REFERENCES

1. K. MIURA and H. K. RECKENDORF, *Progr. med. chem.* **5**, 320 (1967).
2. K. ROSCHEMTHALER, P. KINDLER, P. HERRLICH and J. IGBOKWE, *Zbl. Bakt. I. Abt. Org.* **215**, 203 (1970).
3. M. ASHWELL and J. S. WORK, *Ann. Rev. Biochem.* **39**, 251 (1970).
4. W. E. BARNETT, D. H. BROWN and J. L. EPLEN, *Proc. natn. Acad. Sci. U.S.A.* **57**, 1775 (1967).
5. H. K. DAS, S. K. CHATTERJEE and S. C. ROY, *J. biol. Chem.* **243**, 4089 (1964).
6. J. P. LEIS and E. B. KELLER, *Biochem. Biophys. Res. Commun.* **40**, 416 (1970).
7. J. LUCAS-LENARD and F. LIPMANN, *Ann. Rev. Biochem.* **40**, 434 (1971).
8. D. K. DUBE, S. CHAKRABARTI, P. BHATTACHARYA, B. B. GOSWAMI and S. C. ROY, *Biochem. Pharmac.* **22**, 659 (1973).
9. D. RICHTER and F. LIPMANN, *Biochemistry* **9**, 5065 (1970).
10. B. B. GOSWAMI, S. CHAKRABARTI, D. K. DUBE and S. C. ROY, *Biochem. J.*, **134**, 815 (1973).
11. D. K. DUBE, S. CHAKRABARTI and S. C. ROY, *Cancer* **29**, 1575 (1972).
12. O. H. LOWRY, N. J. ROSEBROUGH, A. I. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
13. R. F. SWANSON, *Nature, Lond.* **231**, 31 (1971).
14. S. CHAKRABARTI, D. K. DUBE and S. C. ROY, *Biochem. J.* **128**, 461 (1972).

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The passage of δ-aminolaevulinic acid across the blood brain barrier of the rat: effect of ethanol

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ONE OF the distinctive features of acute intermittent porphyria (AIP) is the excessive urinary excretion of certain porphyrins and their precursors, δ-aminolaevulinic acid (ALA) and porphobilinogen (PBG). This is a result of the genetically mediated increase in porphyrin biosynthesis characteristic of the disease. One result of this elevated biosynthesis is that there is an associated increase in the plasma concentration of ALA. ALA is virtually undetectable in the plasma of normal subjects, but a level of 24 μg/ml has been reported in an acute porphyric attack. Similarly, normal cerebrospinal fluid (CSF) contains no ALA, yet ALA can be detected in patients with AIP.[1] These observations suggest that at elevated plasma concentrations ALA can pass the blood–brain barrier (BBB), thereby entering the brain tissue and CSF. There is, however, conflicting evidence on this possibility. Thus, although Kramer[2] has indicated that ALA is readily taken up by brain tissue, Musyka[3] has reported that the BBB is impermeable to ALA.

The resolution of these conflicting reports is of importance since there is increasing evidence that ALA may be an aetiological factor in the production of the clinical manifestations of the disease. Although the purified porphyrins and porphobilinogen are pharamcologically inactive,[4] ALA has been shown to inhibit brain ATPase activity[2] and membrane sodium transport *in vitro*.[5] Further, ALA causes behavioural changes in mice[6] and has a hypotensive action in anaesthetised rats.[7] Such actions of ALA could bear some relationship to the fact that some 55 per cent of patients with AIP exhibit psychological symptoms; and post-mortem examination of porphyric patients has revealed central neuropathy.[8] There is also convincing evidence that ALA can increase the susceptibility of experimental animals to drug induced convulsions.[9]

Certain porphyrinogenic drugs are known to provoke attacks in subjects with latent AIP.[10] These drugs, which include ethanol, increase the activity of hepatic ALA synthetase, the enzyme which produces ALA from glycine and succinyl-CoA. There is the possibility, therefore, that they precipitate porphyric attack by increasing the circulating level of ALA and thus, if ALA can pass the BBB, the brain tissue level of ALA. Alternatively, they could cause the BBB to become permeable or more permeable to ALA. Acute attacks of porphyria have been provoked by an episode of over-indulgence in ethanol.[11] This experiment was designed therefore, to confirm whether or not ALA could pass the BBB in normal animals and in animals under acute ethanol intoxication.

