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EFFECTS OF ANTICONVULSANT AND CONVULSANT DRUGS ON THE ATPASE ACTIVITIES OF SYNAPTOSOMES AND THEIR COMPONENTS

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1 The effects of anticonvulsants, and other drugs on the Na^+,K^+-adenosine triphosphatase (ATPase) (ouabain-sensitive) and Mg^{++}-ATPase activities of synaptosomes and their components have been determined.

2 The Mg^{++}-ATPase activity of synaptosomes was not affected by the drugs but the Na^+,K^+-ATPase activity was inhibited by phenytoin (diphenylhydantoin), ethosuximide and diazepam.

3 Fractions containing mainly membranes, mitochondria or synaptic vesicles, were prepared from synaptosomes by osmotic shock and subsequent density gradient centrifugation. Inhibition of Na^+,K^+-ATPase activity by phenytoin, ethosuximide and diazepam was apparent only in the membrane fraction.

4 The fraction containing synaptic vesicles exhibited pronounced Mg^{++}-ATPase but no Na^+,K^+-ATPase activity. In contrast to the enzymes of the membranes and mitochondria, the Mg^{++}-ATPase of the vesicles was inhibited by diazepam and all of the anticonvulsants tested.

Introduction

Mechanisms of action of anticonvulsant drugs are still a matter for speculation and no one effect of the drugs stands out sufficiently to support the contention that one mechanism of anticonvulsant action is common to all members of the group. The theory that the increase in the glucose concentration of the brain caused by all the anticonvulsants tested is involved in their action through membrane stabilization (Gilbert, Gray & Heaton, 1971) has received support (Nahorski, 1972) but it is a difficult one to test. Of the other effects of the drugs the changes which some induce in brain sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na^+,K^+-ATPase, EC.3.6.1.3) activity, thereby influencing ion gradients, are of particular interest. However, whilst there is little doubt that phenytoin (diphenylhydantoin) can influence brain Na^+,K^+-ATPase activity, whether inhibition or stimulation results appears to depend upon the concentrations of sodium and potassium ions in the assay medium (Festoff & Appel, 1968; Rawson & Pincus, 1968; Formby, 1970; Woodbury & Kemp, 1970; Gilbert, Buchan & Scott, 1974a; Gilbert & Wyllie, 1974b; Koostra & Woodhouse, 1974). The anticonvulsant ethosuximide also inhibits Na^+,K^+-ATPase but previous experiments in this laboratory have failed to detect effects of phenobarbitone or acetazolamide on the activity of the enzyme (Gilbert *et al.*, 1974a).

The suggestion of Paton, Vizi & Zar (1971) that acetylcholine release in the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum is regulated through inhibition of Na^+,K^+-ATPase has added new significance to the observations concerning phenytoin action. Recent experiments (Gilbert, Wyllie & Davison, 1975) have provided data supporting this interesting hypothesis but the precise relationships between the activities of the several ATPases of the nerve terminal and transmitter release have yet to be established.

In this paper we describe experiments to determine effects of anticonvulsants, convulsants and other drugs on the magnesium-activated ATPase (Mg^{++}-ATPase) and Na^+,K^+-ATPase activities of synaptosomes prepared from rat cerebral cortex. An attempt has also been made to define the sites of the effects more precisely within the synaptosome by examining separately the mitochondria, membranes and synaptic vesicles prepared from synaptosomes by osmotic disruption and density-gradient centrifugation.

Methods

Male Sprague-Dawley rats weighing 250–450 g were used, the preparation of synaptosomes usually involving eight animals. They were stunned and

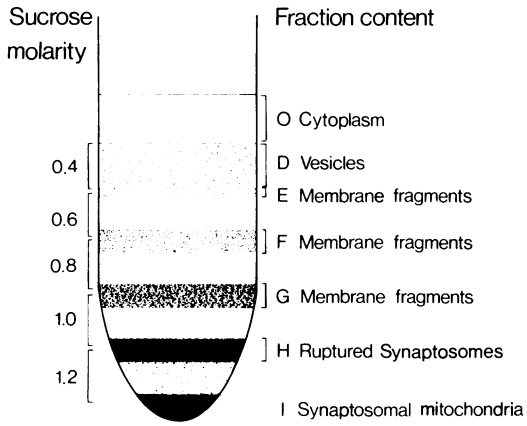

Figure 1 The synaptosome pellet was homogenized in distilled water (2 ml/g of original cortex) and separated on a sucrose gradient. The diagram represents the appearance of the centrifuge tube after centrifugation at 53,500 *g* for 2 hours.

