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EFFECTS OF DRUGS ON THE UPTAKE OF ACETYLCHOLINE IN RAT BRAIN CORTEX SLICES

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Abstract—The following drugs inhibit competitively uptake of acetylcholine against a concentration gradient into rat brain cortex slices incubated aerobically at 37° in a physiological saline–glucose medium containing 20 μM paraoxon: eserine ($K_i = 0{\cdot}9 \times 10^{-5}$M), tetramethylammonium chloride ($K_i = 0{\cdot}4 \times 10^{-5}$M), tetraethylammonium chloride ($K_i = 0{\cdot}05 \times 10^{-5}$M), atropine ($K_i = 1{\cdot}8 \times 10^{-5}$M), cocaine ($K_i = 0{\cdot}33 \times 10^{-5}$M), procaine ($K_i = 0{\cdot}23 \times 10^{-5}$M), lidocaine ($K_i = 0{\cdot}09 \times 10^{-5}$M), chlorprocaine ($K_i = 0{\cdot}05 \times 10^{-5}$M), methacholine ($K_i = 0{\cdot}04 \times 10^{-5}$M), succinylcholine ($K_i = 0{\cdot}6 \times 10^{-5}$M), *d*-tubocurarine ($K_i = 0{\cdot}6 \times 10^{-5}$M), hexamethonium ($K_i = 1{\cdot}2 \times 10^{-5}$M), pilocarpine ($K_i = 0{\cdot}5 \times 10^{-5}$M), nicotine ($K_i = 0{\cdot}6 \times 10^{-5}$M), strychnine ($K_i = 0{\cdot}6 \times 10^{-5}$M), and hemicholinium ($K_i = 0{\cdot}6 \times 10^{-5}$M). The following drugs inhibit noncompetitively: chlorpromazine and amphetamine. A comparison of the inhibitor constants of a number of these drugs, which act competitively on acetylcholine transport, with the inhibitor constants towards acetylcholine esterase or certain neurophysiological acetylcholine receptor sites leads to the conclusion that the transport carrier site for acetylcholine is not identical in chemical structure with the anionic site of acetylcholine esterase or with the acetylcholine receptor sites. The noncompetitive effects of certain drugs suggest the presence of a site on the transport carrier, with an affinity for these drugs, which affects the reactions of acetylcholine at the brain cell membrane.

THE UPTAKE of acetylcholine into brain slices incubated aerobically at 37° in a physiological saline medium containing glucose, and in the presence of a suitable choline esterase inhibitor, consists of several processes—a passive diffusion process independent of metabolism, possibly an exchange diffusion process which may be significant at low external concentrations of acetylcholine, and a process of uptake against a concentration gradient which is energy dependent[1] and probably controlled by operation of the sodium pump.[2] Using mouse brain cortex slices, in the presence of 1 μM Sarin, it was found that the rate of uptake of acetylcholine (above that due to passive diffusion) depends on the acetylcholine concentration according to the Michaelis–Menten equation. It was found, moreover,[1] that the following drugs acted as competitive inhibitors of the uptake: hemicholinium 3 (HC-3) ($K_i = 0{\cdot}5 \times 10^{-5}$), eserine ($K_i = 0{\cdot}7 \times 10^{-5}$), atropine ($K_i = 1{\cdot}6 \times 10^{-5}$), oxotremorine ($K_i = 2{\cdot}3 \times 10^{-5}$) and morphine ($K_i = 3{\cdot}0 \times 10^{-5}$). Pentobarbitone was without effect.

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We have carried out experiments on the effects of local anesthetics, *d*-tubocurarine, amytal and other drugs on the concentrative uptake of acetylcholine in rat brain cortex slices in the presence of 20 μM paraoxon, and the results are described in this communication.

