Hormonal effects on glycine metabolism in rat epididymal adipose tissue

It is now acknowledged that adipose tissue is a sensitive site of direct hormonal regulation of carbohydrate and fat metabolism[1-3]. In order to adapt to the varying requirements of the organism for fat storage, the tissue is capable of rapid and reversible growth, during which synthesis (or involution) of enzymic and structural proteins is likely to occur[4]. The present communication suggests that the regulation of amino acid and protein metabolism in this tissue may well be worthy of extensive study.

The animals used were male Wistar rats weighing between 200 and 240 g. They were fed Purina laboratory chow up to the time of death unless otherwise noted. Killing was by decapitation and the epididymal fat bodies were immediately removed, weighed on a torsion balance, and incubated for 3 h at 37° in Krebs–Ringer bicarbonate buffer. The latter contained 0.5 m*M* [1-^{14}C]glycine, with a specific activity of 0.66 μC/μmole. After incubation, an aliquot of the medium was used for isolating and counting CO_2 as $BaCO_3$, as previously described[5]. The fat bodies were extracted three times with chloroform–methanol (2:1), dried and homogenized in 10% trichloroacetic acid with a known amount of human albumin (Fraction V) as carrier. The precipitate was separated by centrifugation, resuspended in 10% trichloroacetic acid and heated to 90° for 15 min. It was then washed twice with fresh trichloroacetic acid, suspended in ethanol and heated to 60° for 15 min. The final precipitate was washed twice with a mixture of ethanol and ether and once with ether. After drying and weighing the material was planchetted and counted in a windowless flow counter. Appropriate corrections for self-absorption were applied. All results were expressed in terms of μmoles glycine oxidized to CO_2 or incorporated into protein per 100 mg of dry fat-free adipose tissue.

On several occasions the protein precipitate was treated with ninhydrin in the presence of carrier amino acid using the method of VAN SLYKE[6]. The CO_2 evolved was collected and found free of radioactivity. After acid hydrolysis of the protein precipitate, however, ninhydrin treatment resulted in labeling of the collected CO_2, approximately 70% of the activity present in the protein prior to hydrolysis being recovered in this fashion. Analysis of the hydrolysate by paper chromatography located the radioactivity primarily in the glycine area. The specific activity of adipose-tissue protein was approximately 10 times as great as that of the protein isolated from the diaphragm of the same animals incubated under identical conditions.

As shown in Table I, the addition of glucose to the incubating medium enhanced the incorporation of glycine into protein in tissues from both fed and fasted rats, more markedly so in the latter. In the absence of glucose, insulin had no appreciable effect on the incorporation of glycine into protein of tissues from fasted or fed animals. In the presence of glucose, however, insulin clearly stimulated the incorporation of glycine into adipose-tissue protein. Epinephrine markedly depressed glycine incorporation into protein, while accelerating its oxidation to CO_2, both effects being apparent with or without glucose. Cortisol, with or without added glucose, depressed glycine incorporation into protein without altering glycine oxidation.

These hormonal effects on the incorporation of glycine into protein by rat adipose-tissue resemble in general those described under similar conditions for rat

TABLE I

EFFECT OF HORMONES AND/OR GLUCOSE ADDED *in vitro* UPON THE METABOLISM OF [1-^{14}C]GLYCINE BY RAT EPIDIDYMAL ADIPOSE TISSUE

Hormones added in vitro	*No. of expts.*	*Glucose added (16 mM)*	*μmoles [1-^{14}C]glycine carbon per 100 mg dry fat-free tissue incorporated into*					
			Protein			*CO_2*		
			Control	*Hormone effect ± S.E.*	*P*	*Control*	*Hormone effect ± S.E.*	*P*
Rats fed *ad lib*								
1 None*	22*	*	0.798*	+ 0.143 ± 0.037*	< 0.001*	0.152*	+ 0.009 ± 0.011*	> 0.4*
2 Insulin 0.1 unit/ml	6	o	0.742	— 0.119 ± 0.106	> 0.3	0.117	+ 0.016 ± 0.001	< 0.001
3 Insulin 0.1 unit/ml	6	+	0.806	+ 0.333 ± 0.051	< 0.01	0.109	+ 0.047 ± 0.011	< 0.02
4 Epinephrine 18 μg/ml	6	o	0.769	— 0.593 ± 0.061	< 0.001	0.209	+ 0.407 ± 0.081	< 0.01
5 Epinephrine 18 μg/ml	6	+	0.924	— 0.533 ± 0.067	< 0.001	0.185	+ 0.521 ± 0.074	< 0.01
6 Cortisol 30 μg/ml	12	o	0.746	— 0.119 ± 0.037	< 0.01	0.125	0 ± 0.017	—
7 Cortisol 30 μg/ml	6	+	0.802	— 0.154 ± 0.057	< 0.02	0.166	— 0.017 ± 0.026	> 0.5
Rats fasted 48 h**								
8 None*	6*	*	0.569*	+ p.305 ± 0.058*	< 0.01*	0.093*	— 0.008 ± 0.025*	> 0.7*
9 Insulin 0.1 unit/ml	6	o	0.569	+ 0.028 ± 0.036	> 0.4	0.093	— 0.015 ± 0.011	> 0.3
10 Insulin 0.1 unit/ml	6	+	0.874	+ 0.241 ± 0.077	< 0.05	0.101	+ 0.012 ± 0.010	> 0.3

* Addition of glucose is the only variable, *i.e.* hormone effect = glucose effect.
** Results obtained simultaneously in tissue samples from the same 6 rats.

hemidiaphragm with, however, two notable exceptions. WOOL *et al.*[7] have reported an effect of insulin upon histidine incorporation into protein in hemidiaphragm from fasted (not from fed) rats, in the total absence of glucose, while MANCHESTER[8] observed this effect in tissues from both fasted and fed animals. Also, cortisol added *in vitro* was without effect upon glycine incorporation into diaphragm protein[9]. In rat epididymal adipose tissue insulin accelerates the incorporation of pyruvate into tissue protein even in the absence of glucose[10], a discrepancy which might derive from better ability of this substrate to support oxidation and energy production than is the case for glycine itself. Indeed, KRAHL (personal communication) has observed that pyruvate, as well as glucose, restores the insulin response for glycine incorporation into protein by this tissue.

These studies clearly suggest that the metabolism of glycine in adipose tissue is both hormonally controlled and modified by the availability of metabolic substrates such as glucose.

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Isolation of cytidine 5′-monophosphate-bound carboxyl-activated peptides from *Saccharomyces cerevisiae*

After the first reports from this laboratory about the occurrence of nucleotide-bound carboxyl-activated peptides in yeast[1,2] a number of these compounds have been isolated from different organisms, and been identified in some detail[3–14]. Some authors reported the occurrence of nucleotide-peptide compounds without giving sufficient details to conclude if those compounds were carboxyl activated[15–17].

During further investigations with the aid of ion-exchange chromatography, a new carboxyl-activated nucleotide-peptide compound has been isolated from yeast.

Abbreviations: CMP, cytidine monophosphate; AMP, adenosine monophosphate.