Incorporation of Label from [1-¹⁴C]Ethanol into the Glutamate–Glutamine Pools of Rat Brain *in vivo*

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Glutamate and glutamine were isolated from the brains of rats given [1-¹⁴C]ethanol by three different routes of injection with and without carrier ethanol or acetate. Without exception, the specific radioactivity of brain glutamine was 3- to 5-fold that of brain glutamate. The route of injection had no consistent effect on the results. These results indicate that small amounts of ethanol are oxidized by rat brain in the compartment responsible for the so-called 'small metabolic pool' of brain glutamate.

Whereas the specific radioactivity of brain glutamate is higher than that of brain glutamine when labelled glucose, lactate or glycerol are given to rats, the reverse is true when many other ¹⁴C-labelled compounds are used (Waelsch *et al.*, 1964; O'Neal & Koeppe, 1966; Berl & Clarke, 1969, a review; Cremer, 1964; Berl & Frigyesi, 1968; Patel & Balazs, 1970; Van den Berg, 1970). The latter include glutamate, glutamine, bicarbonate, acetate, propionate, butyrate, leucine and phenylalanine. The compartmentation of glutamate metabolism in brain has also been demonstrated *in vitro* (Berl *et al.*, 1968). With the exception of β -D-hydroxybutyrate and acetoacetate (Cremer, 1971), all ketogenic compounds studied (i.e. butyrate, leucine and phenylalanine) have resulted in brain glutamine specific radioactivities that are higher than those of brain glutamate. With the possible exception of propionate, all glucogenic compounds studied (except glutamate and glutamine) give brain glutamate specific radioactivities greater than those of brain glutamine.

Because ethanol is metabolized via acetyl-CoA (Schulman *et al.*, 1957; Meghal *et al.*, 1969) and because rat brain has small but significant amounts of alcohol dehydrogenase (Raskin & Sokoloff, 1970), we have investigated the labelling of brain glutamate and glutamine after the administration of [1-¹⁴C]-ethanol by several routes. The results obtained are in essence identical with those obtained with labelled acetate and suggest that the conversion of ethanol into acetyl-CoA in rat brain occurs in the area responsible for the metabolism of the small metabolic pool of brain glutamate.

Methods

Animals

Male albino rats (180–250g body wt.) obtained from the Holtzman Co. (Madison, Wis., U.S.A.),

were injected with 0.3–0.8 ml of aq. [1-¹⁴C]ethanol. Pyrazole at 6.6 mmol/kg was given intraperitoneally to rats nos. 9–11 (Lester & Benson, 1970); these received [1-¹⁴C]ethanol 30 min later. The rats were killed either by freezing *in situ* or by decapitation into liquid N₂ 10 min after ethanol administration. Intravenously injected rats received Nembutal (60 mg/kg body wt.) 40 min before ethanol was administered in a heparinized syringe (O'Neal & Koeppe, 1966). Frozen brains were removed as described by Minard & Mushahwar (1966). Blood was collected from the decapitated animals into beakers containing heparin (approx. 0.2 mg/ml of blood).

Isolation, assay and degradation of amino acids

The amino acids were isolated from acid-soluble extracts of frozen brain tissue by methods described by O'Neal & Koeppe (1966), except that glutamine was hydrolysed enzymically, by dissolving the freeze-dried effluent of the first Dowex 1 (acetate form) column in 10 ml of 0.1 M-sodium acetate buffer, pH 4.0, and treatment of this solution with glutaminase (250 μ g) at 37°C for 1 h. After adjustment of the pH to 7.0, this solution was passed over the second Dowex 1 (acetate form) column (O'Neal & Koeppe, 1966). Lactate was isolated from the brains of rats nos. 5–9 by the method of Minard & Mushahwar (1966).

The brain glutamate from rats nos. 1–4 was pooled, diluted with carrier glutamate, then degraded (Hill *et al.*, 1958) and assayed for ¹⁴C (Koeppe & Hill, 1955).

Measurement of radioactivity

Exhaled ¹⁴CO₂ was trapped in CO₂-free 1 M-NaOH and assayed with a vibrating-reed electrometer (Koeppe & Hill, 1955). The ¹⁴C content of the isolated brain metabolites and of perchloric acid filtrates of blood and brain was measured in a Packard Tri-Carb

liquid-scintillation spectrometer with Bray's (1960) solution as scintillator.

