Biochem. J. (1989) 264, 597–599 (Printed in Great Britain)

Liver tryptophan pyrrolase

A major determinant of the lower brain 5-hydroxytryptamine concentration in alcohol-preferring C57BL mice

Abdulla A.-B. BADAWY,*‡ Christopher J. MORGAN,* Jason LANE,* Kam DHALIWAL* and Don M. BRADLEY†

*South Glamorgan Health Authority, Biomedical Research Laboratory, Whitchurch Hospital, Cardiff CF4 7XB, Wales, U.K., and † Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, Wales, U.K.

The lower brain 5-hydroxytryptamine concentration in alcohol-preferring C57BL, compared with -non-preferring CBA, mice is caused by a decrease in circulating tryptophan availability to the brain secondarily to a higher liver tryptophan pyrrolase activity associated with a higher circulating corticosterone concentration. Activity or expression of liver tryptophan pyrrolase and/or their induction by glucocorticoids may be important biological determinants of predisposition to alcohol consumption.

INTRODUCTION

There is considerable evidence suggesting that a deficiency in the cerebral indolylamine 5-HT (5-hydroxytryptamine) may be a key factor in predisposition to alcohol (ethanol) consumption in both man and experimental animals (for review, see [1]). A central 5-HT deficiency in human alcoholism has not, however, been investigated directly for methodological reasons, but is strongly suggested by indirect evidence showing that circulating tryptophan availability to the brain [2] and the urinary concentration ratios of the major 5-HT metabolite 5-HIAA (5-hydroxyindol-3-ylacetic acid) to metabolites of the other tryptophan-degradative pathways [3] are decreased in abstinent chronic alcoholics, and that the extent of alcohol consumption by non-abstinent alcoholics is decreased by therapy with selective central 5-HT-reuptake inhibitors [1,4]. In addition to the ability of these uptake inhibitors and of other modulators of central 5-HT metabolism and function to influence ethanol consumption in experimental animals (for references see [1]), there is direct evidence that cerebral 5-HT concentration is lower in alcohol-preferring, than in -non-preferring, strains of mice [5,6] and rats [7,8].

A low brain [5-HT] in alcohol preference could be due to an increase in 5-HT turnover, a decrease in its synthesis, or both. There is no evidence that the activity of monoamine oxidase type A (the form of the enzyme responsible for 5-HT degradation) is higher in alcohol-preferring mice [9] or rats [10]. Impaired 5-HT synthesis is therefore a more likely explanation. Cerebral 5-HT synthesis is controlled by brain tryptophan concentration, because the rate-limiting enzyme of the 5-HT-biosynthetic pathway, tryptophan hydroxylase, is unsaturated with its tryptophan substrate [11–13]. It follows therefore that peripheral factors influencing tryptophan availability to the brain must play important roles in control of cerebral 5-HT synthesis. These factors include tryptophan binding to circulating plasma albumin [14], competition between neutral amino acids (Val, Leu, Ile, Phe and Tyr) and tryptophan for the same cerebral uptake mechanism [11,12], and activity of the major tryptophan-degrading enzyme, liver tryptophan pyrrolase (tryptophan 2,3-dioxygenase; EC 1.13.11.11) [15].

In the present work, we demonstrate that the low cerebral [5-HT] in male mice of the alcohol-preferring C57BL strain is the result of a decrease in circulating tryptophan availability to the brain, secondarily to a higher liver tryptophan pyrrolase activity. A brief summary of part of this work has appeared in abstract form [16].

MATERIALS AND METHODS

Adult male mice of the alcohol-preferring C57BL/10ScSn and -non-preferring CBA/Ca strains (22–25 g each) were purchased from the Animal Unit of the University of Bristol Medical School (Bristol, U.K.) through Dr. P. V. Taberner (Department of Pharmacology), who also kindly provided samples of mouse sera. Because C57BL mice are mildly diabetic [17], some CBA obese mice, derived from the colony of the London Hospital Medical College [18], were also obtained from the above source. Data obtained in the present work with these latter mice did not, however, differ significantly from those obtained in the CBA lean controls, and were therefore not included in the present paper.