decapitated and the cerebral cortices rapidly removed and cleared of white matter. All subsequent operations from the preparation of the cortex homogenate to the recovery of the synaptosomal and other fractions were performed at 0–4°C. The cortices were homogenized in 0.32 M sucrose containing 1 mM ethylenediamine tetraacetic acid (EDTA) using a Potter-Elvehjem homogenizer fitted with a teflon pestle; the clearance was 0.23 mm and the speed of rotation 840 rev/minute.

The homogenate (10%) was separated into the various primary fractions by a modification of the method of Gray & Whittaker (1962). The nuclear, pellet and the crude mitochondrial fraction were spun down at 1000 *g* (10 min) and 10,000 *g* (20 min) respectively, and the synaptosomes were recovered from the mitochondrial fraction by resuspending it in sucrose/EDTA solution (2–3 ml/g original cortex) and centrifuging it at 53,500 *g* for 2 h on a sucrose density gradient (5 ml 1.2 M and 7.5 ml 0.8 M sucrose solution). The synaptosome suspension was removed from between the 0.8 and 1.2 M sucrose solution, the molarity of the sucrose adjusted to 0.4 M and the suspension centrifuged at 100,000 *g* for 30 min to obtain the synaptosome pellet. For studies of synaptosome components the pellet was disrupted by homogenization in distilled water (2 ml/g original cortex) and standing at room temperature for 60 minutes. This suspension was placed on a discontinuous density gradient consisting of successive 3 ml layers of sucrose (Figure 1) as described by Whittaker, Michaelson & Kirkland (1964).

After centifuging at 53,500 *g* for 2 h the bands were removed separately with a needle attached to a 20 ml syringe. The seven fractions obtained were designated O, D, E, F, G, H and I in order of the increasing density of sucrose and these corresponded to the cytoplasm (O), vesicle (D), membrane (E, F, G), ruptured synaptosomes (H) and mitochondria (I) fractions from synaptosomes.

Samples of the fractions were stored at −23°C before determination of the protein contents or enzymatic activities. Results obtained with freshly prepared material were not altered significantly by storage at this temperature.

ATP (disodium salt; Sigma Chemical Company) was converted into the free acid by passing it through Dowex 50 resin (H^+ form, 200–400 mesh) in a cold room and adjusting it to pH 7.40 with 1 M tris solution. Deionized water was added to make the solution 40 mM with respect to ATP, the final concentration being calculated from the extinction of the solution at 260 nm. The concentration of sodium ions in this solution was less than 100 μM and was less than 10 μM in the final ATPase assay medium.

The concentrations of endogenous sodium and potassium ions were determined in some preparations by flame photometry and by atomic absorption spectrophotometry after digestion of samples overnight in 15.8 N nitric acid and subsequent dilution.

For ATPase assays the tissue fractions were resuspended in 50 mM imidazole/HCl buffer pH 7.40 and 0.2 ml samples, each containing 0.1–0.2 mg protein, were added to buffered media. The media contained NaCl (150 mM), KCl (10 mM) and $MgCl_2$ (5 mM) for total ATPase, $MgCl_2$ (5 mM) for Mg^{++}-ATPase and $MgCl_2$ (5 mM) and NaCl (150 mM) for Na^+-ATPase (see below) assays. The mixtures (final volume 0.9 ml) were pre-incubated for 15 min at 37°C before starting the reactions by addition of 0.1 ml Tris ATP solution (4 mM final concentration). Sodium dodecyl sulphate (1 ml, 0.8%) was used to stop the reactions after 10 minutes. The phosphate contents of the clear solutions were determined by the method of Bonting, Simon & Hawkins (1961). When used, drugs were added to the pre-incubation media. Na^+,K^+-ATPase activity was calculated by subtracting the Mg^{++}-ATPase activity from the total ATPase activity.

