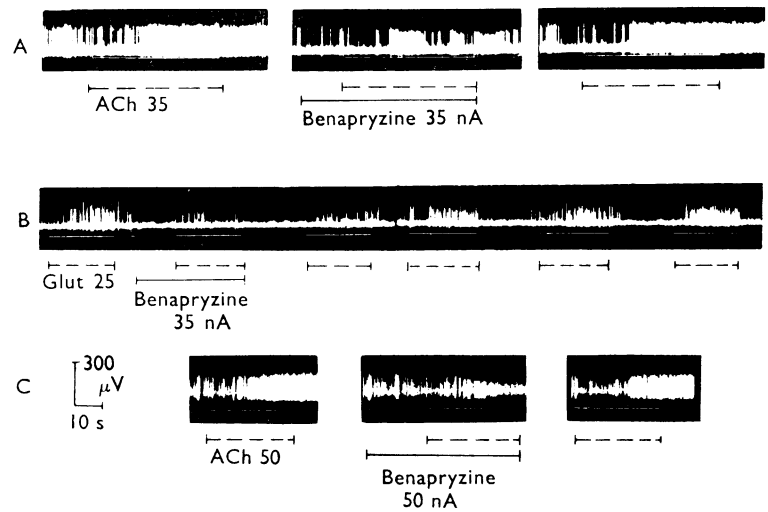


FIG. 1. Effects of benapryzine on single cortical neurones. A. Reduction of acetylcholine (ACh, 35 nA)-induced discharge during the iontophoretic application of benapryzine (middle record); B. depressant action of benapryzine on excitation by L-glutamate, note reduction in spike height during benapryzine application and delayed recovery of firing rate after benapryzine application terminated (c.f. Fig. 1A); C. reduction in spike height and firing rate during application of benapryzine to a neurone excited by acetylcholine 50 nA: periods of application of excitant and depressant are indicated by horizontal broken and full lines respectively.

line or L-glutamate-induced response was often accompanied by a decrease in the amplitude of the extracellular action potential and was particularly evident when large currents (50 nA or more) of benapryzine were used (Fig. 1B & 1C). Characteristically, on stopping the application of benapryzine the spike height recovered more rapidly than the firing rate. Occasionally, the depression of acetylcholine or L-glutamate-evoked excitation by benapryzine was preceded by an increase in the discharge rate of the cell. A similar phenomenon was observed with all the test drugs used. This paradoxical excitation is probably comparable to that reported with a wide range of depressant substances (Krnjević & Phillis, 1963c).

The effects of benapryzine on the response of neurones to acetylcholine or L-glutamate seemed to be independent of the current applying the excitant and of the firing rate of the neurones. This point is illustrated in Fig. 2, which represents the combined data from 18 cells on which the effects of various currents of benapryzine were tested on the excitatory responses to first 25 nA acetylcholine then 50 nA acetylcholine. The mean firing rates of these neurones were $15{\cdot}6 \pm 7{\cdot}1$ (spikes/s $\pm$ S.D.) and $27{\cdot}8 \pm 7{\cdot}9$ (spikes/s $\pm$ S.D.) during applications of 25 and 50 nA acetylcholine respectively. The slopes of the two lines are not significantly different.

Benapryzine more effectively reduced the sensitivity of neurones to the excitatory effects of L-glutamate than to those of acetylcholine. This is illustrated in Table 1

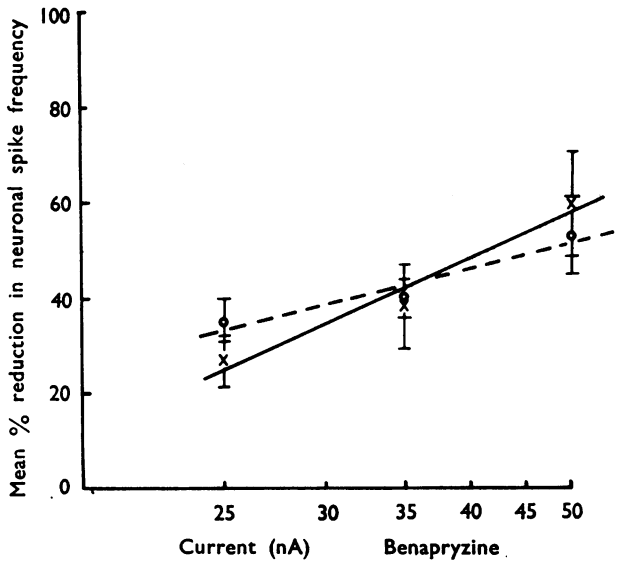


FIG. 2. Log current (dose) response lines to benapryzine on 18 neurones excited by acetylcholine 25 nA (×——×) and 50 nA (○——○). The vertical lines represent the S.D. of each mean. The mean firing rates of the neurones were 12·2±4·5 (S.D.) and 27·0±7·0 spikes/s with excitant currents of 25 and 50 nA acetylcholine respectively.

