produce a characteristic hypnotic state (Marcus, Winters, Roberts & Simonsen, 1971), but which, at doses in excess of 1 g/kg, induce general seizures (Clifford, Taberner, Tunnicliff, Rick & Kerkut, 1972). In the present work we have injected rats with subhypnotic doses of GHB and IMA both simultaneously and at various time intervals and have determined the duration of the loss of righting reflex and the total sleeping time. Criteria for loss of righting reflex and sleeping time were as defined previously (Rick, Benton & Taberner, 1972).

Potentiation of the depressant behavioural effects occurred when 100 mg/kg IMA I.P. was given 30 min prior to 100 mg/kg GHB I.P. This potentiation did not occur when the drugs were administered in the reverse order. When 200 mg/kg GHB and 200 mg/kg IMA were given together, general seizures were induced in all the animals tested which lasted for over 3 hours.

Since we have shown that IMA can inhibit rat brain gamma-aminobutyric acid-2-ketoglutaric acid aminotransferase (GABA-T) (Clifford *et al.*, 1972) we have examined the effect of pre-treating animals with 12·5 mg/kg of amino-oxyacetic acid (AOAA), an established inhibitor of GABA-T, which induces large increases in the brain GABA level in various species (Wallach, 1961). Rats pretreated with 12·5 mg/kg AOAA I.P. slept for over two hours following an injection of 200 mg/kg GHB 30 min later. When the GHB was administered 2 or 4 h after the AOAA the animals slept for up to 4 h and showed loss of righting reflex for about 60 minutes. This effect was not observed with shorter time intervals between the injections.

In addition we have confirmed that pyrazole, a potent inhibitor of alcohol dehydrogenase, potentiates GHB sleeping time and loss of righting reflex (Taberner, Rick & Kerkut, 1972; Bessman & McCabe, 1972). Also, rats pretreated with 100 mg/kg pyrazole I.P. slept for over 4 h following an injection of 200 mg/kg IMA, suggesting that the effect of pyrazole on the inactivation of GHB might not be as specific as has been suggested. At the doses used, none of the drugs, GHB, IMA, AOAA or pyrazole induced sleep when administered alone.

REFERENCES

BESSMAN, S. P. & MCCABE, E. R. B. (1972). 1,4-Butane diol—a substrate for rat liver and horse liver alcohol dehydrogenases. *Biochem. Pharmacol.*, **21**, 1135–1142.

CLIFFORD, J. M., TABERNER, P. V., TUNNICLIFF, G., RICK, J. T. & KERKUT, G. A. (1972). Biochemical and pharmacological actions of imidazoleacetic acid. *Biochem. Pharmacol.* (in press).

MARCUS, R. J., WINTERS, W. D., ROBERTS, E. & SIMONSEN, D. G. (1971). Neuropharmacological studies of imidazole-4-acetic acid actions in the mouse and rat. *Neuropharmacology*, **10**, 203–215.

RICK, J. T., BENTON, D. A. & TABERNER, P. V. (1972). Changes in cerebral metabolism following administration of gamma-hydroxybutyric acid and imidazoleacetic acid. *Proceedings of the First European Congress on Sleep Research, Basel, Switzerland* (in press).

TABERNER, P. V., RICK, J. T. & KERKUT, G. A. (1972). Metabolic factors involved in the interaction between pyrazole and butane-1,4-diol and 4-hydroxybutyric acid. *Life Sciences*, Part 1, **11**, 335–341.

WALLACH, D. P. (1961). 'Studies on the GABA pathway I'. The inhibition of γ-aminobutyric acid-α-ketoglutaric acid transaminase *in vitro* and *in vivo* by U-7524 (amino-oxyacetic acid). *Biochem. Pharmacol.*, **5**, 323–331.

Effect of amino-oxyacetic acid on the accumulation of ^{3}H-γ-aminobutyric acid (^{3}H-GABA) by rat retina

M. J. NEAL and M. S. STARR*

Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX

Retinae were dissected and incubated in Krebs bicarbonate medium at 37° C with radioactive amino acids as described previously (Starr & Voaden, 1972).