We have recently shown (Gilbert & Wyllie, 1974a; 1975) that the Na^+,K^+-ATPase activity so calculated (that is, relative to the total activity determined in the presence of sodium, potassium and magnesium ions) consists of at least two components. One of these is not inhibited by ouabain and appears to be due to a sodium-activated, magnesium-dependent ATPase (Na^+-ATPase). This component contributes some 25–30% to the activity of the apparent Na^+,K^+-ATPase in synaptosomes from rat cerebral cortex and in the present study none of the drugs tested influenced its activity. For simplicity, therefore, we have reported here the activity pertaining to the Na^+,K^+-ATPase

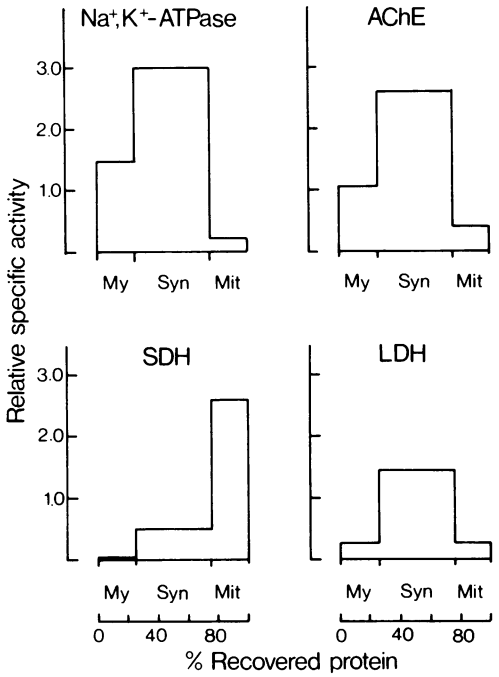

Figure 2 Distribution of the cytoplasmic marker lactate dehydrogenase (LDH), the mitochondrial marker succinate dehydrogenase (SDH), and two membrane fragment markers Na^+,K^+-ATPase and acetylcholinesterase (AChE). The crude mitochondrial fraction was separated into myelin (My), synaptosomes (Syn) and mitochondria (Mit) by density gradient centrifugation. The ordinates (relative specific activity) are the percentages of the enzymes recovered in each fraction divided by the percentages of protein recovered in the same fraction.

(EC.3.6.1.3), the enzyme which requires sodium and potassium ions together for activation in the presence of magnesium ions. The activity was calculated by subtraction of the Mg^{++}-ATPase activity from the total ATPase activity.

The protein contents of solutions were determined by the method of Lowry, Rosenbrough, Farr & Randall (1951). Acetylcholinesterase was assayed manometrically by the method of Aldridge & Johnson (1959). Succinate dehydrogenase was assayed by the manometric technique of Quaestel & Wheatley (1938) as described and modified by Aldridge & Johnson (1959). Lactate dehydrogenase was assayed spectrophotometrically (Johnson, 1960).

The following drugs were used: acetazolamide sodium (Lederle); barbitone sodium (BDH); diazepam (Roche Products); dimethadione (Abbot Laboratories); ethosuximide (Parke Davis); Leptazol (Sigma); ouabain (BDH); phenobarbitone sodium (BP); phenytoin sodium (Sigma); picrotoxin (BDH); SC-13504 (Searle); strychnine sulphate (Sigma); trimethadione (Abbot Laboratories).

Results

The distribution of the enzyme markers Na^+,K^+-ATPase, acetylcholinesterase, succinate dehydrogenase and lactate dehydrogenase in the myelin, synaptosome and mitochondrial fractions prepared from the cerebral cortex homogenates is shown in Figure 2. The identity of the fractions was ascertained from electron micrographs, the preparation and assessment of which were carried out by Dr D.N. Wheatley in the Department of Pathology and the degrees of contamination of fractions were very small. The results illustrate that the synaptosome fraction is enriched in the activities of the enzymes normally associated with nerve terminals, acetylcholinesterase and Na^+,K^+-ATPase in the membranes and lactate dehydrogenase trapped from the cytoplasm of the cell body, as reported by others (Johnson & Whittaker, 1963; Whittaker, 1959, 1965).

