EFFECT OF NORADRENALINE ADDITION ON THE RELEASE OF FATTY ACIDS FROM INCUBATED BOVINE ADIPOSE TISSUE

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Introduction

Noradrenaline, as a lipolytic agent, has been shown to increase the percentage of oleic acid in plasma of ruminant, accompanied by an increase in total free fatty acid(FFA) in the plasma. Further, relatively greater loss of palmitic acid, rather than other fatty acids, has been observed in adipose tissue of sheep and cattle during a fasting period. It was deduced from these observations that there was a preferential release of oleic and palmitic acid from adipose tissue of ruminant. However, relatively greater release of specific fatty acids has not been directly observed. In this experiment, mobilization of six major FFAs from the incubated bovine adipose tissue to incubation medium were measured under two different conditions to see if preferential release of specific fatty acids could be introduced under urgent lipolytic conditions in vitro.

Materials and Methods

Three Japanese Black and two Holstein steers were used. Four animals were slaughtered at a body weight of 650 kg and one Japanese Black steer was slaughtered at a body weight of 500 kg. Immediately after stunning, subcutaneous adipose tissue was sampled.

Several tissue sections (ca. 40 mg per section) were prepared with scissors and approximately 200 mg were weighed and placed in each incubation flask. The incubation medium for measuring basal lipolysis contained 60 mg bovine albumin (essentially fatty acid free, Fraction V, Sigma Chemical Co.) in 3 ml of Krebs-Ringer phosphate buffer (pH 7.3). To introduce urgent lipolysis, 1.5 μl of noradrenaline solution (1 mg/1 ml water) and 0.3 mg ascorbic acid were added to the incubation medium. Triplicate flasks were incubated for 1 hr at 37°C with agitation. Triplicate blanks (without tissue) were also incubated for both basal and urgent lipolysis. After 1 hr, flasks were placed on ice to terminate incubations.

For HPLC measurement of released FFA, heptadecanoic acid (6 μg/30 μl methanol) was added as the internal standard into 0.5 ml aliquot of the incubation medium. FFA were fractionated into n-heptan according to the method of Dole and Meinertz(1960). Fractionated FFAs were derivatized with fluorescent reagent. A 1.5 ml aliquot of resulting solution was dried under air stream, and 50 μl of 9-anthryldiazomethane (ADAM) (Funakoshi Pharmaceutical Co. Japan) solution (0.05% W/V ADAM in ethylacetate) was added. The derivatized sample was dissolved again in 2 ml of acetonitrile-water (9:1 V/V) and 20 μl aliquot was injected into HPLC. The chromatographic separation was performed at 30°C using a column packed with cosmosil 5C8 (4.6x250mm, Nakarai Chemicals Co.). The mobile phase was composed of acetonitrile-water(9:1 V/V), with a flow rate of 1 ml/min. Using a fluorescence detector, the fluorescence of the sample were measured at 412 nm with excitation at 365 nm.

Results and Discussion

Release of total FFA was stimulated by noradrenaline to a rate that was 1.5-fold that of basal lipolysis. Release of myristic, palmitoleic, linoleic, palmitic and oleic acid were stimulated to rates that were 1.5, 1.9, 1.6, 1.5 and 1.6-fold, respectively (to that of basal lipolysis), while the rate of

stearic acid was 1.2-fold that of basal lipolysis (table 1). This suggests that the adipose tissue in the urgent lipolytic condition releases individual FFAs in different ratio than in the normal condition. The fact that the release of myristic or palmitic acid is greater than that of stearic acid is consistent to the deduction from former experiments in vivo. In attention on FFAs of same carbon number, table 1 suggest that the unsaturated fatty

TABLE 1. FFA RELEASE FROM ADIPOSE TISSUE UNDER BASAL AND NORADRENALINE STIMULATED LIPOLYSIS[1]

	[μgram FFA/ (hr x g tissue)]		
FFA	Basal (B) +	Noradrenaline(NA)	NA/B
14:0	15.9+0.6	23.2+0.6	1.5
16:1	29.5+1.8	56.8+1.8	1.9
18:2	10.7+1.0	17.5+1.0	1.6
16:0	89.5+3.6	131.2+3.6	1.5
18:1	131.4+5.8	204.7+5.8	1.6
18:0	38.0+1.4	44.9+1.4	1.2
Total	314.9+13.2	478.3+13.2	1.5

[1] Least squares mean + S.E. for five steers.

acid is released more readily than the saturated one. Further, this appears to indicate some degree of systematic selectivity in the release, which could be due to selective permeability of cell membrane or variety in the affinity of individual FFA for albumin. It is a reasonable hypothesis to state that the difference in the affinity of individual FFA for albumin (Goodman, 1958) brings about preferential removal of specific FFAs from the surface of adipose tissue, because the FFAs were released in great quantity only when bovine albumin was added in the incubation medium (table 2). Yet, this hypothesis does not exclude intracellular controls of preferential preparation of FFAs for the release. Pande and Mead (1968) suggested that the membrane enzyme may have a role in the uptake and transport of fatty acids, and it is possible that the fatty acyl-CoA may be more suitable for movement across the cell membrane than the fatty acid itself. Palmitic acid is more readily derivatized with coenzyme A than stearic acid in many species. In bovine liver microsomal fraction, the reaction rate of palmitic acid for coenzyme A was higher than that of stearic acid (Mitsuhashi et al., 1988), which is consistently with other species. Specificity of acyl coenzyme A synthetase may be related to preferential release of fatty acids from adipose tissue.

TABLE 2. STIMULATION OF FFA RELEASE BY BOVINE ALBUMIN[1]

	[μgram FFA/ (hr x g tissue)]			
	– albumin		+ albumin	
FFA	– NA	+ NA	– NA	+ NA
14:0	4.6+ 1.3	4.2+ 1.3	17.6+ 1.3	24.3+ 1.3
16:1	3.2+ 3.0	4.1+ 3.0	29.8+ 3.0	62.1+ 3.0
18:2	2.0+ 1.9	2.6+ 1.9	12.4+ 1.9	20.2+ 1.9
16:0	15.4+ 5.5	12.6+ 5.5	103.1+ 5.5	139.2+ 5.5
18:1	21.2+10.4	16.6+10.4	125.6+10.4	202.3+10.4
18:0	5.4+ 1.9	3.9+ 1.9	31.6+ 1.9	32.0+ 1.9
Total	51.9+22.7	43.9+22.7	320.1+22.7	480.7+22.7

[1] Least squares mean + S.E. for two steers

(Key Words: Adipose Tissue, Fatty Acid, Release)

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