When retinae were incubated with ^{3}H-GABA (5×10^{-8}M) there was a rapid accumulation of radioactivity by the tissue, resulting in a maximum tissue medium ratio (dpm/g tissue: dpm/ml medium) of 50:1 for control retinae and 100:1 for retinae exposed to amino-oxyacetic acid (AOAA, 10^{-5}M). A double reciprocal plot of the initial velocity

of ^{3}H-GABA uptake against the concentration of GABA in the medium revealed two major components, corresponding to a high and low affinity uptake process with values for the apparent Km of 40 μM and 650 μM and for maximum velocity of $6{\cdot}7\times10^{-8}$ and $6{\cdot}1\times10^{-7}$ (mol/min)/g wet wt. tissue respectively. The same values were obtained in the presence of AOAA (10^{-5}M) indicating that at this concentration AOAA does not affect the initial rate of entry of GABA into the retina.

The maximum potentiation of ^{3}H-GABA accumulation achieved was 80% and this only occurred when GABA aminotransferase (GABA-T) activity was inhibited by 100%. The lowest concentration of AOAA producing this effect on uptake was found to be 10^{-6}M ; below this concentration both the inhibition of GABA-T and the potentiation of ^{3}H-GABA accumulation became progressively smaller.

The inhibition of retinal GABA-T by AOAA was time-dependent and was not reversed by pyridoxal-5-phosphate (10^{-3}M) or by repeated washing of the tissue in fresh medium. AOAA also inhibited glutamic decarboxylase (GAD), but to a lesser extent than GABA-T, and the GAD activity was partially restored by pyridoxal-5-phosphate. The inhibition of GAD *in vitro*, but not *in vivo* (Baxter & Roberts, 1961), can explain why the endogenous levels of GABA and other amino acids were not found to be changed by AOAA *in vitro*. It seems unlikely therefore, that AOAA is able to increase the accumulation of radioactive GABA by the tissue by enhancing the amount of exchange diffusion with endogenous GABA pools. Although AOAA also significantly increased the retinal accumulation of radioactive L-aspartic acid ($P<0{\cdot}001$) presumably by inhibiting aspartate aminotransferase, it did not alter the accumulation of L-glutamic acid, L-glutamine, taurine, glycine, γ-aminoisobutyric acid or DL-dopamine.

The efflux of radioactivity from retinae loaded with ^{3}H-GABA was markedly reduced in the presence of AOAA at a concentration sufficient to inhibit GABA-T by 100%. Under these conditions the radioactivity released by control retinae is in the form of tritiated metabolites, whilst only GABA is released in the presence of AOAA (Goodchild & Neal, 1972). These findings suggest that AOAA potentiates the accumulation of ^{3}H-GABA by isolated retina by reducing the metabolism of the amino acid and hence reducing the efflux of radioactivity from the tissue in the form of radioactive metabolites.

We are grateful to the S.K.F. Foundation and to the University of London Central Research Fund for grants to M.J.N.

REFERENCES

BAXTER, C. F. & ROBERTS, E. (1961). Elevation of γ-aminobutyric acid in brain: selective inhibition of γ-aminobutyric-α-ketoglutaric acid transaminase. *J. Biol. Chem.*, **236**, 3287–3294.

GOODCHILD, M. & NEAL, M. J. (1972). Uptake of [^{3}H]-γ-aminobutyric acid by the rat retina. *Br. J. Pharmac.* (in press).

STARR, M. S. & VOADEN, M. J. (1972). The uptake of [^{14}C]-γ-aminobutyric acid by the isolated retina of the rat. *Vision Res.*, **12**, 549–557.

Increase in brain and spinal acetylcholine levels without antinociceptive actions following morphine administration in the frog

A. NISTRI and G. PEPEU*

Department of Pharmacology, School of Pharmacy, University of Cagliari 09100 and Department of Pharmacology, University of Florence 50134, Italy

The hypothesis that the analgesic effect of morphine and related drugs may involve a cholinergic mechanism has been repeatedly proposed and is supported by several observations. Among these, the rise in brain acetylcholine (ACh) following the administration of analgesic doses of morphine in rats (Maynert, 1967) and in mice (Harris, 1970) and the decrease in ACh output from the cerebral cortex in cats (Jhamandas, Phillis & Pinsky, 1971).