Effects of anticonvulsants and convulsants on synaptosome ATPase activities

None of the drugs tested (Table 1) altered the Mg^{++}-ATPase activity of synaptosomes. Two of the anticonvulsants altered the Na^+,K^+-ATPase activity, phenytoin (0.2 mM) and ethosuximide (2.5 mM) inhibiting it as we have found previously (Gilbert & Wyllie, 1974a, b; Gilbert, Scott & Wyllie, 1974b). Dose-response curves (for instance Figure 3) for the two anticonvulsants indicated that they inhibited the

Table 1 Effects of drugs on synaptosome ATPase activities

Drug (mM)	*% Inhibition of Na^+,K^+-ATPase*	P
Phenytoin (0.2)	65.0 ± 9.1 (6)	<0.001
Ethosuximide (2.5)	64.2 ± 5.3 (5)	<0.001
Diazepam (0.25)	55.1 ± 3.8 (4)	<0.01

Values are the mean with s.e. mean and the number of observations is given in parentheses. Phenobarbitone (1.0), acetazolamide (0.1), trimethadione (1.0), dimethadione (1.0), SC-13504 (2 μg/ml), leptazol (1.0), strychnine (0.2), picrotoxin (1.0) and barbitone (1.0) had no effect and none of the drugs tested altered the Mg^{++}-ATPase activity. Typical control values (μmol Pi.mg protein^{-1} h^{-1}) Na^+,K^+-ATPase 9.70 ± 0.79 (22), Mg^{++}-ATPase 6.60 ± 0.54 (22).

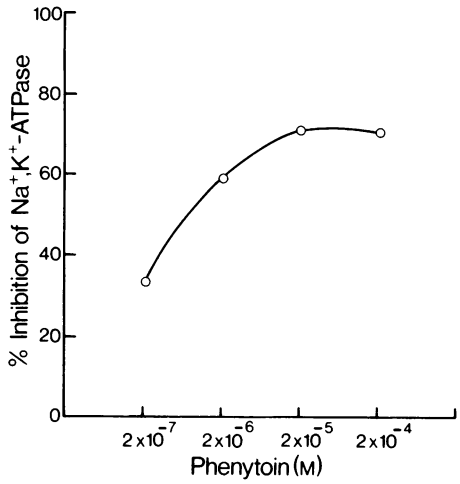

Figure 3 Typical log dose-response plot for the inhibition of synaptosomal Na^+,K^+-ATPase by phenytoin.

activity *in vitro* at concentrations which are similar to those likely to be present *in vivo* during anticonvulsant therapy (Millichap, 1965; Eadie & Tyrer, 1974), that is 0.04 mM for phenytoin and 0.5 mM for ethosuximide. Diazepam (0.25 mM) which has found use in the treatment of status epilepticus (Eadie & Tyrer, 1974) also inhibited the Na^+,K^+-ATPase activity.

Effects of anticonvulsants on the ATPases of synaptosome components

Osmotic disruption of synaptosomes and subsequent separation of the components into fractions containing mainly membranes, synaptic vesicles or mitochondria permitted investigations of the ATPases in these components separately, although a very small degree of contamination of each fraction by material from another was inevitable and was apparent in electron micrographs.

The relative specific activities of Na^+,K^+-ATPase, acetylcholinesterase, succinate dehydrogenase and lactate dehydrogenase in the fractions are shown in Figure 4 and they are in agreement with the results of Whittaker *et al.* (1964) and Hosie (1965) who used similar techniques to isolate components of synaptosomes. Considering Figure 4 in conjunction with Figure 1 it can be seen that the material floating above the least dense sucrose layer (0.4 M), fraction O, contained lactate dehydrogenase activity but little activity of any of the other enzyme markers. These findings are compatible with the proposed cytoplasmic origin of this material (Whittaker, 1965). Fraction D, the material suspended in the 0.4 M sucrose, was shown by electron microscopy to contain the synaptic

