ON THE CONTRIBUTION OF THE TRICARBOXYLIC ACID CYCLE TO THE SYNTHESIS OF GLUTAMATE, GLUTAMINE AND ASPARTATE IN BRAIN

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After intracerebral injection of glutamate-U-^{14}C the specific activity of glutamine in the brain is found to be higher than that of glutamate (Berl et al., 1961). Topical application of glutamate-U-^{14}C (Berl, 1965), intravenous injection (Waelsch et al., 1964), or intraperitoneal injection (Naruse, unpublished), of radioactive bicarbonate leads to similar results. However, when glucose-U-^{14}C is used as a precursor the specific activity of glutamine is never observed to be higher than that of glutamate (Cremer, 1964; Gaitonde et al., 1965; Van den Berg, unpublished).

These results point to the existence of at least two compartments for glutamate and at least two separate reaction pathways from pyruvate to glutamate-glutamine. Roberts et al. (1965) and Berl(personal communication) found that after the injection of leucine-U-^{14}C the specific activity of glutamine is also found to be higher than the specific activity of glutamate. Since the pathway leading from leucine to glutamate-glutamine probably involves acetyl-CoA (see Meister, 1965), it appears likely that the compartmentalization of the glutamate pools that is observed when bicarbonate is injected might also be observed from precursors of acetyl-CoA. Therefore experiments were carried out with acetate-1-^{14}C and -2-^{14}C in comparison with glucose-1-^{14}C, -2-^{14}C and -6-^{14}C to show whether the use of acetate as a precursor would lead to the preferential labeling of glutamine. It was believed that such experiments might suggest a mechanism to explain the

apparent conflicts between the glucose and the glutamate, bicarbonate and leucine data.

Since multiple turns of the tricarboxylic acid cycle lead to the preferential elimination of the C-1 of acetyl-CoA as CO_2 in comparison to the C-2 (Weinman et al., 1957), it is apparent that metabolites derived directly from tricarboxylic acid cycle intermediates should be preferentially labeled by acetate-2-^{14}C as opposed to acetate-1-^{14}C. Similar consideration would hold for glucose-1-^{14}C or -6-^{14}C and -2-^{14}C. Therefore the ratio of incorporation of the C-2 to C-1 of acetate and the C-1 (or C-6) to the C-2 of glucose into a given metabolite is a function of the contribution of cycling in the tricarboxylic acid cycle to the labeling of this metabolite.

The results to be reported in this paper show that glutamine derived from acetate is coupled to the tricarboxylic acid cycle in a different manner than glutamine derived from glucose.

METHODS

Young adult mice (Swiss Albino, 24-26 gram body weight, male) were injected intraperitoneally with 0.2 ml of the radioactive compounds (New England Nuclear Corp.) dissolved in 0.9% NaCl. The animals were killed by immersing them in liquid nitrogen. The hemispheres of the cerebrum were removed, weighed, powdered with dry ice, transferred to centrifuge tubes and extracted with trichloroacetic acid (1.5 ml 0.25 M/300 mg brain). After adding 8 ml water the tubes were centrifuged and the precipitates washed twice with 8 ml water. The combined extracts were neutralized and fractionated on Dowex-1-acetate as described by Naruse et al. (1966). The neutral fraction was hydrolysed (2 N HCl, 1 hr, 100°C) and the glutamate formed from glutamine was isolated on Dowex-1-acetate. Glutamate was determined with glutamate dehydrogenase and aspartate with ninhydrin.

The samples were counted at infinite thinness in a Nuclear Chicago End window counter, with an efficiency of about 30%. The isotopic purity of the fractions was checked with paper chromatography.

RESULTS

Experiments with glucose-1-^{14}C, -2-^{14}C and -6-^{14}C, Table 1

TABLE 1

Specific activity (SA) (cpm/μmole) of glutamate (glu); relative specific activity (to glutamate) (RSA) of aspartate (asp) and glutamine (gln) in brain of mice at different time intervals after the injection of 10 μc D-glucose-1-^{14}C, -2-^{14}C, -6-^{14}C (0.61, 1.0 and 0.4 mg resp). The ratio of the SA of the amino acids after glucose-1-^{14}C and -6-^{14}C to the SA after glucose-2-^{14}C. Each value is the average of two experiments.