TABLE 1. *Depressant effect of benapryzine on single cortical neurones excited by acetylcholine and/or L-glutamate*

Excitant (mean current nA)	No. of cells	Depressant—benapryzine Mean depressant current (nA±S.D.)	Mean % depression (±S.D.)
Acetylcholine	7*	60±28·1	57±14·0
(40)	6†	48±16·3	55± 8·2
L-glutamate	7*	25±10·9	58±11·9
(33)	6†	27±11·7	56± 9·7

* Neurones sensitive to one excitant only. † Neurones sensitive to both acetylcholine and L-glutamate.

which shows the currents of benapryzine required to produce a similar percentage depression in the firing rate of neurones excited by acetylcholine or L-glutamate or both excitants. It can be seen from this table that on a current basis benapryzine is approximately twice as effective in depressing L-glutamate-evoked than acetylcholine-evoked excitation. Moreover, it will be observed that the differential potency of benapryzine on glutamate-evoked and acetylcholine-evoked excitation is similar whether tested on neurones sensitive to only one or both excitants. A typical record from a neurone sensitive to both acetylcholine and L-glutamate is shown in Figure 3.

Atropine

On cholinoceptive neurones the acetylcholine response was particularly sensitive to the depressant action of iontophoretically applied atropine. Thus, even short applications of atropine with currents as small as 5 nA were often sufficient to reduce acetylcholine-evoked excitation for periods up to 10 minutes. Very much higher currents of atropine were required to have any effect on L-glutamate-induced

excitations and these responses, unlike the acetylcholine responses, rapidly recovered on terminating the application of atropine. Figure 4 illustrates some of the characteristic effects of atropine on cortical neurones.

When the depressant effects of benapryzine and atropine expelled from the same electrode were compared on a current basis atropine was consistently more potent than benapryzine in reducing acetylcholine-evoked responses and consistently less potent than benapryzine in reducing L-glutamate-evoked responses (e.g. Fig. 4). Similar results were obtained when atropine and benapryzine were compared on neurones excited by both acetylcholine and L-glutamate and on separate populations

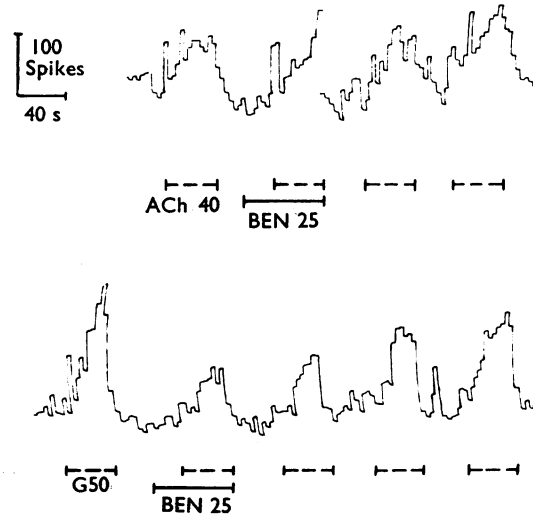


FIG. 3. Ratemeter record showing the effects of benapryzine (BEN 25 nA) on the excitatory responses to acetylcholine (ACh 40 nA) and L-glutamate (G50 nA) on the same neurone. This neurone was discharging spontaneously at a rate of 5–15 spikes/second. Each horizontal peak on the record corresponds to the mean firing rate during a 5 s epoch. Periods of application of excitant drugs are represented by broken lines and those for benapryzine by continuous lines below the records.