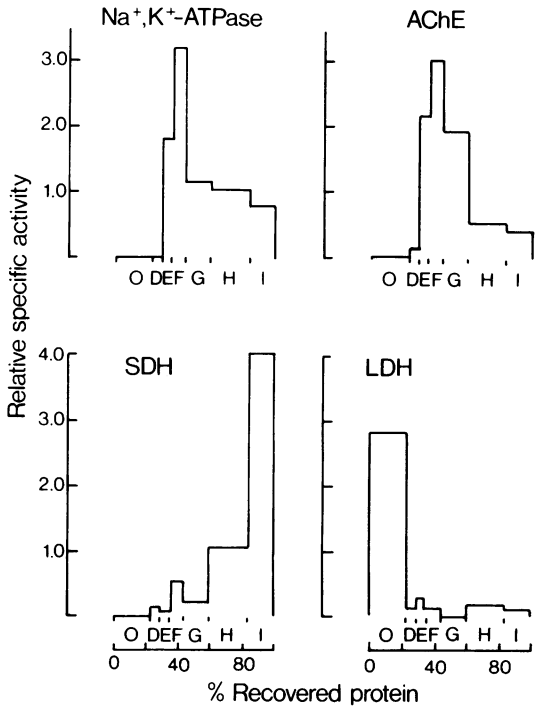

Figure 4 Distribution of the cytoplasmic marker lactate dehydrogenase (LDH), the mitochondrial marker succinate dehydrogenase (SDH) and membrane markers Na^+,K^+-ATPase and acetylcholinesterase (AChE) in fractions (see Figure 1) prepared from disrupted synaptosomes prepared by density gradient centrifugation. The ordinates (relative specific activity) are the percentages of enzyme recovered in the fractions divided by the percentages of protein recovered in the same fraction.

vesicles. It contained no detectable Na^+,K^+-ATPase activity and very little of the activities associated with the other enzymes. Other studies (see later) have shown that this fraction contains Mg^{++}-ATPase.

The activities of acetylcholinesterase and Na^+,K^+-ATPase, enzymes generally associated with membranes, were high in the material deposited at the interphases between the 0.4–0.6 M, 0.6–0.8 M and 0.8–1.0M sucrose solutions, fractions E, F and G respectively. Electron microscopy suggested that the material in the three fractions was derived from membranes. Comparatively little succinate or lactate dehydrogenase activity was detected in these fractions and since no obvious difference, other than the different densities, was apparent between them the fractions were pooled as the 'membrane' fraction for subsequent studies.

Fraction H (between 1.0 and 1.2 M sucrose) contained ruptured synaptosomes, the various

components of the original particles adhering one to another. High acetylcholinesterase, Na^+,K^+-ATPase and succinate dehydrogenase activities were apparent in this fraction but, as one might expect, very little of the activity of the cytoplasmic enzyme lactate dehydrogenase.

The pellet at the base of the centrifuge tube (fraction I) was composed mainly of mitochondria which had been contained originally in the synaptosomes. The mitochondrial enzyme succinate dehydrogenase showed high activity in the fraction and the activities of acetylcholinesterase and Na^+,K^+-ATPase were also marked. Very little lactate dehydrogenase activity was present.

Table 2 shows that none of the anticonvulsants tested altered either the Mg^{++}- or the Na^+,K^+-ATPase activity of the mitochondrial fraction. The lack of an effect of ethosuximide is in agreement with our previous results (Gilbert & Wyllie, 1974a). Ethosuximide (2.5 mM) and phenytoin (0.2 mM) each inhibited the Na^+,K^+-ATPase of the membrane fraction markedly.

Experiments with the synaptic vesicles (fraction D) gave particularly interesting results (Table 3). There was no detectable Na^+,K^+-ATPase activity in the vesicular fraction. Mg^{++}-ATPase was present, however, and its activity was comparable to that of the membrane fraction which was approximately 50% of the activity in the mitochondrial fraction. All of the anticonvulsant drugs inhibited the Mg^{++}-ATPase activity of the vesicles, whereas the Mg^{++}-ATPase activities of the other fractions were not influenced by the drugs. Ouabain, barbitone and the convulsants leptazol, strychnine and picrotoxin did not alter the vesicular Mg^{++}-ATPase activity significantly at the relatively high concentrations indicated, and lower concentrations were also ineffective. Diazepam (0.25 mM) inhibited the enzyme by some 63%. This