The animals were acclimatized to their new environment for 3 weeks, during which they were maintained on cube diet 41B (Oxoid) and water, before being killed by decapitation between 11:00 and 13:00 h. Brains and livers were rapidly removed and cooled by immersion in liquid N_2 and then stored at −20 °C overnight before analysis. Livers were perfused *in situ* with ice-cold 0.9% (w/v) NaCl before immersion in liquid N_2. Liver tyrosine aminotransferase (EC 2.6.1.5) [19] and tryptophan pyrrolase [20,21] activities and concentrations of liver, serum and brain tryptophan [22] and those of brain 5-HT and 5-HIAA [23] and serum corticosterone [24]

Abbreviations used: 5-HIAA, 5-hydroxyindol-3-ylacetic acid; 5-HT, 5-hydroxytryptamine.

‡ To whom correspondence and reprint requests should be addressed.

Table 1. Tryptophan, tyrosine and related metabolic parameters in alcohol-preferring C57BL and -non-preferring CBA/Ca mice

Experimental details are given in the text. In Expt. 1, all parameters listed were determined in individual mouse brains, sera and/or livers, whereas those in Expt. 2 were measured in sera pooled from at least six mice per individual determination. In Expt. 1, concentrations of total serum tryptophan and the sum of its five competitors were determined by auto-analyser, and are therefore expressed in μM, whereas in Expt. 2, both total and free serum [Trp] were determined fluorimetrically and are expressed in μg/ml. Liver tryptophan pyrrolase and tyrosine aminotransferase activities are expressed in μmol of product (kynurenine and 4-hydroxyphenylpyruvate respectively) formed/h per g wet wt. The holoenzyme and total enzyme activities of both enzymes are those obtained respectively in the absence and presence of added cofactor (2 μM-haematin or 40 μM-pyridoxal 5-phosphate respectively). Apo-(tryptophan pyrrolase) activity was obtained by difference. Serum corticosterone is expressed in μg/dl. All other expressions (except the percentage free serum tryptophan and the tryptophan:sum of competitors ratios) are in μg/ml of serum or per g wet wt. of tissue. Values are means $\pm$ S.E.M. for each group of six mice, except for liver tryptophan pyrrolase and tyrosine aminotransferase activities (eight mice per group each), and free and total serum tryptophan concentrations (determined fluorimetrically) and the percentage free serum tryptophan (five samples per group, with each sample representing the pooled sera of six mice). Values in C57BL mice were compared with those in CBA/Ca controls, and the significance of the differences is indicated as follows: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Abbreviations used: CAA, sum of competing amino acids; TAT, tyrosine aminotransferase; TP, tryptophan pyrrolase; Trp, tryptophan.

Expt. no.	Parameter	CBA/Ca mice	C57BL mice
1	Brain [5-HT]	0.94 $\pm$ 0.024	0.72 $\pm$ 0.027***
	Brain [5-HIAA]	0.43 $\pm$ 0.019	0.39 $\pm$ 0.009*
	Brain [Trp]	1.84 $\pm$ 0.09	1.43 $\pm$ 0.07**
	Total serum [Trp] (μM)	123 $\pm$ 8	91 $\pm$ 10*
	Sum of serum Trp [CAA] (μM)	954 $\pm$ 81	857 $\pm$ 24
	Serum [Trp]/[CAA] ratio	0.130 $\pm$ 0.005	0.105 $\pm$ 0.011*
	Liver [Trp]	9.10 $\pm$ 0.83	6.24 $\pm$ 0.39**
	Liver TP activity:		
	Holoenzyme	0.90 $\pm$ 0.05	1.90 $\pm$ 0.07***
	Total enzyme	1.80 $\pm$ 0.11	4.30 $\pm$ 0.18***
	Apoenzyme	0.90 $\pm$ 0.07	2.40 $\pm$ 0.20***
	Liver TAT activity:		
	Holoenzyme	153 $\pm$ 15	161 $\pm$ 11
	Total enzyme	206 $\pm$ 13	222 $\pm$ 9
2	Free serum [Trp]	1.82 $\pm$ 0.10	1.33 $\pm$ 0.12**
	Total serum [Trp]	20.58 $\pm$ 1.22	15.82 $\pm$ 0.46**
	Free serum Trp (%)	8.84 $\pm$ 0.36	8.41 $\pm$ 0.71
	Serum [corticosterone]	8.1 $\pm$ 1.3	16.4 $\pm$ 1.6**

were all determined by standard procedures. When concentrations of the five tryptophan competitors (Val, Leu, Ile, Phe and Tyr) were determined in serum by autoanalyser, that of tryptophan was also determined in the same samples by the same procedure for comparative purposes. Statistical analysis of results was by Student's *t* test.