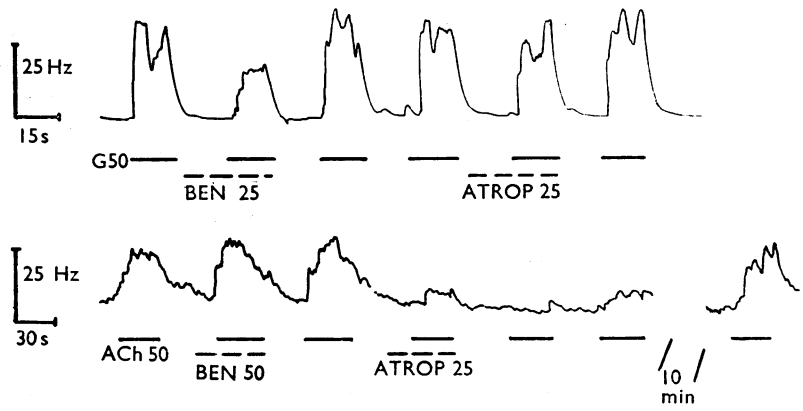


FIG. 4. Ratemeter records showing (upper trace) the effects of benapryzine (BEN 25 nA) and atropine (ATROP 25 nA) on a cell firing in response to L-glutamate (G50 nA), and (lower trace) a second cell in the same microelectrode track firing in response to acetylcholine (ACh 50 nA). Iontophoretic drug applications are indicated by solid and broken lines below the records. Note the difference in time scales and also the time break in the lower record.

of neurones responding mainly to acetylcholine or mainly to L-glutamate. Thus it can be seen from Table 2 that on a current basis atropine was about twice as effective as benapryzine in decreasing the sensitivity of neurones to acetylcholine and two-thirds as effective as benapryzine in decreasing the sensitivity of neurones to L-glutamate. Further, unlike benapryzine, atropine did not cause a reduction in spike heights even when applied with iontophoretic currents of 50 nA or more.

Benzhexol

The anti-Parkinson drug benzhexol depressed the firing rate of both acetylcholine- and L-glutamate-sensitive neurones. The characteristics of these depressant effects including the effects on spike heights were similar in every respect to those previously described with benapryzine. Like benapryzine, benzhexol effectively reduced the excitatory effects of L-glutamate more than those of acetylcholine. On a current basis, benzhexol was not significantly different in potency from benapryzine in reducing the sensitivity of neurones to acetylcholine and L-glutamate (Table 2).

Procaine

When applied iontophoretically to cerebral cortical neurones the depressant effects of procaine on acetylcholine and L-glutamate-evoked responses resembled those of benapryzine and benzhexol. Thus the excitatory responses to L-glutamate were reduced more than the excitatory responses to acetylcholine by procaine and this effect was usually accompanied by a reduction in spike height. Compared with benapryzine, procaine was equipotent, on a current basis, in reducing L-glutamate-evoked responses but approximately half as potent in reducing acetylcholine-evoked responses (Table 2).

TABLE 2. *Relative potencies of drugs in decreasing the excitatory responses induced by acetylcholine and L-glutamate on single cortical neurones (potency of benapryzine taken as* 1·0)

	Relative potency (benapryzine=1·0)			
	Acetylcholine excitations		L-Glutamate excitations	
Drugs	No. of tests	Mean±S.E.	No. of tests	Mean±S.E.
Atropine	10	0·66±0·05†	10	1·48±0·05*
Benzhexol	9	1·14±0·12	8	1·21±0·17
Procaine	7	2·15±0·15†	9	1·19±0·14

* $P<0.05$ and † $P<0.01$ compared with benapryzine using Student's t test.

In one experiment procaine and benapryzine were administered intravenously to a cat, via the femoral vein, while observing the excitatory responses of a single cortical cell to alternate iontophoretic applications of L-glutamate and acetylcholine. The results obtained were qualitatively similar to those obtained when the drugs were applied iontophoretically. Procaine at a dose of 5 mg/kg markedly reduced the sensitivity of the neurone to L-glutamate but had little effect on its sensitivity to acetycholine. This effect of procaine persisted for about 10 minutes. Benapryzine at a dose of 2 mg/kg initially almost completely abolished the excitatory effects of the same neurone to both acetylcholine and L-glutamate. However, the acetylcholine response completely returned within 4 min, whereas the response to L-glutamate required a further 40–60 min before it returned to the pre-injection level (Fig. 5).