METHODS AND MATERIALS

Full details of the methods we have employed and of the materials we have used are given in our accompanying paper.[2] Drugs were dissolved in 0·1 ml Krebs–Ringer phosphate medium (pH 7·4) and added, at an appropriate concentration, to the solution (0·1 ml) of radioactive acetylcholine in the side tube of Warburg manometric vessels. They were tipped into the main vessel at the commencement of the experimental incubation period immediately after temperature equilibrium (37°). The main vessel contained 2·8 ml Krebs–Ringer phosphate medium, in which rat brain cortex slices (approximately 50 mg wet wt.) were suspended, and which also contained 10 mM glucose and 20 μM paraoxon. The vessels were gassed with oxygen and evolved CO_2 was absorbed by potassium hydroxide on rolls of filter paper in the centre well of the manometric vessel. Usually 10–15 min were allowed for temperature equilibration before acetylcholine, with or without the drug, was tipped into the vessel. Details of the manner in which the amount of labeled acetylcholine taken up by the tissue, after 1 hr incubation, was estimated have been described.[2] Corrections were made routinely for tissue swelling.

Representation of results

Results are recorded in Table 1 which give a list of inhibitor constants derived from Lineweaver–Burk curves in which reciprocals of the amounts of uptake of labeled acetylcholine per 100 mg wet wt. tissue, corrected for passive diffusion, and of the

TABLE 1. INHIBITOR CONSTANTS (K_i) FOR VARIOUS DRUGS THAT ACT AS COMPETITIVE INHIBITORS OF THE UPTAKE OF ACETYLCHOLINE (CORRECTED FOR PASSIVE DIFFUSION) IN RAT BRAIN CORTEX SLICES IN THE PRESENCE OF 20 μM PARAOXON, AND ON ACETYLCHOLINE ESTERASE

	K_i (M)		
	Acetylcholine transport carrier	Acetylcholine esterase	Ref.
Eserine	$0{\cdot}9 \times 10^{-5}$	5×10^{-8}	7
Strychnine	$0{\cdot}6 \times 10^{-5}$	$2\text{–}3 \times 10^{-5}$	8
Tetramethylammonium chloride	$0{\cdot}4 \times 10^{-5}$	$16{\cdot}2 \times 10^{-4}$ $23{\cdot}3 \times 10^{-4}$(25°)	10 9
Tetraethylammonium chloride	$0{\cdot}05 \times 10^{-5}$	45×10^{-5}	10
d-Tubocurarine	$0{\cdot}6 \times 10^{-5}$	$2{\cdot}5 \times 10^{-6}$	11
Atropine	$1{\cdot}8 \times 10^{-5}$		
Cocaine	$0{\cdot}33 \times 10^{-5}$		
Procaine	$0{\cdot}23 \times 10^{-5}$		
Lidocaine	$0{\cdot}09 \times 10^{-5}$		
Chlorprocaine	$0{\cdot}05 \times 10^{-5}$		
Methacholine	$0{\cdot}04 \times 10^{-5}$		
Succinylcholine	$0{\cdot}6 \times 10^{-5}$		
Hexamethonium	$1{\cdot}2 \times 10^{-5}$		
Pilocarpine	$0{\cdot}5 \times 10^{-5}$		
Nicotine	$0{\cdot}6 \times 10^{-5}$		
Hemicholinium 3 (HC-3)	$0{\cdot}6 \times 10^{-5}$		

concentrations of acetylcholine are plotted. Under our experimental conditions, for an incubation period of 1 hr, the amount taken up by passive diffusion in 100 mg tissue is 0·89 C_{EX} where C_{EX} is the amount of acetylcholine in 0·1 ml medium. As already found with mouse brain slices,[1] the Lineweaver–Burk plots are linear within experimental error. The standard deviations from the mean are shown in the plots and usually do not amount to values greater than ± 5 per cent. The values recorded are the means of at least four independent observations. Results giving some typical Lineweaver–Burk plots, obtained in the presence of drugs that act as competitive inhibitors of uptake, are shown in Fig. 1.