METHODS

Two groups of 16 male Sprague–Dawley rats (200–250 g) were used. One group was given ethanol (5 g/kg), as a 50 per cent solution in water, by gastric intubation, the other group an equivalent amount of water administered by the same route. They were then left for 0·5 hr at which time the ethanol group was intoxicated. An intraperitoneal (IP) injection of ALA 40, 120 or 200 mg/kg was then given, the water treated and ethanol treated animals being injected alternately. The ALA had previously been adjusted to pH 6–7 with molar sodium bicarbonate, and "spiked" with uniformly labelled tritiated (^{3}H) ALA. The animals were then left for a further 0·5 hr to allow the ALA to equilibrate. Technecium (^{99}Tc) labelled serum albumin was then injected into a tail vein and allowed to circulate for 2 min, at which time the animal was stunned, exsanguinated by cutting its throat, and the blood collected in a heparinized bottle

The brain was then dissected out, blotted dry and weighed. It was then halved, medially, one half being homogenized in water and counted for ^{99}Tc in a gamma counter; the weight of total homogenate was noted. This homogenate was centrifuged at 3000 rev/min for 15 min and a known volume of supernate was counted in a scintillation counter using "Instagel" as a scintillant. In order to establish that the ^{3}H counts were due to ^{3}H-ALA and not some metabolic product of it, a paper chromatogram of a sample of brain supernate was run. It was found that all the ^{3}H activity was present in the spot which corresponded with the ALA standard. The method used here was that described by Elliott.[12]

The ^{99}Tc count of 1 ml whole blood was determined, as was the ^{3}H content of 1 ml blood by the method described for the brain supernate. The blood concentration of ALA in μg/ml was determined colorimetrically.[13]

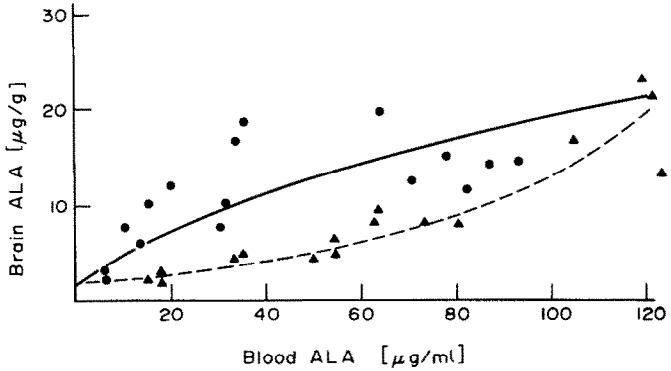

FIG. 1. A comparison of blood and aminolaevutic acid and brain and aminolaevutic acid in ethanol-treated and normal rats—(●) control; (▲) ethanol.

CALCULATION

ALA in brain tissue = Total brain ALA − ALA in blood in brain

$$= \frac{^3\text{H counts in Brain}}{^3\text{H counts/}\mu\text{g ALA}} - \frac{^{99}\text{Tc counts in brain}}{^{99}\text{Tc counts in blood/ml}} \times \text{blood ALA concn } (\mu\text{g/ml})$$

This result was divided by the weight of the brain in order to express the results as μg ALA/g brain tissue.

RESULTS

It was found that as the concentration of ALA in the blood increased so there was an increase in the concentration of ALA in the brain. At most blood concentrations, except at the highest, the content of ALA in the brains of the animals untreated with ethanol, was higher than the content in the brains of the animals treated with ethanol. When these points were plotted it was found that in both cases there were highly significant positive regression lines with the following equations:

(1) Control

Brain ALA (μg/g) = 0·6 (blood ALA (μg/ml)$^{1\cdot27}$.

Where the regression coefficient is 0·82.

(2) Ethanol treated

Brain ALA (μg/g) = 0·02 exp [2·06 (Blood ALA μg/ml)].