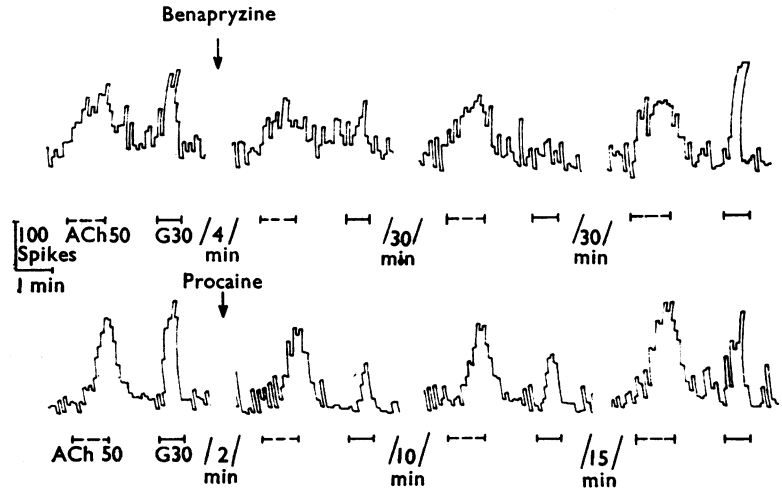


FIG. 5. Ratemeter record showing the effects of an intravenous injection of benapryzine 2 mg/kg (at arrow—upper record) and procaine 5 mg/kg (at arrow—lower record) on a single cortical neurone excited by alternate iontophoretic applications of acetylcholine (ACh 50 nA) and L-glutamate (G30 nA). Iontophoretic drug applications are indicated by broken and solid bars. The time breaks in the record are indicated between oblique lines below the record.

TABLE 3. *Concentrations of drugs causing a 50–60% reduction in the height of the compound action potential of the isolated frog sciatic nerve within 10 minutes*

Drug	No. of tests	Mean Conc. (mM±S.D.)	Potency ratio	Mean recovery time (min)
Benapryzine	11	0·468± 0·28	1	32·4±8·3
Benzhexol	9	1·27 ± 0·5	2·6	21·4±7·8
Procaine	10	1·35 ± 0·62	2·8	21·1±6·0
Atropine	4	62·5 ± 25·0	128·6	18·0±6·7

Local anaesthetic properties

All the drugs used exhibited local anaesthetic properties to a greater or lesser extent on the isolated frog sciatic nerve (Table 3). When compared with procaine, benapryzine was more than twice as effective ($P<0·01$), benzhexol equally effective and atropine less than 50 times as effective in reducing the size of the electrically-evoked action potential in the sciatic nerve. The order of potency of the drugs in this test is roughly similar to their order of potency in reducing the sensitivity of cortical neurones to L-glutamate and (with the exception of atropine) acetylcholine (cf. Table 2).

Discussion

The present results show that the potential anti-Parkinson drug benapryzine is not a specific antagonist of acetylcholine in the cat cerebral cortex since it reduced the local excitatory effects of L-glutamate more effectively than those of acetylcholine. This is in keeping with reports that benapryzine has little anti-cholinergic activity in the periphery compared with other commonly used anti-Parkinson drugs (Brown *et al.*, 1969; Leslie & Conway, 1970). However, the finding that benzhexol was also non-selective in its action on acetylcholine-evoked excitation in the cortex was unexpected since the beneficial effect of this drug in Parkinson's

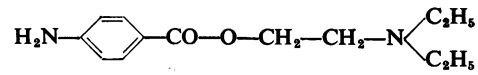
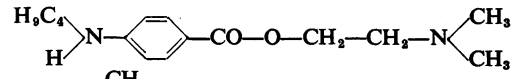
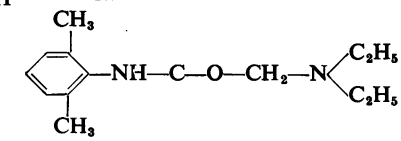
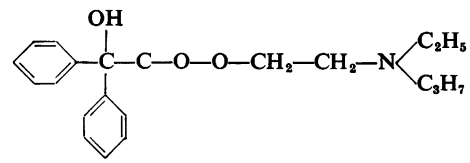
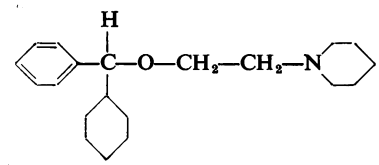
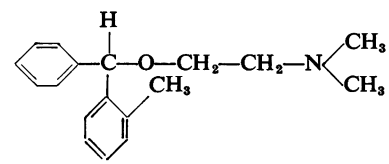
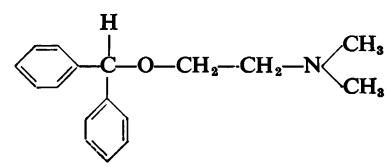
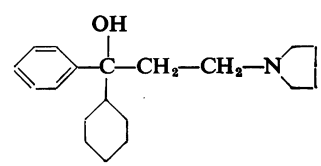
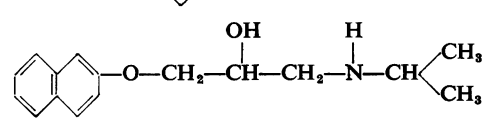
disease has been assumed to be related to its anti-cholinergic properties (Klawans, 1968). It could be argued that the apparent lack of specificity of benapryzine and benzhexol as central acetylcholine antagonists in this study might be attributed to differences between cholinoceptive cells in the cortex and those in the caudate where many of the anti-Parkinson drugs have been suggested to act (Duvoisin, 1967). Though this explanation cannot be excluded it seems unlikely as the characteristics of the neuronal response to acetylcholine in this study are similar to those reported by other investigators in the cortex and caudate nucleus (Krnjević & Phillis, 1963b; Crawford & Curtis, 1966; McLennan & York, 1966).