Materials

[1-¹⁴C]Ethanol (2.4–5.0 mCi/mmol), was purchased from New England Nuclear Corp., Boston, Mass., U.S.A. Glutaminase (grade IV), Nembutal and pyrazole were purchased, respectively, from Sigma Chemical Co. (St. Louis, Mo., U.S.A.), Abbott Laboratories (North Chicago, Ill., U.S.A.), and Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.).

Results and Discussion

The results are presented in Table 1. Although considerable variation was noted from animal to animal, change in the mode of injection or killing caused no consistent differences. Because the specific radioactivity of blood was essentially the same as that of brain, no corrections were made for the approx. 3% of blood in brain and contamination of brain components by blood components is not considered a problem.

As expected, pyrazole, a potent inhibitor of ethanol oxidation (Lester & Benson, 1970), markedly decreased the formation of ¹⁴CO₂ (rats nos. 9–11). Approx. 80–90% of the ¹⁴C in the acid-soluble extract of brains from pyrazole-treated rats passed through the Dowex 1 (acetate form) column, com-

pared with 50–60% for the untreated animals (results not shown), suggesting that the brains of pyrazole-treated rats contained a relatively larger amount of unmetabolized ethanol. These results, in conjunction with the decreased specific radioactivity of the brain amino acids of the pyrazole-treated rats (Table 1) suggest, but do not prove, that pyrazole enters the brain and inhibits ethanol oxidation *in situ*.

The labelling of amino acids in brain may result from ethanol oxidation *in situ* or from prior oxidation of ethanol in another tissue (probably liver) and subsequent transport of a labelled metabolite (acetate or glucose) to the brain. If the brain labelling resulted from prior formation of ¹⁴C-labelled blood glucose the ratio of specific radioactivity of brain glutamate to that of brain glutamine should be greater than 1 (Cremer, 1964; O'Neal & Koeppe, 1966). The glutamine/glutamate specific-radioactivity ratios were from 3 to 5 (Table 1), eliminating this possibility. Moreover, in the 10 min time-period used in these experiments no investigators have reported evidence for substantial labelling of brain metabolites via blood glucose. When a longer time-period was used (1 h) such indirect labelling is a definite probability (Koeppe & Hahn, 1962). Further evidence against blood glucose being the source of brain ¹⁴C is the fact that starvation, which markedly enhances gluconeogenesis and the labelling of blood glucose, did not increase the specific radioactivities of the brain amino acids (Table 1). Also, brain lactate

Table 1. Radioactivity in rat brain, blood, exhaled CO₂ and certain brain amino acids 10 min after administration of [1-¹⁴C]ethanol

The mode of injection was intraperitoneal except for rat no. 6 (intravenous) and rats nos. 7 and 8 (intracisternal). Each rat received 15.5 μ Ci except rats no. 7 and 8, which received 4 μ Ci. With the dose of [1-¹⁴C]ethanol rat no. 3 received 0.5 mmol of unlabelled carrier ethanol and rat no. 4 received 0.25 mmol of sodium acetate. The rats from which blood results are presented were killed by decapitation into liquid N₂; the remaining animals were frozen in liquid N₂ without decapitation. The blood and brain ¹⁴C results refer to acid-soluble material.

Rat no.	Treatment	Exhaled ¹⁴ CO ₂ (% of dose)	Blood ¹⁴ C (nCi/ml)	Brain ¹⁴ C (nCi/g)	Brain amino acids (% of acid-soluble ¹⁴ C)	Specific radioactivity (nCi/ μ mol)		
						Glutamate	Aspartate	Glutamine
1	Fed	10.4	—	13.7	39	0.185	0.098	0.507
2	Fed	7.1	—	13.6	35	0.171	0.133	0.601
3	Fed plus 0.5 mmol of carrier ethanol	15.7	—	30.7	18	0.241	0.137	0.701
4	Fed plus 0.25 mmol of sodium acetate	4.0	—	26.4	45	0.396	0.230	1.97
5	Starved 24 h	11.6	17.9	16.6	18	0.129	0.158	0.381
6	Starved 48 h	3.2	22.8	19.6	10	0.067	0.204	0.201
7	Starved 24 h	6.8	5.4	19.5	52	0.237	0.362	1.09
8	Fed	5.8	8.3	14.1	19	0.073	0.093	0.242
9	Starved 24 h plus pyrazole	0.5	8.3	9.3	8	0.024	0.043	0.097
10	Fed plus pyrazole	0.6	—	11.9	11	0.044	0.038	0.184
11	Fed plus pyrazole	0.3	—	6.6	21	0.047	0.041	0.157