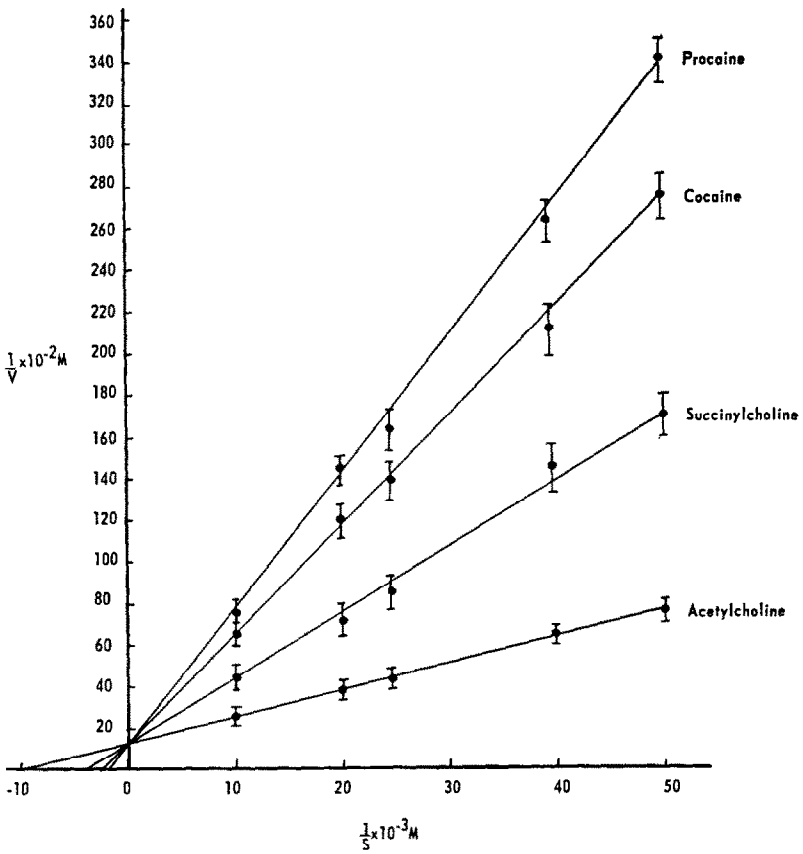

FIG. 1. Variation of the reciprocal of uptake of acetylcholine (corrected for passive diffusion) into rat brain cortex slices, in the presence of 20 μM paraoxon, with the reciprocal of the corresponding acetylcholine concentration. Effects of succinylcholine, cocaine and procaine, each at 10 μM.

RESULTS

Results given in Fig. 1 show a linear relationship between the reciprocals of the uptake of labeled acetylcholine corrected for passive diffusion and of the corresponding labeled acetylcholine concentration in the medium. This is in accordance with

the results of Schuberth and Sundwall[1] who indicated that the relationship follows the Michaelis–Menten equation:

$$\frac{1}{v} = \frac{K_s}{VS} + \frac{1}{V}$$

where v = uptake of labeled acetylcholine corrected for passive diffusion, K_s = apparent Michaelis constant, S = concentration of labeled acetylcholine in the medium, and V = maximum uptake of labeled acetylcholine as given by the intercept on the y axis. The apparent Michaelis constant is given by the intercept on the x axis, from which $K_s = 10{\cdot}8 \times 10^{-5}$M. The value found with mouse brain cortex slices in the presence of 1 μM Sarin is $K_s = 8{\cdot}25 \times 10^{-5}$M.[1]

In the presence of eserine (10 μM), the Lineweaver–Burk curve, derived from the equation:

$$\frac{1}{v} = \frac{K_s}{VS}\left(1 + \frac{I}{K_i}\right) + \frac{1}{V}$$

where I = concentration of inhibitor, S = concentration of acetylcholine, and K_i = inhibitor constant, crosses the ordinate at the same point as that in the absence of eserine, indicating competitive inhibition, as already demonstrated with mouse brain cortex slices.[1] K_i, for eserine, as determined from the intercept on the X axis equals $0{\cdot}9 \times 10^{-5}$M (Table 1). The value found for mouse brain cortex slices, in the presence of 1 μM Sarin, equals $0{\cdot}7 \times 10^{-5}$M.[1]

