After perfusion with oxygenated Krebs bicarbonate solution for 20 min the preparations were either stimulated electrically (5 ms, 100 Hz, 20 mA) or exposed to high potassium ion concentrations (50 and 100 mM). The release of the radioactive taurine was monitored by scintillation spectrometry and expressed as the fractional rate constant (f, min^{-1}).

Both high potassium ion concentrations and electrical stimulation significantly increased the release of [^{14}C]-taurine from slices of cerebral cortex but not from spinal cord (see Table 1). In addition, this evoked release was calcium-dependent and was not accompanied by an increased efflux of the inert marker [^{3}H]-inulin. Experiments were carried out under similar conditions in which the release of [^{14}C]-glycine and [^{3}H]-γ-aminobutyric acid were measured; electrical stimulation and high potassium ion concentrations evoked release of [^{14}C]-glycine from cord but not from cerebral cortex, whereas [^{3}H]-γ-aminobutyric acid was preferentially released from slices of cortex. The results suggest that if indeed taurine is a neurotransmitter, it is more likely to subserve this function in the cerebral cortex rather than in the spinal cord.

Miss S.H. Topiwala is an M.R.C. student.

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Effects of ethosuximide on adenosine triphosphatase activities of some subcellular fractions prepared from rat cerebral cortex

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The report of Woodbury, Koch & Vernadakis (1958) that phenytoin (diphenylhydantoin) increased the rate of extrusion of sodium ions from cells in the central nervous system raised the possibility that the anticonvulsant action of the drug results from stimulation of sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na,K-ATPase). Direct evidence concerning this point is conflicting, however, and such an effect is probably not common to all anticonvulsants (Gilbert, Buchan & Scott, in press). We report here the effects of the anticonvulsant drug ethosuximide on both the Na, K-ATPase and the magnesium=adenosine triphosphatase (Mg-ATPase) activities of subcellular fractions prepared from rat cerebral cortex.

Cerebral cortex tissue was obtained from male, Sprague-Dawley rats and homogenized in ice-cold 0.32 M sucrose solution using a glass homogenizer fitted with a teflon pestle. Primary fractions were obtained by centrifuging the homogenate at 900 *g* for 15 min (nuclei and debris), 18,000 *g* for 30 min (crude mitochondrial fraction) and 100,000 *g* for 60 min (microsomal fraction). Fractions enriched in either synaptosomes or mitochondria were prepared from the crude mitochondrial fraction as described previously (Balfour & Gilbert, 1971). Fractions were washed once with ice-cold 0.32 M sucrose solution and resuspended in a similar solution prior to assaying enzymic activity. ATPase activities were determined by measuring the release of inorganic phosphate from either Tris ATP or disodium ATP in 20 mM imidazole/HCl buffer pH 7.4 at 37°C in the presence of either $MgCl_2$ (5 mM) for Mg-ATPase or of $MgCl_2$ (5 mM), NaCl (150 mM) and KCl (10 mM) for total ATPase. The difference between the total and the Mg-ATPase activities was taken to represent Na,K-ATPase activity.

Ethosuximide had no effect upon the Mg-ATPase activities of the fractions under any of the conditions tested. However, the Na,K-ATPase activities of microsomal and synaptosomal fractions prepared from rats which had received ethosuximide (20 mg/kg, i.p.) daily for three days were lower than those of controls. Upon incubation with microsomal and synaptosomal fractions from untreated rats the drug (0.25-25 mM) again inhibited the Na,K-ATPase activities.

The Na,K-ATPase activity of the synaptosomal fraction increased approximately linearly with increases in the medium sodium concentration from 50 mM to 150 mM, the potassium concentration being maintained at 10 mM. Ethosuximide (2.5 mM) was without effect upon the activity of the enzyme when the medium contained 50 mM sodium, but prevented the increase in activity normally associated with the increase in the sodium concentration to 150 mM. Approximately 20% of the activity of the Na,K-ATPase was not sensitive to ethosuximide under any of the conditions tested, and other experiments suggested that a corresponding proportion was insensitive to ouabain (0.1 mM). These observations suggest that at least two components contribute to the Na,K-ATPase activity.

