EFFECT OF GLUCAGON AND DIBUTYRYL CYCLIC AMP ON THE TISSUE DISTRIBUTION OF [^{14}C] α-AMINOISOBUTYRIC ACID IN RATS

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1. Introduction

The uptake into liver of [^{14}C]α-aminoisobutyric acid (^{14}C-AIB), an amino acid that is transported like naturally occurring neutral amino acids but is not metabolized, has been shown to be increased by glucagon [1]. This effect of glucagon. like others, appears to be mediated by cyclic AMP, indeed, dibutyryl cyclic AMP itself caused a similar effect [1]. We were interested in knowing whether the entry of the amino acid into other tissues besides liver is influenced by glucagon or by dibutyryl cyclic AMP and consequently have studied in more detail the distribution of ^{14}C-AIB in rats treated with these agents.

2. Methods

Male albino rats from the Wistar strain were obtained locally (Harlan Industries, Cumberland, Indiana). The rats were given 1–[^{14}C] AIB (New England Nuclear Corporation, specific activity 12.2 μCi/μmol) by subcutaneous injection at a dose of 10 μCi/kg 24 hr before they were killed. Food was withheld after [^{14}C] AIB injection. Two hr before the rats were killed, they were given glucagon (Lilly) at a dose of 1 mg/kg s.c. or N^6,O^2-dibutyryl cyclic AMP (Sigma) at a dose of 50 mg/kg i.p. Rats were killed by cervical dislocation and all tissues were removed rapidly and frozen on dry ice. The tissues were stored frozen prior to analysis. They were then homogenized in 4 vol of 0.1 N HCl, and 1 vol of 30% prechloric acid was added to precipitate proteins. After centrifugation, aliquots of the supernatant fluid were taken for liquid scintillation spectrometry.

Table 1
Distribution of radioactivity in tissues of rats given [^{14}C] α-aminoisobutyric acid

Tissue	Treatment group		
	Control	Glucagon	Dibutyryl cAMP
	dpm/g (in thousands)		
Kidney	207 ± 24	193 ± 4 (93%)	151 ± 14* (73%)
Liver	47 ± 4	107 ± 5* (228%)	78 ± 3* (166%)
Spleen	31 ± 2	30 ± 2 (97%)	33 ± 3 (106%)
Heart	24 ± 1	23 ± 2 (96%)	26 ± 2 (108%)
Brain	19 ± 1	18 ± 0.5 (95%)	19 ± 0.4 (100%)
Skeletal muscle	17 ± 0.5	14 ± 0.7 (82%)	16 ± 0.6 (94%)
Lung	17 ± 0.7	14 ± 1 (82%)	18 ± 1 (106%)
Adrenal	15 ± 1	13 ± 1 (87%)	18 ± 1 (120%)
Epididymal fat	9 ± 0.8	6 ± 0.3* (67%)	6 ± 0.3* (67%)
Blood plasma	9 ± 0.5	5 ± 0.3* (56%)	7 ± 0.3 (78%)

*Significantly different from control group $P < 0.01$. Numbers in parenthesis represent percent of control value for that tissue.

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3. Results

Table 1 lists the content of [^{14}C] AIB in rat tissues in order of decreasing concentration. The enhancement of [^{14}C] AIB uptake into liver as reported previously by Scott et al. [1] after glucagon or dibutyryl cyclic AMP injection is confirmed in our experiment. The ratio of liver/plasma concentration was 5 in control rats, 11 in rats treated with dibutyryl cyclic AMP, and 21 in rats treated with glucagon. Only the liver showed enhanced amino acid uptake. The concentration of radioactivity in epididymal fat paralleled exactly that in plasma, hence was decreased slightly but significantly in rats treated with glucagon or with dibutyryl cyclic AMP. The concentration of radioactivity in kidney was decreased significantly in dibutyryl cyclic AMP-treated rats.

4. Discussion

One objective in this study was to determine if the increase of amino acid uptake into liver was associated with a decreased amino acid concentration in a specific tissues. Glucagon did not cause a significant decrease in amino acid concentration in any tissue other than epididymal fat, a tissue with very low concentration of the amino acid. AIB levels in skeletal muscle and in lung tended to be decreased; considering the large mass of muscle tissue, one suspects that the small statistically insignificant decrease in AIB concentration in that tissue could well have supplied the amount of amino acid that caused the increase in liver concentration. Dibutyryl cyclic AMP injection caused a decrease in amino acid concentration in the kidney.

The results suggest that perhaps only the liver has a cyclic AMP-dependent amino acid uptake mechanism. The failure of glucagon to stimulate [^{14}C] AIB into tissues other than liver could reflect a lack of glucagon-sensitive adenyl cyclase in most tissues. However, even dibutyryl cyclic AMP did not stimulate amino acid uptake into any other tissue, and a possible interpretation of this finding is that cyclic AMP does not mediate amino acid uptake in tissues other than liver.

An alternative explanation for the elevation of hepatic AIB content besides the existence of a cyclic AMP-dependent amino acid uptake mechanism in liver could be enhanced blood flow to the liver. Glucagon is known to increase hepatic blood flow [2–4], and it would be unreasonable to think that dibutyryl cyclic AMP might have a similar action. However, Chambers et al. [5] have shown that glucagon stimulates AIB uptake in the isolated, perfused liver, hence the increase of AIB content in liver of glucagon-treated rats (table 1) is probably partly if not entirely due to stimulation of amino acid transport by glucagon.

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