Tetramethylammonium and tetraethylammonium ions

Both tetramethylammonium chloride (10 μM) and tetraethylammonium chloride (10 μM) bring about competitive inhibition of the uptake of acetylcholine (corrected for passive diffusion) in rat brain cortex slices in the presence of 20 μM paraoxon. The data obtained from the linear Lineweaver–Burk plots yield the inhibitor constants recorded in Table 1. It is evident that the affinity of tetraethylammonium ion for the transport carrier is about 8-fold that of the tetramethylammonium ion and about 18-fold that of eserine.

Atropine

Atropine inhibits competitively acetylcholine uptake into rat brain cortex slices in the presence of 20 μM paraoxon. Such competition also occurs with mouse brain cortex slices in the presence of 1 μM Sarin.[1] K_i (inhibitor constant) for atropine equals $1{\cdot}8 \times 10^{-5}$ (Table 1). The value for K_i for atropine, for mouse brain cortex slices, equals $1{\cdot}6 \times 10^{-5}$M.[1]

Local anesthetics

The following local anesthetics have been investigated: cocaine, procaine, lidocaine and chloroprocaine. They all inhibit competitively uptake of acetylcholine into rat brain cortex slices in the presence of 20 μM paraoxon. Lineweaver–Burk plots for cocaine and procaine are shown in Fig. 1. The inhibitor constants (K_i) for the local anesthetics are recorded in Table 1.

Methacholine and succinylcholine

Both these substances inhibit competitively the uptake of acetylcholine into rat

brain cortex slices in the presence of 20 μM paraoxon, their inhibitor constants being recorded in Table 1. The Lineweaver–Burk plot for succinylcholine is shown in Fig. 1.

Choline, itself, is a relatively poor inhibitor of acetylcholine uptake into rat brain cortex slices in the presence of 20 μM paraoxon. The results of two experiments gave an inhibitor constant (K_i) for choline equal to 41×10^{-5}M. This may be compared with the value $22{\cdot}6 \times 10^{-5}$ obtained with mouse brain cortex slices in the presence of 1 μM Sarin.[1] In view of the relatively small inhibitory effect of choline on the uptake of acetylcholine into rat brain cortex slices, it is evident that paraoxon must block possible hydrolysis of succinylcholine or methacholine in the tissue, for otherwise the release of choline from these esters, at the concentration investigated (10 μM), would give rise to very little inhibition of the acetylcholine uptake.

d-Tubocurarine

d-Tubocurarine inhibits acetylcholine uptake into rat brain cortex slices competitively, the inhibitor constant (K_i) being $0{\cdot}6 \times 10^{-5}$M (Table 1).

It is known that the uptake of carbamylcholine into rat brain cortex slices is inhibited by *d*-tubocurarine, the inhibition of influx being described as competitive, with an inhibitor constant equal to $0{\cdot}3 \times 10^{-5}$M.[3]

The fact that the inhibitor constants for *d*-tubocurarine on carbamylcholine uptake in rat brain slices and for acetylcholine uptake in rat brain slices in the presence of 20 μM paraoxon are of the same order suggests that both acetylcholine and carbamylcholine are taken up at the same site on the brain cell membrane.

Other competitive inhibitors of acetylcholine uptake

The following drugs inhibit competitively the uptake of acetylcholine into rat brain cortex slices in the presence of 20 μM paraoxon: hexamethonium, pilocarpine, nicotine, strychnine and hemicholinium, their inhibitor constants being recorded in Table 1. The inhibitor constant for hemicholinium with mouse brain slices in the presence of 1μM Sarin equals $0{\cdot}5 \times 10^{-5}$M.[1]

Noncompetitive inhibitors of acetylcholine uptake

Ouabain and 2:4 dinitrophenol have already been mentioned as noncompetitive inhibitors of acetylcholine uptake into rat brain cortex slices in the presence of 20 μM paraoxon.[2] The following substances also prove to be noncompetitive inhibitors: chlorpromazine, amphetamine.

