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Anticonvulsant drugs and brain glucose

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STUDIES of the effects of phenobarbitone and dimethadione on the uptake of D-xylose by guinea pig cerebral cortex slices have shown that after a 10-min incubation period a greater concentration of xylose is present in the non-raffinose compartment (total water minus raffinose space) of the slices than in the corresponding compartment (which was similar in volume) of slices from animals receiving saline.[1] Xylose and glucose appear to be transported by the same mechanism in brain tissue *in vitro*[2] and *in vivo** so that our results might account, at least in part, for the finding of Mayman, Gatfield and Breckenridge[3] that the glucose content of mouse brain was significantly elevated by phenobarbitone. We have recently examined other anticonvulsants for their effects on sugar transport and metabolism *in vitro* and on the brain glucose level *in vivo*.

The brain and serum glucose levels of mice were determined using the glucose oxidase technique,[4] in animals injected intraperitoneally with 0·9% saline, acetazolamide (150 mg/kg), phenobarbitone sodium (40 mg/kg and 250 mg/kg), diphenylhydantoin (20 mg/kg), ethosuccimide (250 mg/kg) or the diphenylhydantoin vehicle (43% ethanol in water). The animals were decapitated 1 hr after the injections except in the cases of ethosuccimide and its controls (15 min) and the heads immediately frozen in liquid nitrogen while blood was collected from the neck. Figure 1 shows that in each case, at a dose of approximately twice the ED_{50}, the anticonvulsant significantly elevated the brain glucose level. This effect was also obtained at the toxic dose of phenobarbitone (250 mg/kg). Figure 2 shows that the serum glucose concentrations tended to be higher in animals receiving some anticonvulsants. This effect has been reported to be significant for phenobarbitone and diphenylhydantoin but in our experiments it was only significant for the latter.

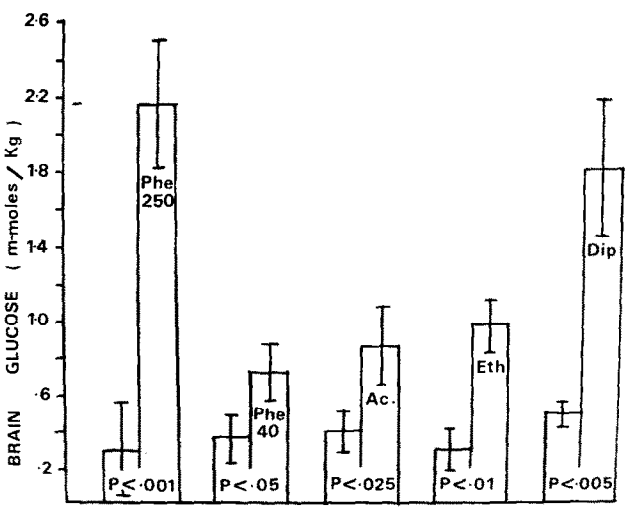

FIG. 1. The brain glucose levels of mice injected with saline, acetazolamide (Ac, 150 mg/kg), phenobarbitone sodium (Phe, 40 mg/kg and 250 mg/kg), diphenylhydantoin (Dip, 20 mg/kg), ethosuccimide (Eth, 250 mg/kg), or the diphenylhydantoin vehicle. The animals were decapitated after 1 hr or 15 min (ethosuccimide and its controls), so that the heads fell into liquid N_2. The left hand column of each pair represents the controls. Each result is the mean of seven observations. The vertical lines indicate standard errors of the means.

* J. C. GILBERT, unpublished observations.

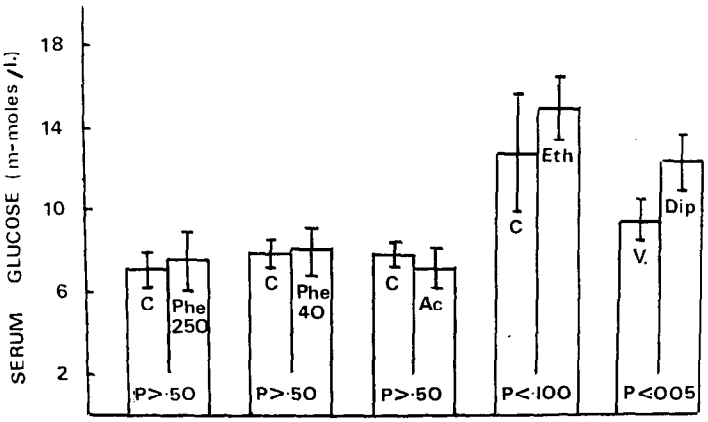

FIG. 2. The serum glucose levels of mice injected with saline (column C in each of the first four pairs), the diphenylhydantoin vehicle (v) or anticonvulsant drugs as in Fig. 1. The ethosuccimide experiments, with their controls, were performed under different conditions than the others. (For details, see text.)

