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The entry of sugars into bone cells. Independence from parathyroid extract and thyrocalcitonin effects

The importance of glucose as a substrate for bone made it of interest to examine whether it is actively accumulated in bone cells, as are amino acids, and whether parathyroid hormone and thyrocalcitonin affect its metabolism by modifying its transfer across the bone cell membrane. Although ADAMSON, LANGELUTTIG AND ANAST[1] could find no evidence of active transport of 3-*O*-methylglucose in embryonic bone, a transport system important in adult life might not appear until later in development. The kinetics of entry of 3-*O*-methylglucose, a non-metabolizable analog of glucose, into isolated bone cells from adult animals was, therefore, determined and the effect of parathyroid extract and thyrocalcitonin on it explored.

Intact bone cells were isolated from the femoral and tibial metaphyses of 38–41-day-old male rats of the Charles River strain following the procedure of WOODS AND NICHOLS[2]. The technique for studying transport phenomena in these cells was based on methods used by CHRISTENSEN AND RIGGS[3].

The cell pellet obtained from 12 rats was suspended in 3.5 ml of Krebs–Ringer bicarbonate buffer (pH 7.4). Aliquots of this suspension (0.5 ml) were equilibrated

in 10-ml erlenmeyer flasks sealed with rubber stoppers for 5 min at 37° under an atmosphere of 95 % O_2–5 % CO_2 in a Dubnoff metabolic shaker. Then 0.5 ml of Krebs–Ringer bicarbonate buffer containing radioactive substrate, 0.4 μC/ml, was rapidly injected through the stoppers. After varying periods, incubation was terminated by cooling in ice after which flask contents were transferred by Pasteur pipette to Kolmer–Brown tubes. Cells were packed by immediate centrifugation at 1750 $\times$ *g* and 2° for 15 min. After decanting the incubation medium the tubes were quickly drained, wiped dry and weighed. Cell pellets were resuspended in 1 ml distilled water by vigorous mechanical agitation and the tubes placed in a 100° bath for 5 min. Cell debris was spun down at 1750 $\times$ *g* for 15 min, the supernatant clear cell extract was removed for radioisotope analysis, and the tubes were dried in an oven and weighed. Tissue water was calculated as the difference between the weight of the wet cell button and its dry residue after extraction. No attempt to correct for trapped medium was made. Radioactivity was measured by placing 0.1-ml samples of cell extract and incubation medium in vials containing 1.5 ml hydroxide of hyamine and 10 ml scintillation fluid (4 g PPO and 50 mg POPOP in 1 l toluene). Vials were counted in a liquid scintillation spectrometer with an efficiency of 65 % for ^{14}C.

Table I shows the time course of 3-*O*-methylglucose entry into isolated bone cells. The distribution ratio between tissue water and medium did not exceed 1.0, indicating that these cells did not concentrate this glucose analog. The same cells, however, did concentrate α-aminoisobutyric acid, with a distribution ratio between 7 and 8.

TABLE I

TIME COURSE OF 3-*O*-METHYLGLUCOSE ENTRY INTO BONE CELLS

Distribution ratio = counts/min per ml tissue water:counts/min per ml incubation medium. Note that the ratio 0.50 found at zero time indicates that as much as 50 % of the counts in the tissue water could be trapped extracellularly in the cell button. Results of duplicate experiments are shown.

Substrate	*Incubation period (min)*	*Distribution ratio*
1 mM 3-methylglucose	0	0.50
		0.50
	2	0.67
		0.67
	4	0.79
		0.83
3 mM methylglucose	30	0.97
		0.92
	45	0.93
		0.96
	60	1.00
		0.96
	75	1.00
		0.97
25 μM α-aminoisobutyric acid	60	7.7
		7.1

Fig. 1 shows the relationship between the rate of influx and the concentration of 3-*O*-methylglucose. The rate was directly proportional to concentration for values as high as 50 mM. Competitive inhibition of this influx could not be demonstrated as shown in Table II. Neither 28 mM glucose nor $5 \cdot 10^{-4}$ M phloretin (a potent inhibitor of carrier-mediated glucose transport[4,5]) appeared to inhibit the influx of 1 mM 3-*O*-methylglucose.