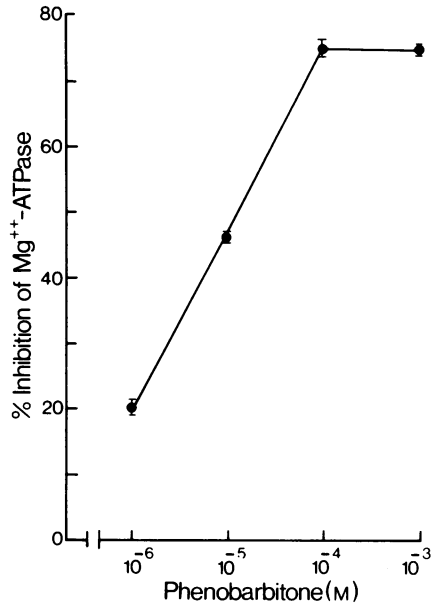

Figure 5 Log dose-response plot for the inhibition of vesicular (D fraction) Mg^{++}-ATPase by phenobarbitone. Vertical bars indicate s.e. mean of 3 or 4 experiments.

Table 2 The effects of anticonvulsants on the ATPase activities of the membrane and mitochondrial fractions prepared by osmotic lysis of synaptosomes

	Membrane fraction ATPase activity (μmol Pi.mg protein^{-1} h^{-1})	
Drug (mM)	*Na^+,K^+-*	*Mg^{++}-*
Control	11.56 ± 1.19 (33)	5.26 ± 0.56 (31)
Phenytoin (0.2)	2.89 ± 0.79 (9)**	5.30 ± 0.80 (9)
Ethosuximide (2.5)	3.61 ± 0.81 (12)**	4.82 ± 0.61 (12)
Phenobarbitone (1.0)	10.38 ± 2.30 (6)	5.48 ± 1.15 (5)
Acetazolamide (0.1)	9.71 ± 1.78 (7)	4.93 ± 1.39 (5)
	Mitochondrial fraction	
Control	10.40 ± 1.70 (9)	10.32 ± 0.39 (9)
Phenytoin (0.2)	6.82 ± 2.61 (3)	10.80 ± 0.79 (3)
Ethosuximide (2.5)	8.51 ± 3.30 (4)	10.40 ± 1.42 (2)
Phenobarbitone (1.0)	12.98 ± 0.12 (2)	8.87 ± 2.46 (2)
Acetazolamide (0.1)	12.82 ± 0.44 (2)	8.82 ± 2.65 (2)

The number of observations is given in parentheses. Values are the mean with s.e. mean. For tests of significance only paired drug and control results were included in the calculations.
** $P < 0.001$.

Table 3 Effects of drugs on the Mg^{++}-ATPase activity of the vesicle-containing fraction

Drug (mM)	*% Inhibition of Mg^{++}-ATPase activity*	P
Control		
Phenytoin (0.2)	54.9 ± 3.9 (3)	<0.001
Ethosuximide (2.5)	73.7 ± 7.0 (4)	<0.001
Phenobarbitone (1.0)	74.9 ± 1.6 (2)	<0.025
Acetazolamide (0.1)	52.8 ± 8.1 (3)	<0.025
Trimethadione (1.0)	70.9 ± 2.8 (3)	<0.005
Dimethadione (1.0)	62.8 ± 6.2 (3)	<0.010
SC 13504 (0.05)	79.5 ± 4.5 (3)	<0.025
Diazepam (0.25)	62.2 ± 7.5 (3)	<0.025

Values are the mean with s.e. mean and the number of observations is given in parentheses. Leptazol (1.0), strychnine (0.25), picrotoxin (1.0), barbitone (1.0) and ouabain (1.0) had no effect. Typical control value (μmol Pi.mg protein^{-1} h^{-1}) 5.10 ± 0.31 (16).

was the maximum degree of inhibition shown by diazepam in a dose-response curve.