The extra glucose contained in the blood of the brains (30 μl/g) of animals receiving diphenylhydantoin does not, *per se*, account for the increase in total brain glucose—there is also a significant extravascular increase. Furthermore, differences in glycolytic rates[6] between control and drug-treated animals during the brief period of ischaemia following decapitation are also too small to account for the results. It is of interest, therefore, to determine if the increased brain glucose results from increased uptake of sugar or from inhibited metabolism or from both. We have determined the uptake of xylose by the non-raffinose compartment of guinea pig cerebral cortex slices using methods similar to those described previously.[1] Slices from the untreated animals were incubated for 30 min at 37° in an oxygenated bicarbonate medium containing sodium pyruvate (4 mM) as nutrient and raffinose (10 mM) and the drug under test, in a concentration not very different from that likely to be reached in the brain *in vivo* (diphenylhydantoin 0·1 mM, acetazolamide 0·02 mM or ethosuccimide 0·5 mM). The slices were then transferred to a similar medium containing 50 mM xylose for 9 min and at the end of this incubation they were weighed, freeze dried and reweighed to determine the total water contents. After analysis of tissues and media,[7] the concentrations of xylose in the non-raffinose compartments of the slices were then determined using the equation shown:

$$\text{Concentration} = \left\{ \frac{\text{xylose space—raffinose space}}{\text{total water—raffinose space}} \right\} \times \text{medium xylose conc.}$$

It should be noted that the non-raffinose and raffinose compartments of the slices behave physiologically as intracellular and extracellular compartments.[7] They do not necessarily correspond morphologically to those compartments. Table 1 shows that acetazolamide and ethosuccimide significantly increase the concentration and therefore, like phenobarbitone and dimethadione, may be assumed to stimulate xylose transport into the non-raffinose compartment. Diphenylhydantoin did not significantly stimulate transport under our conditions and this finding is compatible with our previous work.[1]

To test the effects of the drugs on the overall glucose metabolism of brain tissue, cerebral cortex slices were pre-incubated for 10 min at 37° in an oxygenated bicarbonate medium with or without the drug and then a known volume of a similar medium containing glucose was added to give a final glucose concentration of 2 mM, and the slices incubated for a further 30 min. Residual glucose was then determined in ice-cold protein-free solutions. Table 2 shows that inhibition of metabolism was only detected when the higher concentration of phenobarbitone was tested and acetazolamide actually stimulated metabolism. These results were, on the whole, not unexpected. The cortical slice is assumed to exhibit a lower level of functional activity than the tissue *in vivo*, and, in addition, anticonvulsant doses of drugs tend to prevent any increase in the metabolic activity of the slice when it is stimulated and not to influence detectably the resting level of activity.[8] Reports of inhibition of the respiration of slices by anticonvulsants have involved concentrations unlikely to be reached *in vivo*[9]—concentrations, in fact, comparable to the higher level of phenobarbitone used here. Evidence

TABLE 1. EFFECTS OF ANTICONVULSANTS ON XYLOSE UPTAKE

Condition	Volume of NRC (μl/g wet wt.) $\pm$ S.E.M.	Xylose concentration in NRC (mM) $\pm$ S.E.M.
Control	$248 \pm 18_{(7)}$	$31 \pm 3_{(7)}$
Acetazolamide	$269 \pm 18_{(7)}$†	$38 \pm 3_{(7)}$*
Diphenylhydantoin	$212 \pm 13_{(5)}$	$38 \pm 4_{(5)}$
Vehicle		
Diphenylhydantoin	$196 \pm 10_{(5)}$	$46 \pm 4_{(5)}$
Control	$232 \pm 17_{(7)}$	$32 \pm 2_{(7)}$
Ethosuccimide	$231 \pm 10_{(7)}$	$37 \pm 2_{(7)}$*

NRC, Non-raffinose compartment.

* Significantly different from the control.

