early (peak 40–75 ms) and late facilitation (peak 700–2,000 ms), neither of which are significantly reduced by atropine (Brimble, Wallis & Woodward, 1972). The present results indeed suggest some variable increase in early facilitation in the presence of atropine, possibly due to an increase in the subliminal fringe. Increasing the number of conditioning stimuli increased the magnitude of late facilitation from 10±1% (n=6), following single stimuli to a value of 29±3% (n=5), following a 1 s, 30 Hz train. After a 1 s, 10 Hz train of stimuli a maximal facilitation of 20±2% (n=19), was obtained. Atropine, 2·9 μM, caused a marked though variable reduction (26±6%, n=6) in this facilitation. The facilitation following a 30 Hz train was also substantially reduced. However, we have been unable to demonstrate post-train facilitation with heterosynaptic testing.

In contrast to the lack of effect of atropine on the late facilitation following single conditioning stimuli, the LN wave was reduced after approximately 25 min by 70±4%, (n=5), in the presence of 2·9 μM atropine, but 29 μM atropine caused no further reduction. 0·29 μM atropine was also effective in reducing the LN wave. After trains of stimuli, measurement of the LN wave is difficult, as it is partially or totally submerged by the P wave (Libet, 1964). It appears superimposed on the declining phase of the P wave and, although 2·9 μM atropine reduced this LN wave, it was not possible to estimate the extent of the reduction.

We conclude that post-train facilitation is partly mediated via muscarinic receptors, but that these receptors apparently play no part in the late facilitation seen after a single conditioning stimulus. Experiments in progress using heterosynaptic testing suggest presynaptic mechanisms are primarily involved.

REFERENCES

BRIMBLE, M. J., WALLIS, D. I. & WOODWARD, B. (1972). Facilitation and inhibition of cell groups within the superior cervical ganglion of the rabbit. *J. Physiol., Lond.*, **226**, 629–652.

LIBET, B. (1964). Slow synaptic responses and excitatory changes in sympathetic ganglia. *J. Physiol., Lond.*, **174**, 1–25.

Some studies on the convulsant action of folic acid

M. G. BAXTER, A. A. MILLER* and R. A. WEBSTER

Pharmacology Laboratory, Wellcome Research Laboratories, Beckenham, Kent BR3 3BS and Department of Pharmacology, University College, London WC1E 6BT

It has been suggested that the anti-folate effect of anticonvulsants may be related to their therapeutic action (Reynolds, 1967) and it is known that folic acid (FA) is a convulsant when given intraventricularly to dogs (Hayashi, 1959) and rats (Hommes & Obbens, 1972; Noell, Magoss, Cohen, Holland & Walters, 1960). We have compared FA and some known convulsants by intracerebroventricular (I.C.V.) and intravenous injection in mice.

TABLE 1. *ED50 values to induce convulsions and hind-limb extension (HLE), and convulsion latencies, for folic acid and some known convulsants by intracerebroventricular and intravenous injection in mice. In the calculation of each ED50 a minimum of 50 mice was used disposed over at least 5 doses*

Response	Folic acid	Leptazol	Picro-toxin	Strych-nine	Bicucu-lline	Glutamic acid	Ouabain
A. Intracerebroventricular (i.c.v.) ED50							
μg/mouse							
Convulsions	17·8	330·0	1·3	2·2	1·1	145·0	1·3
HLE	20·5	617·0	6·8	6·3	6·5	780·0	>20
Conv. latency (secs)	104·0	18·8	134·8	83·1	26·8	6·3	140·4
B. Intravenous ED50							
mg/kg							
Convulsions	846·0	29·4	5·8	0·5	0·5	>1800	>20*
HLE	1,024·0	41·8	11·5	0·6	0·8	—	—
Conv. latency (secs)	5,843·0	3·1	157·0	53·1	4·3		
C. Ratio of doses i.v./i.c.v. × 0·05 (1 μg/mouse = approx. 0·05 mg/kg)							
Convulsions	950·0	1·7	89·2	4·5	9·1	—	—
HLE	996·0	1·3	33·8	1·8	2·4		
Conv. latency (secs)	56·2	0·2	1·1	0·6	0·2		

* Lethal dose.

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

B. E. LEONARD* and H. RIGTER

Pharmacology Department, N.V. Organon, Oss, the Netherlands

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.