RESULTS AND DISCUSSION

Tryptophan metabolism and disposition in alcohol preference

As shown in Table 1, brain [5-HT] and [5-HIAA] in C57 mice were lower (by 23% and 10% respectively) than those of the CBA strain. Brain tryptophan concentration was also lower (by 22%) in the former mice. These results are therefore consistent with 5-HT synthesis, and possibly also turnover, being lower in brains of alcohol-preferring C57BL mice, and suggest that this is because of a lower cerebral concentration of the tryptophan precursor.

Brain tryptophan concentration is controlled by the three peripheral factors mentioned in the Introduction. One of these, the ratio of concentration of circulating tryptophan to the sum of those of its five competitors (Val, Leu, Ile, Tyr and Phe), was 19% lower in sera of C57 mice (Table 1). This lower ratio appears to be caused by the lower tryptophan concentration (26%) and not by a higher sum of the five competitors. In fact, there were no significant strain differences in the sum of (Table 1), or the individual (results not shown), concentrations of these competitors. The above changes in tryptophan availability refer to the total (free plus protein-bound) circulating amino acid. Free (ultrafiltrable) tryptophan concentration (determined fluorimetrically in five samples, each of which represents serum pooled from six mice) was also lower (by 27%) in C57 mice. Total serum tryptophan concentration, also determined fluorimetrically in the same five samples, was 23% lower in C57 mice. These proportionate decreases in free and total serum tryptophan concentrations in C57 mice therefore were not associated with altered binding of the amino acid to circulating proteins, as is suggested by the absence of any significant strain difference in the percentage free serum tryptophan (Table 1).

A decrease in circulating tryptophan availability to the brain in the absence of altered protein binding of the amino acid is a typical effect of elevated liver tryptophan pyrrolase activity [25]. The results in Table 1 demonstrate that this is also the case in the present work. Thus liver tryptophan pyrrolase activity of C57 mice was more than twice that of the CBA strain and this was associated with a 31% lower liver tryptophan concentration, presumably because of accelerated hepatic degradation of the amino

acid secondarily to this higher pyrrolase activity, as is also the case in similar circumstances [25]. Tryptophan pyrrolase activity can be increased by a number of mechanisms [20,26]. In the glucocorticoid-dependent hormonal induction mechanism, the increase in activity of the haem-containing holoenzyme is relatively similar to, or slightly lower than, that in the haem-free apoenzyme, whereas the other two mechanisms (substrate activation and stabilization by tryptophan and cofactor activation by haem) are associated with a greater increase in holoenzyme, relative to that in apoenzyme, activity. The results in Table 1 of the present work show that the pyrrolase holoenzyme and apoenzyme activities in C57 mice were 111% and 167% respectively greater than the corresponding values in CBA controls, and thus implicate hormonal induction as the most likely mechanism of this strain difference. This is further supported by the observation (Table 1) that C57 mice have twice as much circulating corticosterone as do CBA controls.

General conclusions and comments

The present results strongly suggest that the lower brain [5-HT] in alcohol-preferring C57BL mice, compared with -non-preferring CBA controls, is caused by a decrease in circulating tryptophan availability to the brain secondarily to a higher liver tryptophan pyrrolase activity associated with a higher concentration of circulating corticosterone. Brain [5-HT] is also known to be lower in some [7,8], but not all [27], alcohol-preferring rat strains, and it therefore remains to be seen if a pyrrolase-dependent mechanism could also be implicated in alcohol preference in this species.

A noteworthy observation in the present work is that of the absence of any significant strain differences in basal liver tyrosine aminotransferase activity between C57 and CBA mice (Table 1), despite the higher circulating corticosterone concentration in the former strain. Tyrosine aminotransferase of C57BL mice has previously been shown [28] to have basal levels similar to, and to be much less sensitive to induction by glucocorticoids than, that of other mouse strains. By contrast, liver tryptophan pyrrolase of C57 mice exhibits a greater sensitivity to induction by glucocorticoids [28]. The present results are consistent with these earlier findings [28]. More recently [29], a new glucocorticoid-receptor species has been detected in rat liver under a variety of conditions and has been implicated specifically in the glucocorticoid induction of tryptophan pyrrolase, but not tyrosine aminotransferase. It therefore remains to be seen if this new glucocorticoid receptor could be demonstrated preferentially or in greater amounts in livers of C57BL mice, than in other strains, and whether differences in the genetic and other characteristics of this and the classical glucocorticoid receptor could be shown to alter the expression of tryptophan pyrrolase in the former strain. Further work on these and related aspects may help advance our knowledge of the biological basis (including the genetics) of alcohol preference and alcoholism.