Inhibition of the Mg^{++}-ATPase by anticonvulsants did not exceed a maximum of approximately 80% of the enzymatic activity in dose-response curves and this is seen, for example, in Figure 5 which shows the curve obtained with phenobarbitone. All the anticonvulsants exerted near maximum effect at concentrations reported to be effective therapeutically.

Discussion

Any attempts to evaluate effects of drugs on nerve terminals isolated from the central nervous system and studied *in vitro* must depend for their validity upon the integrity of the preparation derived from the tissue. With this in mind, certain criteria were selected as indices of the quality of the synaptosomes used in the present work.

Electron microscopy, performed and assessed in another department (see results section) showed that the synaptosome fraction was reasonably homogeneous and largely uncontaminated by free mitochondria. The synaptosomes were between 0.45 and 0.6 μm in diameter and often retained attached membrane material which was presumably postsynaptic in origin. The fraction was enriched in the activity of acetylcholinesterase, an enzyme localized in presynaptic membranes of cholinergic neurones (Lewis & Shute, 1964). The activity of succinate dehydrogenase in the synaptosome fraction was presumably due to the presence of intra-synaptosomal mitochondria. Lactate dehydrogenase, a cytoplasmic enzyme which becomes trapped to some extent in the synaptosome when the nerve terminal ruptures and reseals during the preparative procedure was retained in the synaptosome fraction as reported by others (Johnson & Whittaker, 1963). Na^+,K^+-ATPase, a membrane bound enzyme which has been reported to be more highly localized in the synaptosome fraction, showed a distribution compatible with that reported by other workers who have used similar techniques to isolate the nerve ending particles (Hosie, 1965).

We have assumed, in view of these observations, that the effects of the drugs on the synaptosomes reliably reflect effects on nerve terminals *in vitro* rather than effects on material contaminating the nerve terminal fractions and derived from other, less discrete regions of the cell.

With regard to the synaptosome fraction itself only ethosuximide and phenytoin significantly inhibited Na^+,K^+-ATPase activity whilst none of the drugs influenced Mg^{++}-ATPase activity. Similar results have been obtained previously (Gilbert *et al.*, 1974a, b; Gilbert & Wyllie, 1974a, b). Formby (1970) and Escueta & Appel (1970) have also reported inhibition of cerebral cortex Na^+,K^+-ATPase by phenytoin but Festoff & Appel (1968) found that when the ratio of sodium to potassium ions in the assay medium was above 25:1 phenytoin stimulated the enzyme. The present results do not confirm that report but it may be that it is the absolute concentrations of the ions, rather than the relative concentrations which are important.

Just how inhibition of Na^+,K^+-ATPase might be involved in anticonvulsant activity is a matter for conjecture. The reported inhibition of post-tetanic potentiation by phenytoin (Esplin, 1957) could well result from inhibition of the enzyme with the result that sodium ions could accumulate in the presynaptic region during repetitive stimulation. Thus sodium ions would not be actively extruded and no hyperpolarization would occur at a time when this would otherwise be the case due to increased Na^+,K^+-ATPase activity. One result could be depressed neurotransmitter release from the nerve terminal.

Like ethosuximide and phenytoin, diazepam inhibited synaptosome Na^+,K^+-ATPase. This result is in agreement with the work of Davis & Brody (1966) and Ueda, Wada & Ballinger (1971) who suggested that the pharmacological activity of diazepam was mediated through this effect. However, this view is not generally accepted (see for instance Landmark & Øye, 1971) and its relationship to the value of diazepam in the treatment of status epilepticus is obscure.

A possible role of Na^+,K^+-ATPase in the regulation of neurotransmitter release merits discussion here. Paton *et al.* (1971) and Vizi (1972) obtained circumstantial evidence to suggest that acetylcholine release at peripheral and central cholinergic synapses can be triggered by a depression of Na^+,K^+-ATPase activity. In their experiments, conditions which, in other tissues, have been shown to inhibit Na^+,K^+-ATPase activity were found to stimulate acetylcholine release from myenteric plexus and from