Lineweaver–Burk plots showing the relation between the reciprocals of the rates of active uptake of acetylcholine in the presence of 10 μM inhibitor and of the corresponding acetylcholine concentrations are given in Fig. 2.

Drugs having no effect on acetylcholine uptake

Amytal has no effect on acetylcholine uptake by rat brain cortex slices in the presence of 20 μM paraoxon, except at those concentrations ($> 0{\cdot}5$ mM) which bring about inhibition of the rate of respiration of the isolated brain. It has already been shown that the rate of uptake of acetylcholine may be proportional to the respiratory rate.[2] It is known that pentobarbitone does not affect acetylcholine uptake in mouse brain slices in the presence of 1 μM Sarin.[1]

Tetrodotoxin has no effect at concentrations (e.g. 10 μM) that block electrical stimulation of respiration in rat brain cortex slices.[4] The following substances have no significant effect on acetylcholine uptake in rat brain cortex slices in the presence of 20 μM paraoxon, at the concentrations quoted: reserpine (10 μM), caffeine (100 μM), chloralhydrate (0·3 mM), adrenaline (50 μM), noradrenaline (50 μM), sodium salicylate (0·5 mM).

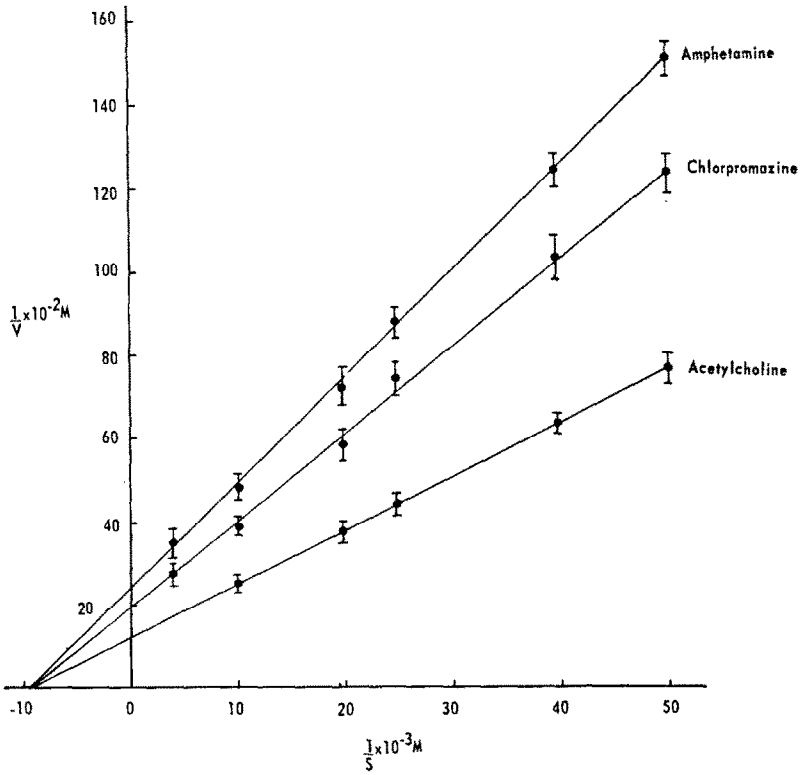

FIG. 2. Variation of the reciprocal of uptake of acetylcholine (corrected for passive diffusion) into rat brain cortex slices, the the presence of 20 μM paraoxon, with the reciprocal of the corresponding acetylcholine concentration. Effects of amphetamine and chlorpromazine, each at 10 μM.