We thank Dr. P. V. Taberner for provision of mouse sera and Miss Gillian A. Brown for animal maintenance.

REFERENCES

1. Naranjo, C. A., Sellers, E. M. & Lawrin, M. O. (1986) J. Clin. Psychiatry **47** (Suppl.), 16–22
2. Branchey, L., Branchey, M., Shaw, S. & Lieber, C. S. (1984) Psychiatry Res. **12**, 219–226
3. Thomson, S. M., Jr. & McMillen, B. A. (1987) Alcohol **4**, 1–5
4. Naranjo, C. A., Sellers, E. M., Sullivan, J. T., Woodley, D. V., Kadlec, K. & Sykora, K. (1987) Clin. Pharmacol. Ther. **41**, 266–274
5. Serri, G. A. & Ely, D. L. (1984) Behav. Brain Res. **12**, 283–289
6. Yoshimoto, K. & Komura, S. (1987) Pharmacol. Biochem. Behav. **27**, 317–322
7. Murphy, J. M., McBride, W. J., Lumeng, L. & Li, T.-K. (1986) Alcohol Drug Res. **7**, 33–39
8. Murphy, J. M., McBride, W. J., Lumeng, L. & Li, T.-K. (1987) Pharmacol. Biochem. Behav. **26**, 389–392
9. Zimmer, J. & Geneser, F. A. (1987) Neurosci. Lett. **78**, 253–258
10. Pispa, J. P., Huttunen, M. O., Sarviharju, M. & Ylikahri, R. (1986) Alcohol Alcohol. **21**, 181–184
11. Fernstrom, J. D. & Wurtman, R. J. (1971) Science **173**, 149–152
12. Carlsson, A. & Lindqvist, M. (1978) Naunyn-Schmiedeberg's Arch. Pharmacol. **303**, 157–164
13. Curzon, G. (1979) J. Neural Transm. **15**, 81–92
14. Curzon, G. & Knott, P. J. (1974) Br. J. Pharmacol. **50**, 197–204
15. Badawy, A. A.-B. (1977) Life Sci. **21**, 755–767
16. Badawy, A. A.-B., Morgan, C. J., Lane, J., Dhaliwal, K. & Bradley, D. M. (1989) Alcohol Alcohol. **24**, 366
17. Taberner, P. V., Unwin, J. W. & Connelly, D. M. (1983) Alcohol Alcohol. **18**, 293–299
18. Campbell, I. L. & Das, A. K. (1982) Biochem. Soc. Trans. **10**, 392
19. Badawy, A. A.-B. (1972) Biochem. J. **126**, 347–350
20. Badawy, A. A.-B. (1975) Biochem. J. **150**, 511–520
21. Badawy, A. A.-B. (1981) J. Pharmacol. Methods **6**, 77–85
22. Badawy, A. A.-B. & Evans, M. (1976) Biochem. J. **158**, 79–88
23. Curzon, G. & Green, A. R. (1970) Br. J. Pharmacol. **39**, 653–655
24. Glick, D., Von Redlich, D. & Levine, S. (1964) Endocrinology (Baltimore) **74**, 653–655
25. Badawy, A. A.-B. & Evans, M. (1983) Alcohol Alcohol. **18**, 369–382
26. Badawy, A. A.-B. (1979) Biochem. Soc. Trans. **7**, 575–583
27. Korpi, E. R., Sinclair, J. D., Kaheinen, P., Viitamaa, T., Hellevuo, K. & Kiianmaa, K. (1988) Alcohol **5**, 417–425
28. Monroe, C. B. (1968) Am. J. Physiol. **214**, 1410–1414
29. Hirota, T., Hirota, K., Sanno, Y. & Tanaka, T. (1985) Endocrinology (Baltimore) **117**, 1788–1795

Received 17 August 1989/25 September 1989; accepted 6 October 1989