isolated from rats nos. 5–9 had only 0.3–3.3% of the total acid-soluble ^{14}C , whereas lactate from the brains of rats given labelled glucose had approx. 20% of the acid-soluble ^{14}C (I. K. Mushahwar & R. E. Koeppe, unpublished work). Finally, degradation of the pooled brain glutamates from rats nos. 1–4 showed 78% of the total ^{14}C to be in C-5, a situation impossible if liver and blood glucose were intermediates between $[1-^{14}\text{C}]$ ethanol and brain glutamate (Koeppe & Hahn, 1962).

If the brain labelling resulted from the metabolism by brain of $[1-^{14}\text{C}]$ acetate formed in the liver from $[1-^{14}\text{C}]$ ethanol, high glutamine/glutamate specific-radioactivity ratios are expected (and found, Table 1). The administration of a substantial load of carrier acetate (rat no. 4) along with the trace dose of $[1-^{14}\text{C}]$ ethanol did not result in a lowering of the specific radioactivity of the brain amino acids. This observation, together with the fact that the specific radioactivities of the brain amino acids from animals given labelled acetate or ethanol intravenously are similar (O'Neal & Koeppe, 1966; I. K. Mushahwar & R. E. Koeppe, unpublished work), makes it highly improbable that acetate formed from ethanol in the liver is the major source of brain labelling after administration of $[1-^{14}\text{C}]$ ethanol. Thus the observed labelling of brain metabolites apparently resulted from the oxidation of ethanol *in situ*. If substantial brain labelling was proceeding via liver formation of acetate and/or glucose, one would expect that after intraperitoneal injection the specific radioactivities of brain amino acids would be much higher than after intracisternal injection. Such was not the case (Table 1).

Therefore we conclude that ethanol is oxidized in the brain and labels the so-called 'small metabolic pool' of brain glutamate, because the specific radioactivity of glutamine is always several-fold that of glutamate (Berl & Clarke, 1969). This further suggests that the oxidation of ethanol to acetaldehyde and/or the conversion of acetaldehyde into acetyl-CoA occurs in the same area as the small glutamate pool. The results reported by Cremer (1971) indicate that the oxidation of acetyl-CoA formed from acetoacetate is more widely distributed in brain than is oxidation of acetate or acetyl-CoA formed from a variety of other ketogenic precursors (i.e. leucine, phenylalanine and butyrate). The results reported herein mean that ethanol can be added to this list of acetyl-CoA precursors that label primarily the small glutamate pool.

As can be calculated from the results in Table 1, all the animals except rats nos. 3, 7 and 8 received 3–6 μmol of ethanol, and 0.1–0.2% of this was found

in brain (based on ^{14}C). Thus 3–12 nmol of ethanol or its metabolites was found and of this amount 10–50% had been converted into the three amino acids isolated (Table 1). Therefore, during the 10 min after injection a few nmol of ethanol were oxidized by brain and used to label the various amino acid pools. The addition of 500 μmol of carrier ethanol (rat no. 3) had no substantial effect on the labelling of brain amino acids. It may be calculated that the brain of rat no. 3 converted 0.2 μmol of ethanol into the amino acids in 10 min, or the rate of oxidation of ethanol by the brain of rat no. 3 was at least 20 nmol/min per g. Raskin & Sokoloff (1970) have clearly demonstrated the presence of alcohol dehydrogenase in rat brain. They report alcohol dehydrogenase activities of approx. 30 nmol/min per g. Our results are in accord with the results of Raskin & Sokoloff (1970) and support their contention that rat brain has small but significant quantities of alcohol dehydrogenase.

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