† Significantly different from the control but difference nullified by correcting for the osmotic effect of the increased NRC xylose concentration. Cerebral cortex slices were incubated in a bicarbonate medium containing raffinose, with or without the drug, for 30 min, and then transferred to a similar medium containing 50 mM xylose for 9 min.

TABLE 2. THE EFFECTS OF DRUGS ON THE GLUCOSE UTILISATION OF CEREBRAL CORTEX SLICES

Series	Condition	Glucose utilisation (μmoles/g wet wt./hr) $\pm$ S.E.M.
A	Control	$26{\cdot}4 \pm 1{\cdot}9_{(4)}$
	Acetazolamide (0·02 mM)	$28{\cdot}9 \pm 2{\cdot}3_{(4)}$*
B	Control	$26{\cdot}3 \pm 1{\cdot}5_{(4)}$
	Ethosuccimide (0·5 mM)	$27{\cdot}3 \pm 1{\cdot}5_{(4)}$
C	Vehicle	$27{\cdot}8 \pm 2{\cdot}0_{(6)}$
	Diphenylhydantoin (0·1 mM)	$25{\cdot}8 \pm 1{\cdot}5_{(6)}$
		$33{\cdot}3 \pm 2{\cdot}9_{(6)}$
D	Control	
	Phenobarbitone (0·1 mM)	$32{\cdot}9 \pm 1{\cdot}8_{(6)}$
	Phenobarbitone (5·0 mM)	$28{\cdot}9 \pm 1{\cdot}9_{(6)}$*

* Significantly different from controls of the same series of experiments. In series D, the cerebral cortex slices were prepared with minimal contact with medium (less medium than in the other series—this decreases the wet weights and increases the glucose utilisation/g). Slices were incubated in a bicarbonate medium, with or without the drug, for 10 min before adding glucose (final conc. 2 mM) and continuing the incubation for 30 min.

suggests,[8] that *in vivo* also, anticonvulsant doses of the drugs have little effect upon the normal metabolic activity of brain. It therefore seems unlikely that the elevation of brain glucose in our experiments simply reflects a decreased utilisation of the main oxidisable substrate of brain due to depression of cerebral activity by the drugs tested. This explanation would also not account for the effects of the drugs on xylose transport *in vitro*, due presumably to direct physicochemical effects on the cell membrane. It is more likely that the anticonvulsants stimulate glucose transport into brain. The possibility should not be overlooked that an elevation of glucose in specific regions of the brain might be implicated in the mechanisms of anticonvulsant activity of the drugs and not simply result from such activity, i.e.

that glucose may play a role independent of its more obvious one of substrate. There is evidence that glucose and other sugars can influence the activity of enzymes.* In addition, glucose might be capable of stabilising an orderly arrangement of water molecules in lattice,[10] and it has been suggested that water molecules play a vital role in the structure and function of the cell membrane.[11] Our own preliminary studies suggest that glucose, (2 mM) under conditions unfavourable for glucose metabolism, reduces the free acid phosphatase activity of cerebral lysosomes subjected to mild osmotic shock. Coldman and Good[12] have observed, using the haemolysis of erythrocytes as a test system, that glucose can increase the energy of activation necessary to convert water molecules to the free (disordered) state. The convulsant metrazol had the opposite effect. Phenobarbitone did not increase the energy of activation and the authors concluded that it must increase the degree of "apolar hydration" in order to account for its narcotic effect. Our work suggests that whatever direct physico-chemical effect phenobarbitone and other anticonvulsants have on the cell membrane may be supplemented by their effect of elevating the cell glucose concentration which, itself, could be involved in the activity of the drugs by influencing water structure at the membrane. This mechanism could be implicated in the biological activity of a variety of drugs.

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Department of Biochemistry,
University of St. Andrews,
St. Andrews, Scotland

J. C. GILBERT†
P. GRAY
G. M. HEATON

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* J. C. BIRD, personal communication.

† Present address: Department of Pharmacology, University Medical Buildings, Foresterhill, Aberdeen, Scotland.

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Effect of versicolorin C, rosenonolactone and cyclopiazonic acid on bovine pancreas deoxyribonuclease

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VERSICOLORIN C, a metabolite of *Aspergillus versicolor*,[1] rosenonolactone, a metabolite of *Trichothecium roseum* Link[2–4] and cyclopiazonic acid, a metabolite of *Penicillium cyclopium* Westling[5] are toxic compounds* of which at least the first two are potential carcinogens according to their chemical structures.

* I. F. H. Purchase, personal communication.