DISCUSSION

Comparison of the cerebral transport carrier for acetylcholine with acetylcholine esterase

In considering the possible physiological significance of the uptake of acetylcholine against a concentration gradient into rat brain cortex slices, the question arises as to whether there is any relation between the transport carrier for acetylcholine and acetylcholine esterase. The affinity of acetylcholine for the transport carrier (apparent $K_m = 1{\cdot}08 \times 10^{-4}$) is of the same order as that for acetylcholine esterase whose K_m is quoted as 10^{-4}M[5] or 6×10^{-4}M.[6] Values are given in Table 1 of the inhibitor constants (K_i) of various drugs that are competitive inhibitors of

acetylcholine uptake in brain slices, in the presence of paraoxon, and of acetylcholine esterase. It will be seen that the affinity of eserine for the transport carrier is much less than that for acetylcholine esterase. On the other hand, the affinities of tetramethyl- and tetraethyl-ammonium ions for the transport carrier greatly exceed that for acetylcholine esterase. The affinity of strychnine for the transport carrier also exceeds that for acetylcholine esterase.

On the assumption that the transport carrier and acetylcholine esterase have the same or similar anionic binding sites for acetylcholine, and that the affinity of eserine for the anionic binding site of the enzyme is lower than it is for the esteratic site,[11] it may be thought that the lower affinity of eserine for the transport carrier than for acetylcholine esterase is simply due to its lower affinity for the anionic binding site. The latter site may control the activity of the transport carrier. However, it is difficult to understand, on this assumption, how the affinities of tetramethylammonium and tetraethylammonium ions, or of strychnine, for the transport carrier can be greater than that for acetylcholine esterase, when the esteratic site of the enzyme has been immobilized by treatment with paraoxon. The affinity of *d*-tubocurarine for the transport carrier also exceeds that for the anionic centre of an acetylcholine esterase preparation from *Torpedo marmorata* (Table 1).[11] For these reasons, it is considered unlikely that the transport carrier for acetylcholine in rat brain cortex is identical with the anionic site of brain acetylcholine esterase.

Comparison of the cerebral transport carrier for acetylcholine with other acetylcholine receptor sites

The apparent dissociation constant of *d*-tubocurarine for the acetylcholine receptor site on the electroplax of *Electrophorus electricus* is $2{\cdot}4 \times 10^{-7}$M.[12] This differs very considerably from the inhibitor constant of *d*-tubocurarine for the acetylcholine transport carrier ($0{\cdot}6 \times 10^{-5}$M) and indicates that there must be a difference in chemical structure between the acetylcholine receptor site on the electroplax and that on the cerebral acetylcholine transport carrier.

Similar differences exist between the dissociation constants of acetylcholine and methacholine with parasympathetic receptors in rabbit stomach smooth muscle ($2{\cdot}08 \times 10^{-6}$M and $2{\cdot}48 \times 10^{-6}$M respectively)[13] and those with the acetylcholine transport carrier.

The results lead to the conclusion that the combining site for acetylcholine on the acetylcholine transport carrier differs in structure from the acetylcholine receptor sites so far examined. A conclusion similar to this was made when the active sites of the acetylcholine receptor in the electroplax and of acetylcholine esterase were compared.[14]

It is evident, however, when the reversible inhibitory effects of local anesthetics (that are acetylcholine analogues), as well as those of atropine or nicotine, are considered, that, in spite of differences in the magnitudes of the apparent affinity constants, there must be much in common between the binding sites of acetylcholine on the cerebral transport carrier and those of acetylcholine esterase or of the neurophysiological receptors.

The fact that chlorpromazine and amphetamine at low concentrations (10 μM) can bring about inhibitions (noncompetitive with acetylcholine) of the uptake of acetylcholine into rat brain cortex slices, treated with 20 μM paraoxon, leads to the

conclusion that there exists a site on the transport carrier, with affinity for certain basic compounds of pharmacological importance, that affects the reactions of acetylcholine at the brain cell membrane.

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