Lipolysis-induced magnesium uptake into fat cells

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Zusammenfassung

10^{-5} M Isoproterenol und 10^{-5} M Adrenalin steigerten die Lipolyse von epididymalen Ratten-Fettgewebe um 64 % und 43 % und erhöhten den Mg-Gehalt um 17 % und 13 %. Diese Wirkungen wurden durch einen β-adrenergen Effekt hervorgerufen. Der α-Agonist Phenylephrin war mit und ohne Isoproterenol wirkungslos.

Zugabe von 10^{-4} M Mn^{2+} hemmte die Mg-Aufnahme in Fettgewebe, ohne dessen Lipolyse und Isoproterenolstimulierbarkeit zu beeinflussen.

Summary

10^{-5} M isoproterenol and 10^{-5} M adrenalin increased lipolysis in rat epididymal fat by 64 % and 43 % as well as Mg content by 17 % and 13 % respectively.

These effects are due to a β-adrenergic action. The α-agonist phenylephrine exhibited no significant effect in the presence or absence of isoproterenol.

Addition of 10^{-4} M Mn^{2+} inhibited Mg^{2+} uptake in adipose tissue without affecting lipolysis and isoproterenol-stimulation of lipolysis.

Résumé

10^{-5} M d'isoprotérénol et 10^{-5} M d'adrénaline ont élevé la lipolyse de tissu gras épididymal des rats de 64 % et 43 % et ont augmenté la teneur magnésique de 17 % et 13 %.

La cause a été un effet β-adrénergique. L'α-agoniste phényléphrine a été sans effet avec et sans l'isoprotérénol.

10^{-4} M Mn^{2+} a inhibé la réception magnésique en tissu gras sans influençant la lipolyse et la stimulation de la lipolyse par l'isoprotérénol.

Introduction

In serum, the concentration of total Mg was found to be reduced when the concentration of free fatty acids [FFA] was increased [3]. This was explained by formation of insoluble Mg soaps. However, binding of Mg^{2+} to FFA should lower the concentration of free Mg^{2+} but not that of total Mg. Therefore, the inverse correlation between total Mg and FFA in serum must be brought about by other mechanisms. One possible mechanism could be cellular uptake to Mg^{2+}, when lipolysis becomes increased.

There is evidence that under conditions of increased lipolysis, e. g. by cold exposure of rats, the Mg content in white [6] and brown [5] adipose tissue was increased. Also, after in-vitro incubation of adipose tissue with adrenalin, Mg content was increased [2]. In these experiments lipolysis and receptor specificity were not determined.

There are controversial results on the action of isoproterenol or adrenalin on membrane transport of Mg^{2+}. Thus, with heart muscle [10] or lymphoma cells [7] isoproterenol inhibited net Mg^{2+} uptake [10] or $^{28}Mg^{2+}$ uptake [7]. In the experiments with lymphoma cells $^{28}Mg^{2+}$ uptake may represent $^{28}Mg^{2+} - {}^{24}Mg^{2+}$ exchange. On the other hand, with fat cell membrane vesicles in the presence of extravesicular ATP net Mg^{2+} uptake and $^{28}Mg^{2+}$ uptake were stimulated and $^{28}Mg^{2+}$ release was inhibited by adrenalin [2]. However, in these experiments, the sidedness of the adipocyte membrane vesicles was not defined.

Probably, catecholamines have two separate effects: 1. alteration of Mg^{2+} membrane permeability and 2. increase in Mg content of fat cells. In the present communication we report on the correlation of the increase in Mg content in adipose tissue to lipolysis and on its β-receptor specificity.

Materials and Methods

Normal fed male Wistar rats weighing ca. 180 g were injected at 10 a. m. with 50 mg/kg nembutal as an anesthetic and 18.5 mg/kg hexamethonium bromide as an autonomic blocking agent according to [2]. Ten minutes later epididymal fat was taken, immediately sliced and washed in incubation medium. The incubation medium consisted of [in mM] 30 Tris HCl, pH 7.4, 145 NaCl, 5 KCl, 1 $MgCL_2$, 1.2 $CaCl_2$, 3 phosphate, 10 glucose, 0.01 ascorbic acid and 3 % bovine serum albumin. About 200 mg adipose tissue per ml medium were incubated at 37 °C for 30 min by gassing with O_2. Catecholamines and propranolol were added before addition of the tissue as indicated in Tab. 1. After incubation, the tis-

sue slices were washed 2 × in cold 0.3 M sucrose to remove extracellular Mg^{2+} and freeze-dried.

The freeze-dried tissue was extracted 3 times with 1 ml petroleum ether [60—80 °C]. Control experiments have shown, that by this procedure no Mg^{2+} is extracted. The extracted fat free tissue was dried at 96 °C and ashed in a low temperature asher. Mg^{2+} was determined by atomic absorption spectrophotometry.

Lipolysis was measured by means of glycerol released to the medium during the 30 min incubation. In deproteinized medium glycerol was determined in an optical test with glycerokinase and glycerophosphate-dehydrogenase according to [12].

L-isoproterenol, hexamethonium bromide and D,L-propranolol were obtained from Sigma, München, L-adrenalin, L-phenylephrine, and nembutal from Serva, Heidelberg, bovine serum albumin [purified] from Behringwerke, Marburg, glycerokinase, glycerophosphate-dehydrogenase, ATP, NAD^+ from Boehringer, Mannheim, and EGTA [ethylene glycol bis [β-amino ethylether]-N, N, -N′, N′-tetraacetic acid] from Fluka, Buchs [Switzerland].

