By either route the convulsion pattern (flexor-extensor) produced in mice by FA resembled that with all other convulsants except strychnine (extensor) and ouabain (fore-limb extension only). Convulsions and EEG changes also followed intraventricular administration of FA to rats and rabbits.

Folic acid was more effective and quicker acting after I.C.V. than I.V. administration which may reflect greater metabolism or poor brain penetration following peripheral injection. The relatively long convulsant latency of FA might be attributable to its reduction to an active form but one reduced analogue, folinic acid, had a similar convulsant potency and latency to FA. Glutamic acid, part of the FA molecule, was convulsant I.C.V. although weaker than FA.

Of the convulsants tested picrotoxin is most like FA in being considerably more effective I.C.V. than I.V. and in having a long latency (I.C.V.). Picrotoxin and FA were also the only convulsants (I.C.V.) that precipitated convulsions to auditory stimuli. In threshold electroshock studies the incidence of HLE was significantly increased by subconvulsive doses of FA, picrotoxin and ouabain but not by strychnine or bicuculline.

Oral dosing 2 h previously with phenobarbitone, phenytoin or troxidone abolished HLE induced by 40 μg (I.C.V.) FA (ED50's: 2·9, 3·8 and 436 mg/kg respectively), by maximum electroshock (ED50's: 7·2, 5·9, 812 mg/kg) and by leptazol I.V. (ED50's: 10·0, 5·0, 270 mg/kg).

Our findings are consistent with the suggestion that high localized folate concentrations could form epileptic foci (Hommes & Obbens, 1972).

REFERENCES

HAYASHI, T. (1959). Neurophysiology and neurochemistry of convulsion. Tokyo: Dainihon-Tosho Co.

HOMMES, O. R. & OBBENS, E. A. M. T. (1972). The epileptogenic action of Na-folate in the rat. *J. Neurol. Sci.*, **16**, 271–281.

NOELL, W. K., MAGOSS, M. S., COHEN, L. H., HOLLAND, J. F. & WALTERS, G. C. (1960). Cerebral effects of folic acid, pyrimidines, amino-acids and their antimetabolites. *Electroenceph. Clin. Neurophysiol.*, **12**, 238.

REYNOLDS, E. H. (1967). Effects of folic acid on the mental state and fit-frequency of drug-treated epileptic patients. *Lancet*, **1**, 1086–1088.

Changes in brain monoamine metabolism associated with CO_2-induced amnesia in rats

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Rats will learn to remain on a brightly lit runway instead of entering a darkened box if entry into the box is associated with an electrically induced footshock. Experiments in our laboratories have shown that this conditioned response is no longer retained if the rats are exposed to an atmosphere of CO_2 immediately after they have been given the footshock.

The object of the present study was to see if the changes in behaviour observed in the amnesia test could be correlated with changes in monoamine metabolism in those regions of the brain which have been implicated in memory.

Four groups of 20 rats (S.P.F., albino, male weighing 230–240 g) were used. The first group was exposed to an atmosphere of CO_2 until respiratory arrest occurred. They were then revived by artificial respiration. The second group was exposed to footshock (0·50 mA for 3 s). The third group was given the footshock treatment followed by CO_2 and the last group was the untreated (control) group. These treatments corresponded exactly to those used in the amnesia test. All animals were killed by decapitation 24 h after treatment and the brains were dissected into the cortex, hippocampus, mid-brain (striatum, hypothalamus, thalamus and amygdala), the brain stem and the cerebellum. Brain areas from 4 rats were pooled for the determination of tyrosine, dopamine, noradrenaline, homovanillic acid, normetanephrine, tryptophan, 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid and γ-aminobutyric acid by standard fluorimetric methods.

The results show that the most marked changes in amine metabolism occur in the hippocampus. This is of particular interest because the hippocampus has been implicated in the control of memory and behavioural inhibition (Douglas, 1972; Milner, 1959). No significant change in any of the parameters could be found in the cortex or cerebellum.

A slight increase in the hippocampal dopamine and homovanillic acid concentration was found in both the shock and in the CO_2+ shock treated groups This could be indicative of an increased dopamine turnover as a consequence of the shock treatment. Dopamine metabolism was unaffected in the group treated with CO_2 alone. However, the most marked change was found in the 5-HT concentration of the hippocampus and brain stem. In the shock treated group there was a rise in the 5-HT concentration but there was no significant change in the concentration of this amine in the group treated with CO_2 + shock.

The results suggest that, as CO_2 treatment blocks the conditioned response, and as the rise in the 5-hydroxytryptamine concentration following shock is also prevented by subsequent treatment with CO_2, there might be a correlation between amnesia and changes in hippocampal 5-hydroxytryptamine metabolism. The changes in dopamine metabolism do not appear to be correlated with the behavioural changes as CO_2 treatment did not reduce the elevation of this amine which occurred following shock.

REFERENCES

DOUGLAS, R. J. (1972). Pavlovian conditioning and the brain. In *Inhibition and Learning*. Boakes R. A. & Halliday, M. S. (eds.). *Academic Press, London*, 529–553.

MILNER, B. (1959). The memory defect in bilateral hippocampal lesions. *Psychiat. Res. Rep.*, **11**, 43–52.

Drugs influencing plasma and brain tryptophan

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Synthesis in the brain of 5-hydroxytryptamine (5-HT) is influenced by the concentration of tryptophan and the rate limiting step in 5-HT synthesis is the hydroxylation of tryptophan by tryptophan hydroxylase which is normally unsaturated with substrate. In circumstances such as food deprivation or immobilization (Curzon, Joseph & Knott, 1972 ; Perez-Cruet, Tagliamonte, Tagliamonte & Gessa, 1972) increased rat brain tryptophan appears to be associated with increased brain 5-HT turnover as indicated by raised concentrations of its metabolite 5-hydroxyindolylacetic acid (5-HIAA). Though brain tryptophan is presumably derived from plasma, the brain and plasma concentrations were not significantly correlated. However, only the small free fraction of plasma tryptophan is directly available to brain as the greater part of the tryptophan as conventionally determined is bound to plasma albumin. Increased brain tryptophan upon food deprivation or immobilization is associated with increased plasma free tryptophan (Knott & Curzon, in the press).

The mechanism by which plasma tryptophan increases has been investigated. It is known that food deprivation increases plasma unesterified fatty acid concentration and that, in common with tryptophan these substances are largely bound to albumin. It was found that other treatments which increased unesterified fatty acid concentration also increased plasma free (i.e. ultrafilterable) tryptophan i.e. intravenous injection of isoprenaline (0·04 mg/kg) ($P<0·01$), intraperitoneal injection of aminophylline (150 mg/kg) ($P<0·001$) and intravenous injection of heparin (5,000 i.u./kg) ($P<0·01$). Aminophylline also increased brain tryptophan, 5-HT and 5-HIAA ($P<0·001$). Conversely intraperitoneal injection of nicotinic acid (50 mg/kg), which decreases plasma unesterified fatty acid concentration, opposed the increases of plasma free tryptophan and brain tryptophan upon food deprivation. Furthermore, addition of fatty acids to plasma *in vitro* caused increased free tryptophan. Insulin (2 U/kg) decreased both plasma unesterified fatty acid and tryptophan concentrations ($P<0·02$).