cerebral cortex slices. Treatment of the myenteric plexus-longitudinal muscle preparation of the guinea-pig ileum with ouabain or *p*-hydroxymercuribenzoate, or alternatively subjecting the tissue to sodium ion depletion, increased acetylcholine release and these conditions were assumed to inhibit Na^+,K^+-ATPase. Although no determinations of the activity of the enzyme were made, there is little reason to doubt the validity of the assumption and it is also reasonable to assume that the enzyme concerned is in the nerve terminal. If acetylcholine release is regulated by this enzyme, then the effects of ethosuximide, phenytoin and diazepam could also cause transmitter release at certain central nervous system cholinergic synapses if the drugs gain access to the necessary sites. Experiments to test this possibility are in progress. However, in considering the possible roles of Na^+,K^+-ATPase in the nerve terminal it should be remembered that anticonvulsants other than ethosuximide and phenytoin did not influence the activity of the enzyme when preparations containing intact synaptosomes were added to the ATPase assay medium. It could be argued that any effect of the anticonvulsants on the membrane ATPase of the synaptosome is masked when the synaptosome bursts to release mitochondria and other components on contact with the hypotonic imidazole/HCl buffer. This emphasizes the importance of the experiments designed to test the effects of the drugs on the membranes, mitochondria and synaptic vesicles, isolated from the synaptosomes and studied separately. The three general components were found to have quite different enzyme characteristics. Na^+,K^+ATPase activity was pronounced in both the membrane and mitochondrial components but, as reported previously (Gilbert & Wyllie, 1974a), only the former was sensitive to ethosuximide. In contrast to the other components the vesicles contained no detectable Na^+,K^+-ATPase activity.

In agreement with the results obtained with synaptosomes, ethosuximide and phenytoin were the only anticonvulsants to alter Na^+,K^+-ATPase activity in any of the subfractions, the effect being to inhibit the enzyme in the membrane fraction.

The most striking finding is that all of the anticonvulsant drugs tested inhibited the Mg^{++}-ATPase activity of the vesicles. The Mg^{++}-ATPase activities of other fractions were not influenced by the drugs and since the activity of the enzyme in the vesicles is similar to that in the synaptosome itself, effects of the drugs on total synaptosome Mg^{++}-ATPase may not be detectable because the vesicles contribute only some 5% to synaptosomal protein. The degree of inhibition of the Mg^{++}-activated enzyme in the vesicles did not exceed 80%, however, and it may be that this reflects a degree of heterogeneity of the fraction, either the 20% of activity which remains is contained in material which contaminates the fraction and which is not vesicular in origin, or this 20% could represent the activity of vesicles of a synaptic type which is different from the type contributing the 80% of activity which is sensitive to the anticonvulsants. This intriguing possibility requires investigation. Clearly, the significance of the Mg^{++}-ATPase has to be established. There is evidence to suggest that a relationship exists between the activity of a Mg^{++}-ATPase and the ability of chromaffin granules of the adrenal medulla to store noradrenaline. Banks (1965) and Kirshner, Smith & Kirshner (1966) showed that the membrane of the storage vesicles contains Mg^{++}-ATPase activity and Taugner & Hasselbach (1968) observed that the degree of inhibition of the enzymes by *N*-ethylmaleimide correlated well with the degree of inhibition of catecholamine uptake into the vesicles. The uptake process appears to be one of active transport, but the pH-dependence of the process suggests that it may not be linked directly to Mg^{++}-ATPase activity (Taugner, 1971; Phillips, 1974). This work does not have a direct bearing upon the relationship between vesicle Mg^{++}-ATPase and transmitter uptake and release in the central nervous system but it does suggest the processes may be related. In this connection the finding that the vesicles exhibit Mg^{++}-ATPase activity but negligible Na^+,K^+-ATPase activity is important in that it will permit studies of the role of the former. In most tissues Mg^{++}-ATPase activity is very closely associated with Na^+,K^+-ATPase activity although it can be separated from it to some extent.

Brief comment should be made concerning inhibition of Mg^{++}-ATPase and anticonvulsant activity. The two phenomena may be unrelated and certainly no relationship was apparent between the clinical uses of the individual anticonvulsants and their ability to inhibit the enzyme. Preliminary studies do suggest, however, that the relative inhibitory potencies of the drugs are of the same order as their potencies with regard to elevation of seizure thresholds in mice and rats. Further experiments are necessary to clarify this point.

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