Results and Discussion

After incubation of epididymal fat with 10^{-5} M isoproterenol or 10^{-5} M adrenalin there was increased glycerol release [lipolysis] by 64 % or 43 % and increased Mg content by 17 % or 13 % respectively [Tab. 1]. These effects are due to a β-adrenergic response. With 10^{-4} M propranolol the increase in lipolysis and Mg content is blocked. With propranolol in absence of added catecholamines lipolysis was somewhat lower than in the controls because of blocking the small β-adrenergic effect of the remaining endogenous catecholamines.

The α-agonist phenylephrine had no significant effect on lipolysis and Mg content. The isoproterenol-induced increase of lipolysis and Mg content was also not significantly influenced by 10^{-4} M phenylephrine. In human and hamster fat tissue, stimulation of α-receptors [by phenylephrine] is known to inhibit the β-adrenergic effect [4]. However, rat adipocytes do not have functional α_2-adrenoceptors [4]. Therefore, the missing effect of phenylephrine on the β-adrenergic stimulation of lipolysis and Mg^{2+} uptake can be explained.

Our experiments have shown that the increases in lipolysis and Mg^{2+} uptake by the β-agonists behaved proportionally [3.5:1], indicating that both effects are coupled.

To test the ion specificity of the lipolysis-induced Mg^{2+} uptake, the interaction of Ca^{2+} and Mn^{2+} was investigated. Omission of Ca^{2+} and addition of 0.1 mM EGTA reduced lipolysis and Mg^{2+} uptake by 30 % or 10 % respectively when compared with the controls. Other authors [9] also found a reduction of lipolysis when Ca^{2+} was lacking. This effect was explained by cell damage as shown by decreased ATP content of the cell suspension. However, stimulation of lipolysis by isoproterenol was independent of extracellular Ca^{2+}. This result agrees with the finding of *Schimmel* [8] that Ca^{2+} influx is not essential for activation of lipolysis although the possible role of redistribution of intracellular Ca^{2+} remains open.

Isoproterenol-stimulated Mg^{2+} uptake in the absence of Ca^{2+} was about the same as in its presence. This result shows that there is no competition between Ca^{2+} and Mg^{2+}. In the presence of 0.1 mM Mn^{2+} stimulation of lipolysis by isoproterenol was not affected, however, Mg^{2+} uptake was inhibited by Mn^{2+}. It has not yet been defined whether the inhibition by Mn^{2+} is competitive or non-competitive, that means, whether the Mg^{2+} channel is unspecific and also transports Mn^{2+} or whether the Mg^{2+} channel is specific and is blocked by Mn^{2+}.

In our experiments lipolysis [glycerol release to medium] was only linear with time for 30 min [not shown]. There is evidence that besides regulation by lipase, lipolysis is additionally regulated by feedback-inhibition of intracellular FFA concentration [11]. Part of the FFA are transported out of the cell and bound to serum albumin. The remaining intracellular fraction of FFA would be responsible for binding of intracellular Mg^{2+} and formation of Mg soaps. By this reaction concentration of intracellular free Mg^{2+} could be reduced. Since net Mg^{2+} influx seems to be regulated by feedback inhibition via intracellular free Mg^{2+} [1], a reduction of intracellular free Mg^{2+} would lead to a net Mg^{2+} uptake. There may be an additional effect of β-agonists on the velocity of Mg^{2+} uptake as reviewed in the introduction. By the lipolysis-induced Mg^{2+} uptake in animals or humans a transient decrease in total extracellular Mg concentration may occur, when at the same time during intracellular Mg^{2+} uptake not enough Mg^{2+} is absorbed in the intestinum for compensation.

Tab. 1: Lipolysis [glycerol release] and Mg content of rat epididymal adipose tissue.
Mean ± S.E.M., n = number of experiments

	n	Glycerol release mmol/kg fat free dry wt.	Mg content mmol/kg fat free dry wt.
control	17	22.9 ± 2.2	9.6 ± 0.4
10^{-5}M isoproterenol	17	37.5 ± 4.0	11.2 ± 0.2
10^{-5}M adrenalin	6	32.8 ± 2.1	10.8 ± 0.2
10^{-5}M isoproterenol + 10^{-4}M propranolol	4	18.1 ± 1.3	9.0 ± 0.8
10^{-4}M propranolol	4	16.3 ± 1.8	9.4 ± 0.2
10^{-4}M phenylephrine	4	20.2 ± 1.0	9.8 ± 0.4
10^{-5}M isoproterenol + 10^{-4}M phenylephrine	4	36.4 ± 1.6	10.8 ± 0.4
Ø Ca, 10^{-4}M EGTA	5	16.0 ± 1.8	8.2 ± 0.9
Ø Ca, 10^{-4}M EGTA + 10^{-5}M isoproterenol	5	28.2 ± 1.8	10.8 ± 0.7
10^{-4}M Mn^{2+}	7	19.2 ± 1.6	10.0 ± 0.5
10^{-4}M Mn^{2+} + 10^{-5}M isoproterenol	7	42.6 ± 4.8	10.5 ± 0.7

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