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Effect of electrode positions on contractions of guinea-pig isolated ileum to electrical stimulation

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Tetrodotoxin (TTX) reportedly abolishes contractions of guinea-pig isolated intestine to electrical field stimulation, but we recently obtained TTX-resistant contractions apparently due to direct muscle stimulation (Bennett & Stockley, 1973). We had a different electrode arrangement (one above and one below the tissue–'longitudinal' field stimulation–somewhat analogous to electrodes tied into each end of the tissue; Bucknell, 1965), so we compared responses with four other arrangements: uninsulated platinum (0.46 mm gauge) wires, one each side of the ileum (Ambache & Freeman, 1968) or wires insulated on entry to the bath but not opposite the tissue (similar to Crema, del Tacca, Frigo & Lecchini, 1968) ('transverse' stimulation); intralumenal and extralumenal electrodes ('transmural' stimulation, Paton, 1955); ring electrodes 2 mm apart around one end of the segment (similar to Furness, 1970).

Segments of guinea-pig ileum in Krebs solution (37° C, 5% CO_2 in O_2, load 0.5 g) were stimulated with 'longitudinal' field stimulation, at a voltage giving just-supramaximal stimulation of longitudinal muscle isotonic contractions at 1 or 2 Hz (10 s trains of alternating square wave pulses). Voltages for the other electrodes were those matching responses to 'longitudinal' stimulation at 1 or 2 Hz, 1 ms duration. Approximately equivalent voltages were: 'longitudinal', 100 V≡17 V/cm; 'transverse' insulated, 10 V≡20 V/cm; 'transverse' uninsulated 50 V≡100 V/cm; 'transmural' 10 V≡20 V/cm; ring electrodes 100 V≡500 V/cm.

Frequency-response curves were obtained at 0.1-64 Hz with 'longitudinal' and another type of stimulation, first without and then with TTX 0.5 μg/ml. Reduction of responses to 'longitudinal' stimulation with TTX was less than with other electrodes (Figure 1). Threshold frequencies for TTX-resistant contractions were sometimes as low as 0.1, 2, 4, 4 and 16 Hz respectively for 'longitudinal', 'transmural', ring electrodes, uninsulated

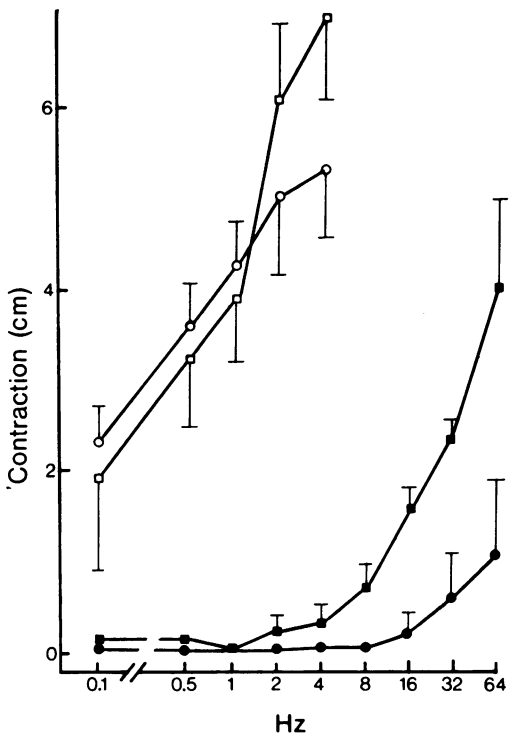

Fig. 1 TTX-resistant contractions were obvious at 2-64 Hz with 'longitudinal' field stimulation (squares) and sometimes occurred at even lower frequencies, but they occurred only at 16-64 Hz with 'transverse' stimulation (circles) using part-insulated electrodes (see text). Each tissue was studied with both electrode arrangements. Open symbols, before TTX; filled symbols, in the presence of TTX 0.5 μg/ml. Vertical bars, 1 s.e. mean. The other electrode arrangements (results not shown) also had less tendency than 'longitudinal' stimulation to produce TTX-resistant contractions.