Synaptic vesicle magnesium-adenosine triphosphatase as a possible site of action of anticonvulsant drugs

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Phenytoin and ethosuximide inhibit a magnesium-activated adenosine triphosphatase (Mg-ATPase) located in the vesicle-containing fraction derived from nerve terminals prepared from rat cerebral cortex (Gilbert & Wyllie, 1974a,b). Mg-ATPase activities in the membrane and mitochondrial fractions derived from the nerve terminals were not influenced by the drugs.

We have recently tested the effects of diazepam and the anticonvulsants phenobarbitone, trimethadione, acetazolamide, sodium valproate and SC 13504 (Searle) on the Mg-ATPase activity of synaptic vesicles using techniques similar to those used previously to prepare synaptosomes, to disrupt them by osmotic shock, to recover the sub-synaptosomal fractions and to assay ATPase activities (Gilbert & Wyllie, 1974a,b; 1975).

All the anticonvulsants and diazepam, when added to preparations *in vitro*, inhibited the Mg-ATPase contained in the fraction enriched in synaptic vesicles but not the enzyme contained in the other fractions prepared from the synaptosomes. Other drugs tested, including convulsants, did not inhibit the enzyme in the vesicle fraction. Administration of phenytoin (50 mg/kg and 100 mg/kg) or phenobarbitone (25 mg/kg and 100 mg/kg) *in vivo* to rats and subsequent preparation of the vesicle-containing fractions showed that the Mg-ATPase was inhibited to the maximum degree which had been observed in the experiments involving the addition of the drugs *in vitro*.

Dose response curves indicated that the relative potencies of the anticonvulsants with regard to inhibition of the enzyme were compatible with their relative potencies in preventing seizure activity in mice.

Studies of the distribution of phenytoin using the assay technique of Dill & Glazko (1972) suggested that the drug was present in the synaptosome cytoplasm and vesicle fractions.

This work suggests that the Mg-ATPase of nerve terminal vesicles may be implicated in the mechanism of action of anticonvulsants.

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Interaction of carbenoxolone sodium with other drugs bound to plasma proteins

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Displacement from plasma protein binding sites has been suggested as one of the causative factors in increased efficacy or toxicity during concomitant administration of two or more drugs (O'Reilly & Levy, 1970; Mason & McQueen, 1974; Pugh, Geddes & Yeoman, 1975). Carbenoxolone sodium is an ulcer-healing drug which is highly bound to plasma proteins. Its efficacy, mode of action and low toxicity may even depend upon this binding (Parke & Lindup, 1973).

We have carried out a series of *in vitro* experiments with the fluorometric probe 1-anilino-8-naphthalene sulphonic acid using the method of Jun, Luzzi & Hsu (1972). Carbenoxolone sodium was studied together with several other protein-bound drugs which may be clinically encountered in carbenoxolone therapy. These included, phenylbutazone, flufenamic acid, prednisolone, aspirin, warfarin, tolbutamide, phenytoin and imipramine. The parent triterpenoid moeity of

carbenoxolone, namely enoxolone, and an analogue, cicloxolone were also included. To enable comparison with other workers we used both crystalline bovine (BSA) and human (HSA) serum albumin solutions (Jun, *et al.*, 1972; Mason & McQueen, 1974).

Initially we found a difference in binding potential between HSA and BSA. With BSA only one class of binding site was seen for the probe whereas two classes were seen with HSA. Moreover, carbenoxolone and phenylbutazone were both bound at the same class of site to BSA but at different classes of sites to HSA. For the remainder of the study HSA was used.

The binding characteristics of HSA of the drugs used formed three distinct groups;

Group 1 consisted of drugs with strong binding affinities to the class I site and included the three triterpenoids of which carbenoxolone was the most strongly bound.

Group 2 also included strongly bound drugs, but these were binding at the class II sites. Of these flufenamic acid was the most strongly bound, followed by phenylbutazone warfarin and tolbutamide, respectively.

Group 3 comprised the weakly bound drugs binding at both class I and II sites. Of these aspirin was the most strongly bound, followed by phenytoin, prednisolone, and imipramine, respectively.

Thus it is probable that interaction, due to displacement from binding sites, between drugs of groups 1 and 2 is unlikely. The weak binding affinities, to both classes of binding sites, of drugs in group 3 indicate that they may be susceptible to displacement by the drugs in both groups 1 and 2. Within each of the three groups of drugs, displacement may be expected of the drugs with the lowest binding affinities by those with a higher affinity within the same group.

Further studies are in progress designed to examine the specificity of carbenoxolone binding and to examine the indicated possible *in vivo* interactions.

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Tryptophan metabolism by the isolated perfused rat liver

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Increased rat liver tryptophan pyrrolase activity following hydrocortisone (5 mg/kg) injection decreases tryptophan concentrations in liver and brain and is accompanied by decreased brain 5-hydroxytryptamine synthesis (Green, Woods, Knott & Curzon, 1975). In this and other experiments investigating the effect of drugs on pyrrolase activity the enzyme has been measured *in vitro* following prior treatment of the animals *in vivo*. In an attempt to observe whether these *in vitro* observations have any relevance to the activity of the enzyme *in vivo*, we have investigated the effect of certain drugs on the activity of the enzyme in the isolated perfused rat liver. The concentration of kynurenine in the medium has been used as an index of enzyme activity. A small lobe of liver was also removed at the beginning and end of the perfusion and enzyme activity measured *in vitro*.

Rat livers were perfused by the method of Hems, Ross, Berry & Krebs (1966) with a semi-synthetic medium (Woods, Eggleston & Krebs, 1970); *in vitro* enzyme activity was measured as described by Curzon & Green (1969).

Perfusion of the basal medium without the addition of tryptophan resulted in a small amount of kynurenine appearing in the medium at the end of a 60 min perfusion (2.0 ± 0.1 μg kynurenine/g liver (wet weight)). The concentration of tryptophan in the basal medium was 0.84 ± 0.15 μg