	SA glutamate			RSA aspartate			RSA glutamine		
Position of label in glucose	C-1	C-2	C-6	C-1	C-2	C-6	C-1	C-2	C-6
t (min)									
5	1961	2525	2981	0.62	0.54	0.60	0.49	0.43	0.50
15	11142	11443	14346	0.82	0.70	0.83	0.74	0.71	0.68
30	17704	12191	22641	0.98	0.70	0.86	0.84	0.85	0.84

	SA after glucose-1-^{14}C / SA after glucose-2-^{14}C			SA after glucose-6-^{14}C / SA after glucose-2-^{14}C		
	Glu.	Asp.	Gln.	Glu.	Asp.	Gln.
t (min)						
5	0.78	0.88	0.90	1.18	1.31	1.11
15	0.97	1.14	1.01	1.25	1.49	1.20
30	1.45	2.02	1.43	1.86	2.27	1.84

It is clear from Table 1 that the specific activity of glutamate is always higher than the specific activity of aspartate and glutamine, although the difference 30 min after the injection is not large. The ratios of the specific activities of the amino acids after glucose-1-^{14}C and -6-^{14}C to the specific activities after glucose-2-^{14}C increase with time. The parallel increase of these ratios for glutamate, aspartate and glutamine indicates that all three compounds are derived from tricarboxylic acid cycle intermediates.

Experiments with acetate-1-^{14}C and -2-^{14}C, Table 2

TABLE 2

SA (cpm/μmole) of glutamate; RSA of aspartate and glutamine in brain of mice at different time intervals after the injection of 15 μc (0.6 mg) sodium acetate-1-^{14}C and -2-^{14}C. The ratios of the SA of the amino acids after acetate of -2-^{14}C to the SA after acetate-1-^{14}C. Each value is the average of three experiments.

	SA glutamate		RSA aspartate		RSA glutamine	
Position of label in acetate	C-1	C-2	C-1	C-2	C-1	C-2
t (min)						
5	1779	2726	0.46	0.44	4.8	3.6
15	1946	3800	0.59	0.70	3.3	1.5
30	1339	4647	0.47	0.70	2.5	1.2

$$\frac{\text{SA after acetate-2-}^{14}\text{C}}{\text{SA after acetate-1-}^{14}\text{C}}$$

	glutamate	aspartate	glutamine
t (min)			
5	1.53	1.46	1.11
15	1.95	2.27	1.02
30	3.47	4.30	1.66

The specific activity of glutamine was always higher than that of glutamate and aspartate (Table 2). The ratio of the specific activities of glutamate and aspartate after acetate-2-^{14}C to the specific activities after acetate-1-^{14}C goes up with time. Therefore, with acetate as a precursor the major amount of glutamate and aspartate are synthesized from intermediates of the tric ··xylic acid cycle. The increase in the ratios with time is more pronounced with acetate as a precursor compared with glucose, an effect which is probably due to the more rapid decrease of the acetate precursor pool.

The behavior of glutamine however is different from that of glutamate

and aspartate. The ratio is always lower than that of the other amino acids and increases more slowly with time. This indicates that the contribution of cycling in the tricarbodylic acid cycle to the labeling of glutamine with acetate as a precursor is less than in the analogous experiments with glucose.

DISCUSSION

It is evident from the results quoted in the introduction and the results reported in this paper that there is a special pathway from acetate and CO_2 to glutamine. One explanation for this might be the existence of more than one type of tricarboxylic acid cycle, reflecting the existence of different populations of mitochondria. Such mitochondrial heterogeneity in brain has already been postulated on other grounds (Salganicoff et al., 1965; Van Kempen et al., 1965).

An alternative explanation is that the pathway from acetate and CO_2 to glutamine occurs outside the mitochondria, although the condensing enzyme has never been reported to be present in the cytoplasm and the acetate activating enzyme in brain is present mostly in the mitochondria (Schuberth, 1965). The small contribution of cycling to the labeling of glutamine which does occur after the use of acetate as a precursor could be explained by transfer of a four carbon unit (oxaloacetate or aspartate) formed in a tricarboxylic acid cycle to the glutamine synthesizing compartment. This mechanism could also explain the contribution of cycling to the labeling of glutamine after the injection of glucose, since the effect of cycling is clearly evident in aspartate.

It can be seen easily that in a normal tricarboxylic acid cycle the distribution of the radioactivity in the oxaloacetate molecule after glucose-U-^{14}C should be uniform and independent of time. If this oxaloacetate or aspartate is used for glutamine synthesis in a special pathway and if the acetyl CoA used for this pathway is relatively cold, one would expect that the amount of radioactivity in the C-1 of glutamine is about 33% of the

total radioactivity in the glutamine molecule and independent of time. Such a labeling pattern has been reported by Gaitonde (1965).

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