It was frequently observed that the reduction in sensitivity of neurones to the excitatory effects of acetylcholine and L-glutamate in the presence of benapryzine or benzhexol was accompanied by a reduction in the amplitude of the extracellular action potentials. Such an effect is typical of local anaesthetics whether applied to peripheral nerves or central neurones (Moore & Narahashi, 1967; Curtis & Phillis, 1960; Krnjević & Phillis, 1963a; Galindo, 1969). This indicates that the effects of benapryzine and benzhexol may be due to a local anaesthetic type action. In this respect, it is noteworthy that benapryzine is structurally similar to procaine (Table 4). This proposal is further reinforced by the demonstration that benapryzine and benzhexol exhibited local anaesthetic actions respectively greater than, and equivalent to, those of procaine on the frog sciatic nerve and that the order of potency of all three drugs in this test was similar to their order of effectiveness, on a current basis, in reducing the sensitivity of cortical neurones to L-glutamate and acetylcholine. However, it should be stressed that, in iontophoretic experiments, rates of release of different drug ions from microelectrodes are not necessarily similar despite the use of equal electrophoretic currents (Curtis, 1964). Consequently, the true order of effectiveness of the drugs used in this investigation in reducing the sensitivity of central neurones to excitants in terms of their extracellular concentrations might well be different from that given by the ratios of ejecting currents.

A further similarity between the effects of procaine and those of benapryzine and benzhexol was that they were all more effective depressants of L-glutamate-induced excitation than acetylcholine-induced excitation. This effect could be explained on the basis of a local anaesthetic action since the depolarizing action of L-glutamate on neuronal membranes has been suggested to be mainly due to an increased permeability of the membrane to sodium ions (Krnjević, 1970), which would be antagonized by a local anaesthetic (Rothstein, 1968). In contrast, acetylcholine-induced excitation has been suggested to involve a reduction in potassium conductance (Krnjević, Pumain & Renaud, 1971), which would be less sensitive to a local anaesthetic (Hille, 1966; Rothstein, 1968). On the other hand, the differential potencies of these drugs on the excitatory responses caused by acetylcholine and L-glutamate may be a reflection of the distribution of acetylcholine and L-glutamate receptive areas on the neurones relative to the orifice of the microelectrode tip (Crawford & Curtis, 1966).

Though atropine has been reported as having local anaesthetic properties in high doses in the central nervous system (Curtis & Phillis, 1960; Krnjević & Phillis, 1963a) in the present experiments depression of the sensitivity of cortical neurones to acetylcholine or L-glutamate by atropine was not accompanied by a reduction in spike height. Further, its local anaesthetic potency was about one-fiftieth that of

TABLE 4. *Chemical structure of some local anaesthetics and anti-Parkinson drugs*

Drug	Chemical structure	Classification
Procaine		Local anaesthetic
Tetracaine		Local anaesthetic
Lignocaine		Local anaesthetic
Benapryzine		Anti-Parkinson drug
Benzhexol		Anti-Parkinson drug
Orphenadrine		Anti-Parkinson drug
Diphenhydramine		Anti-Parkinson drug (anti-histaminic)
Procyclidine		Anti-Parkinson drug
Propranolol		Anti-Parkinson drug (β-adrenoceptor blocker)

procaine in the present tests. This suggests that the depressant effects of large doses of atropine in the cerebral cortex involved effects unrelated to its local anaesthetic properties.

Whether the beneficial effects of benapryzine or benzhexol in Parkinson's disease are related to their local anaesthetic properties is not known. It is interesting to note, however, that local injections of procaine into the brain have been employed

empirically to dissect out the areas involved in Parkinson's disease (Cooper, 1954). In addition, intracaudate injections of procaine and tetracaine have been shown to abolish physostigmine-induced tremors in cats (Lalley, Rossi & Baker, 1971). A further point of interest is that, if benapryzine and benzhexol are acting as local anaesthetics in Parkinson's disease, it is possible that many of the other drugs used in the treatment of this disease would exert a similar action since they have structural features in common with local anaesthetics (Table 4).

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