Influence of ouabain, DNP and DMSO on amino-acid transfer out of the subarachnoid space

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Wright, Nogueira & Levin (1971) found that when ^{14}C-GABA and ^{14}C-leucine were left in contact with the cerebral cortex of cats for 5 h, a higher concentration of radioactivity was found in the pia than in the underlying cortex. It seemed of interest, therefore, to find out whether a transport process was operating at the level of this membrane. The purpose of these experiments was to study the effect of metabolic inhibitors and dimethyl sulphoxide (DMSO) which increases transport across biological membranes, on the disappearance of ^{14}C-L-leucine from the subarachnoid space.

Plastic cups 1 cm in diameter containing 0·4 ml of artificial CSF, 10 mM ^{14}C-L-leucine and the substance under investigation were placed on the cortex of cats anaesthetized with pentobarbitone. Chromatographic and radioactivity assays in

TABLE 1. *Influence of metabolic inhibitors and DMSO on the disappearance of* L-*leucine 10 mM from cortical cups and its passage into the tissue after 5 h*

Compounds	Remaining† in cup		Passage to tissue† (pia–cortex 3 mm depth)
Ouabain (7)‡ (10^{-5} M)	^{14}C−	^{12}C−	
	+30±0·9	+32±1·1	−71 ±7·9
	$p<0·01$	$p<0·05$	$p<0·001$
Dinitrophenol (7) (10^{-4} M)	−19±3·0	+5±0·2	+10±2·0
DMSO [10%] (10)	−17±1·9	−10±1·2	—
DMSO [20%] (3)	−57+10·9	−46±12·1	—
	$P<0·05$		

†Figures represent percentages compared to control cups.
‡Figures in parentheses after substances represents number of determinations.

samples of fluid remaining in the cup after 5 h were then carried out. Tissue samples of the underlying cortical tissue were measured for radioactivity content.

Ouabain (10^{-5} M) caused a 71% reduction in the concentration of ^{14}C-leucine in the cortex, with a corresponding increase in the amount recovered in the cups. DNP (10^{-4} M), which would be expected to suppress aerobic phosphorylation, did not alter the uptake in the same way as ouabain. The lack of effect of DNP on our *in vivo* model might be explained by the availability of alternative energy sources or ATP stores not present in incubation procedures. However, DMSO increased the disappearance of leucine from the cups and this effect could be due to a partial breakdown of the blood-brain barrier (Brink & Stein, 1967).

The effect of ouabain could be explained by its action on Na-K stimulated ATP-ase, which might be involved in cation transport (Garrahan & Glynn, 1965). Kostyo & Schmidt (1963), have shown coupling between ionic and amino-acid transport in the rat diaphragm. Our results suggest that a similar mechanism could be operating at the pial membrane.

REFERENCES

BRINK, J. J. & STEIN, D. J. (1967). Pemoline levels in brain: Enhancement by dimethyl sulphoxide, *Science, N. Y.*, **158**, 1479–1480.

GARRAHAN, P. J. & GLYNN, J. M. (1965). Uncoupling the sodium pump. *Nature, Lond.*, **207**, 1098–1099.
KOSTYO, J. L. & SCHMIDT, J. E. (1963). Inhibitory effects of cardiac glycosides and adrenal steroids on amino acid transport. *Am. J. Physiol.*, **204**, 1031–1038.
WRIGHT, P. M., NOGUEIRA, G. J. & LEVIN, E. (1971). Role of the pia mater in the transfer of substances out of the cerebrospinal fluid. *Exp. Brain Res.*, in the Press.

Effect of drugs used in status-epilepticus on the potassium fluxes of cerebrospinal fluid in the conscious dog

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Zuckerman & Glazer (1968) demonstrated that localized high concentrations of potassium in the region of the inferior horn of the lateral ventricle could cause convulsions originating in the hippocampal system.

The potassium concentration in the cerebrospinal fluid (C.S.F.) is maintained within narrow limits independent of large alterations in plasma concentrations (Kemeny, Boldizsar & Pethes, 1961). The mechanisms whereby this is achieved have been investigated and characterized by ventriculo-cisternal perfusion techniques (Heisey, Held & Pappenheimer, 1962; Cserr, 1965; Katzman, Graziani & Ginsberg, 1968; Bradbury & Stulcova, 1970). In the experiments described here, drugs useful in the treatment of status epilepticus were investigated for their effect on the potassium fluxes of C.S.F.

Ventriculo-cisternal perfusions were performed in adult beagle dogs with chronically implanted guide tubes, using inulin and ^{42}K contained in a sterile salt solution which was comparable to dog C.S.F. The perfusion fluid was pumped into one lateral ventricle and out of the cisterna magna at a rate of 0·3 ml/min using two equally calibrated modules of a multichannel pump. The experiments were carried out while the animals were conscious and free moving, and pressure alteration within the cerebrospinal fluid system still appeared to be governed by normal physiological variables such as head movement, respiration and blood pressure. The effluent from the perfusion system was collected by means of a fraction collector and the samples were subsequently analysed for inulin, ^{42}K and total potassium. This data, in conjunction with the results of comparable analyses on the inflowing fluid, allowed calculation of bulk flow of C.S.F. from the system and the appropriately corrected rate constants (meq/min) governing the influx and efflux of C.S.F. potassium. All these parameters could thus be assessed on a continuous basis throughout the experiments.

When a stable base line of control values had been established after a period of at least 90 min, the drug under study was administered by either intravenous or intramuscular injection. Of the drugs investigated, the barbiturate anaesthetics, sodium thiopentone and sodium pentobarbitone, when given in doses sufficient to achieve light anaesthesia, had the most striking effects, producing highly significant decreases of up to 40% in both potassium rate constants. Phenytoin and diazepam both produced significant decreases of between 6–14% in efflux and influx rates of C.S.F. potassium in subanaesthetic doses while paraldehyde, even when given in sufficiently high doses to produce light anaesthesia, had no significant effect on potassium fluxes.

These preliminary results would seem to indicate that while the potassium fluxes of