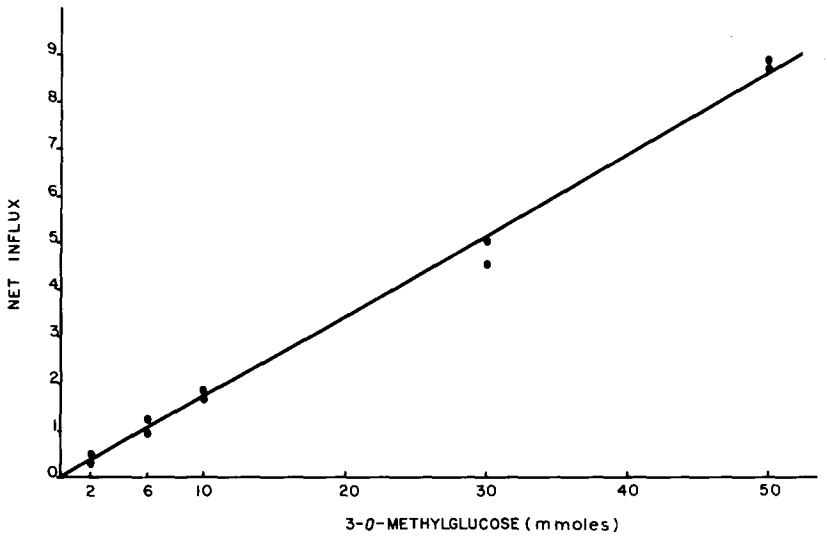

Fig. 1. Effect of substrate concentration on rate of influx of 3-*O*-methylglucose ^{14}C into bone cells incubated for 4–5 min. Results of duplicate experiments are plotted to show the high degree of reproducibility. Net rate of influx = μmoles/min per ml tissue water = (counts/min per ml tissue water per min)/(counts/min per μmole).

TABLE II

EFFECT OF PHLORETIN AND GLUCOSE ON THE INFLUX OF 3-*O*-METHYLGLUCOSE

Bone cells were incubated 4 min only in order to keep metabolic breakdown of labelled glucose at a minimum. Results of duplicate experiments are shown in each case.

Substrate	*Distribution ratio*
1 mM 3-methylglucose	0.79 0.83
1 mM 3-methylglucose + $5 \cdot 10^{-4}$ M phloretin	0.85 0.71
1 mM 3-methylglucose + 28 mM glucose	0.74 0.73

Neither parathyroid extract nor thyrocalcitonin altered the rate of influx of 3-*O*-methylglucose as shown in Table III.

These findings indicate that in contrast to amino acids[6] no concentrative transport of 3-*O*-methylglucose occurs in rat bone cells under the conditions used thus

TABLE III

EFFECT OF PARATHYROID HORMONE AND THYROCALCITONIN ON 3-*O*-METHYLGLUCOSE INFLUX IN BONE CELLS

Rats were injected subcutaneously with parathyroid extract (Lilly), 1 unit/g body weight, or thyrocalcitonin (kindly donated by Dr. PAUL L. MUNSON, Harvard School of Dental Medicine), 25 units/animal. Control animals received appropriate volumes of diluent (1.6% glycerin–0.2% phenol and 0.001 M HCl, respectively). Bone cells were isolated from rats decapitated at varying intervals after injection, and incubated with 5 mM 3-*O*-methylglucose for 3 min. All animals treated with parathyroid extract showed a decrease in serum P_i, measured before and after treatment with hormone. The thyrocalcitonin preparation used lowered serum calcium in rats after 15 min. Results of duplicate or triplicate experiments are shown.

Time between injection and sacrifice	*Distribution ratio*		
	Control	*Parathyroid extract*	*Thyrocalcitonin*
10 min	0.79, 0.65	0.64, 0.76	0.74, 0.73
2 h	0.77, 0.77, 0.76	0.82, 0.83, 0.79	
8 h	0.70, 0.66	0.76, 0.66	

extending previous observations[1]. Moreover, the kinetics of entry of 3-*O*-methylglucose into these cells support the idea that the bone cell membrane is passively permeable to this glucose analog and therefore probably to glucose. Finally, it was noteworthy that neither of the hormones tested had any apparent effect on sugar uptake, suggesting that their modes of action on glucose metabolism should be sought further along its metabolic pathways.

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