Where the regression coefficient is 0·95.

DISCUSSION

The results obtained indicate that ALA does pass the BBB at plasma concentrations known to occur in AIP. As the relative distribution of [^{3}H]ALA activity between the intracellular and extracellular fluids was not determined, these results represent only the total brain tissue content. This is seen to increase with the plasma concentration. Acute ethanol intoxication inhibits the entry of ALA into the brain. This effect is probably due to a competitive antagonism between the ethanol and ALA. The uncharged, lipid soluble ethanol molecules, would be expected to pass the blood brain barrier more readily than the polar ALA molecules, thus decreasing the passage of the latter.

The fact that ALA can enter the brain, strengthens the possibility that it could, directly or indirectly, contribute to the signs and symptoms of AIP. Since acute ethanol pretreatment inhibits this process, acute ethanol consumption would not appear to precipitate attacks by increasing the permeability of the brain to ALA. It is known, however, that ethanol does induce hepatic ALA synthetase.

The emergence of ALA as a possible aetiological factor in the neuropsychiatric syndrome of porphyria, stimulates further hypothetical considerations. When present in abnormally high amounts in the CNS, ALA could exert a direct action on central processes. It could also exert such an action indirectly by release, activation, or inhibition of a naturally occurring central substance. Another possibility is that abnormal amounts of ALA, or one of its normal metabolic products, such as PBG, could undergo an aberrant metabolism to some centrally active metabolite. In this respect it is of interest that the so-called "mauve factor", which appears to be significantly related to psychosis, has been identified as a trialkyl pyrrole (2,4-dimethyl-3-ethyl pyrrole) (Kryptopyrrole).[14] Russell[15] has proposed several biosynthetic schemes by which this substance could be produced from PBG.[15] Further Krischner and Pfeiffer[16] propose that Kryptopyrrole, and *trans*-methyl-2-hexanoic acid, a substance present in the sweat of chronic schizophrenics, could be produced from a common metabolite which originates from ALA.

It would seem, therefore, that further investigations into the central actions and metabolic fate of excessive amounts of ALA could contribute to the elucidation of the underlying mechanisms which express themselves in acute porphyria and associated diseases.

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REFERENCES

1. V. P. Sweeney, M. A. Pathak and A. P. Astbury, *Brain* **93,** 369 (1970).
2. S. Kramer, D. Viljoen, D. Becker and J. Metz, *S. Afr. J. Lab. clin. Med.* **17,** 103 (1971).
3. V. I. Musyka, *Bnop. Med. Nauk.* **15,** 521 (1969).
4. A. Goldberg, W. D. M. Paton and J. W. C. Thompson, *Br. J. Pharmac.* **9,** 91 (1954).
5. L. C. Issacson, R. Douglas and L. Eales, *S. Afr. J. Lab. clin. Med.* **17,** 97 (1971).
6. F. B. McGillion, M. R. Moore and A. Goldberg, *Scot. Med. J.* **18,** 133 (1973).
7. F. B. McGillion and A. Goldberg, *Br. J. Pharmac.* in press. (1973).
8. J. B. Gibson and A. Goldberg, *J. Path. Bact.* **71,** 495 (1956).
9. N. S. Kosower, E. M. Kosower, A. B. Zimm and R. Carraway, *Biochem. Med.* **2,** 381 (1969).
10. F. de Matteis, *Pharmac. Rev.* **19,** 523 (1967).
11. J. C. Ferguson, A. D. Beattie, S. G. McAlpine and H. Conway, *Postgrad. Med. J.* **46,** 717 (1970).
12. W. H. Elliot, *Biochem. J.* **74,** 90 (1960).
13. B. Haeger-Aronsen, *Scand. J. clin. Lab. Invest.* **12,** Suppl. 47,33 (1960).
14. D. G. Irvine, W. Bayne, H. Miyashita and J. R. Majer, *Nature, Lond.* **224,** 811 (1969).
15. C. S. Russell, *J. Theor. Biol.* **35,** 277 (1972).
16. K. Krischer and C. C. Pfeiffer, *Res. Commun. Chem. Path. Pharmac.* **5,** 19 (1973).