EFFECT OF ANOXIA ON THE RATE OF INCORPORATION OF ACETATE-1-C^{14} AND GLYCINE-1-C^{14} INTO SOLUBLE RAT BRAIN AND LIVER PROTEINS AT DIFFERENT BODY TEMPERATURES

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The intensity of incorporation of acetate-1-C^{14} and glycine-1-C^{14} into soluble (in 0.14 M NaCl) rat brain and liver proteins under normal conditions and after a stay of 2 h in a pressure chamber at 240 mm Hg was studied. Anoxia inhibited the incorporation of labeled acetate and glycine into the tissue proteins of both organs. Depression of metabolism of soluble liver proteins, unlike that of brain proteins, clearly depends on the degree of lowering of the body temperature.

KEY WORDS: anoxia; hypothermia; brain and liver protein metabolism.

Previous investigations in the writers' laboratory [3, 4] showed that during acute anoxia produced by reducing the air pressure the rate of incorporation of orthophosphate-P^{32} is definitely reduced into various phospholipid (PL) fractions of the rat brain; the degree of inhibition depends not so much on the degree of lowering of the partial oxygen pressure in the inspired air as on the depth of the hypothermia accompanying the anoxic state. A similar relationship between the degree of depression of brain PL metabolism and the body temperature has also been found in another form of hypoxia induced by cyanide poisoning and also during thiopental anesthesia. In these cases the rate of incorporation of orthophosphate-P^{32} into rat brain PL also was reduced even in the absence of hypothermia, but if the body temperature was lowered the depression of PL metabolism became more marked still [1, 5].

It has also been shown [2] that in anoxic anoxia the intensity of metabolism of soluble rat brain tissue proteins is also clearly depressed, as shown by a decrease in the rate of incorporation of carbon-labeled acetate and glycine into them.

The object of this investigation was to study the extent to which the observed depression of soluble brain protein metabolism during anoxia depends on the degree of hypothermia, as was observed previously with respect to the brain PL. Parallel with the study of brain protein metabolism, the rate of incorporation of carbon-labeled acetate and glycine into soluble liver proteins also was investigated in all the experimental animals. The object of this part of the work was to discover to what extent the principles established for nerve tissue are specific for that tissue and to what extent they can be regarded as nonspecific and common to other tissues.

EXPERIMENTAL METHOD

Acetate-1-C^{14} or glycine-1-C^{14} was injected intraperitoneally in a dose of 1 Ci/g into adult male Wistar albino rats. The animals were decapitated 2 h after injection of the isotope. Proteins were extracted

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TABLE 1. RSR of Soluble Rat Brain and Liver Proteins under Normal and Anoxic Conditions (M ± m)

Conditions	Parameters	Acetate-1-C^{14}		Glycine-1-C^{14}	
		brain	liver	brain	liver
Control	number of animals	20	20	21	21
	RSR	5,06±0,44	5,13±0,44	6,19±0,64	10,00±0,78
Hypoxia Group 1	number of animals	20	19	16	16
	RSR (in % control)	68,12±3,04	85,74±6,18	59,12±4,18	67,18±3,94
	P	<0,01	<0,05	<0,001	<0,001
	Fall of temperature	1,25°		1,44°	
Hypoxia Group 2	number of animals	22	20	17	17
	RSR (in % of control)	58,58±3,07	58,55±5,08	58,33±3,22	48,98±3,92
	P	<0,001	<0,001	<0,001	<0,001
	Fall of temperature	4,92°		4,58°	

Legend. P calculated relative to control.

from the cerebral hemispheres and liver in the cold for 4 h with 0.14 M NaCl solution and then precipitated by TCA in a final concentration of 5%. The acid-soluble fraction (ASF) was extracted from separate weighed samples of tissue with 5% TCA. The protein residue was washed and freed from lipid impurities by a mixture of chloroform and methanol (2 : 1), and then dissolved in 0.5 N NaOH. Aliquot samples were taken from the solution for determination of the protein content by the method of Ryth and Gill [7] and measurement of radioactivity (with a windowless flow counter). Radioactivity of the ASF of the tissues was determined in separate samples. The rate of incorporation of labeled acetate and glycine into the proteins, taken as a measure of the intensity of their metabolism, was estimated from the relative specific radioactivity (RSR) of the proteins, calculated as the ratio between the specific activity (SR) of the protein (in counts/min/mg protein) and the SR of the ASF (in counts/min/quantity of ASF extracted from 1 mg wet weight of tissue).

Acute anoxia was created by keeping the rats in a chamber for 2 h under a pressure of 240 mm Hg. The rectal temperature was measured before and after. The experimental animals were divided into two groups: 1) animals whose body temperature fell during a stay of 2 h in the pressure chamber only very slightly – by less than 3°C compared with its initial level and 2) animals with marked hypothermia, whose temperature fell by 3°C or more. The writers showed previously [3, 4] that under anoxic conditions a fall of body temperature by less than 3°C in rats is not accompanied by any appreciable decrease in the rate of incorporation of orthophosphate-P^{32} into brain PL. Since most of the animals, when they were kept under these conditions in the pressure chamber (2 h, 240 mm Hg), belonged to group 2, to increase the number of animals in group 1 the development of marked hypothermia in some series of experiments was prevented artificially, by heating the air in the chamber. Group 1 thus included rats in which the fall of body temperature under anoxic conditions was not significant, either with or without heating the inspired air.

EXPERIMENTAL RESULTS

In the control animals SR of the ASF of brain tissue was identical when both labeled compounds were used [8, 7]. This indicates equal permeability of the blood-brain barrier for acetate and glycine. A different picture was observed in the liver; the values of SR for the ASF of the liver tissue were much higher than for the brain tissue, whether labeled acetate or (in particular) glycine was used (29.7 and 194.7, respectively). Consequently, these compounds penetrate more easily from the blood into liver tissue than into brain tissue. The sharp difference in the values of SR for the ASF of the liver when acetate and glycine were used may be explained by the more rapid utilization of acetate than of glycine in the synthesis of the various compounds, chiefly higher fatty acids, in the liver cells and also, possibly, by the more intensive dilution of the label as a result of high activity of catabolic processes leading to the formation of unlabeled acetate.

As Table 1 shows, in the control rats RSR of the soluble brain and liver proteins with respect to acetate-1-C^{14} was the same. Glycine-1-C^{14} was incorporated much more intensively into liver proteins than

into brain proteins. This difference was possibly the result of the preferential utilization of glycine by the liver cells for the synthesis of soluble proteins.

Under anoxic conditions the decrease in the intensity of metabolism of the soluble proteins was greater in the brain than in the liver in the rats of group 1 (body temperature fell by less than 3°C, on the average by 1.25-1.44°C). In every case the difference relative to the control was statistically significant.

In the rats of group 2 (mean fall of temperature 4.6-4.9°C) the depression of protein metabolism in the liver was more clearly marked still and it differed statistically significantly from that observed in group 1. In the brain, increased depression occurred only when labeled acetate was used.

It can be concluded from these results that a decrease in the partial oxygen pressure in the inspired air leads to definite depression of the intensity of metabolism of soluble brain and liver proteins, just as was observed for the PL metabolism of these organs. The degree of the hypothermia accompanying the anoxia corresponded to a definite extent to the degree of depression of protein metabolism, although the relationship was much less close than for the PL of the investigated tissues. Incidentally, correlation between the degree of hypothermia and the depth of depression of protein metabolism is much stronger in the liver than in the brain.

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