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Saturation kinetics of palmitate uptake in perfused skeletal muscle

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We investigated the kinetics of palmitate uptake in a physiological skeletal muscle preparation by using the isolated perfused rat hindquarter. When plotted against the unbound plasma palmitate concentration, palmitate uptake displayed a simple Michaelis-Menten relation with a calculated V_{max} and K_m of 16.3 nmol·min^{-1}·g^{-1} and 0.06 μmol·l^{-1}, respectively. These results show that, as in isolated cell systems, uptake of free fatty acids in perfused skeletal muscle follows saturation kinetics consistent with carrier-mediated membrane transport of free fatty acids.

Free fatty acid; Muscle uptake

1. INTRODUCTION

Free fatty acids (FFA) are a major fuel source for skeletal muscle. Studies in men and dogs have suggested a linear relationship between utilization of free fatty acids and the total FFA concentration in plasma [1,2] suggesting that uptake of FFA into muscle occurs by passive diffusion. However, more than 99.9% of the FFA are carried in plasma bound to albumin. Thus, according to the traditional theory of cellular uptake for protein bound ligands, uptake of FFA should be dependent upon the unbound rather than the total FFA concentration. Along these lines, it has recently been shown that FFA uptake in cultured hepatocytes, adipocytes and cardiomyocytes is a saturable function of the unbound FFA concentration [3]. Such a relationship suggests that transport across the cell membrane does not follow simple diffusion. In accordance with such a hypothesis, a fatty acid binding protein has been isolated from the plasma membrane of hepatocytes [4], adipocytes [5] and cardiac myocytes [6] and addition of antibodies to these binding proteins inhibits FFA uptake [4–6] suggesting that fatty acid binding proteins may function as carriers for FFA. Studies of physiologically intact muscle preparations are, however, lacking. The purpose of the present study was to describe the kinetics for the uptake of FFA in an intact physiological in vitro muscle preparation.

2. MATERIALS AND METHODS

2.1. *Animal preparation*

Male Wistar rats were maintained on a 12-h light/dark cycle and received regular rat chow and water ad libitum. On the day of the experiment, fed rats weighing between 180 and 220 g were anesthetized by an i.p. injection of sodium pentobarbitol (5 mg/100 g body weight) and prepared surgically for hindquarter perfusion as previously described [7].

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2.2. *Experimental procedures*

The initial perfusate (300 ml) consisted of Krebs-Henseleit solution, 1–3-day-old washed bovine erythrocytes (hematocrit, 30%), 5% BSA (Cohn fraction V, Sigma, St Louis, MO), 6 mM glucose, varying amounts of albumin-bound palmitate (final perfusate concentration = 130–2200 μmol) and 100 μl of albumin-bound (1-^{14}C)palmitate (New England Nuclear, Boston MA). No insulin was added. The perfusate (37°C) was continuously gassed with a mixture of 35% oxygen, 3% carbon dioxide and 62% nitrogen, which yielded an arterial pH value of 7.4 and partial pressures for carbon dioxide and oxygen of 25–30 mm Hg and 150–200 mm HG, respectively.

The first 25 ml of perfusate that passed through the hindquarter was discarded, whereupon the perfusate was recirculated at a flow of 12.5 ml/min. After an equilibration period of 20 min, the blood was recirculated at the same flow rate for another 40 min during which time resting arterial and venous perfusate samples were taken at 10, 25 and 40 min.

2.3. *Analyses, calculations and statistics*

Samples for FFA (1.5 ml) were put in EGTA (30 μl, 200 mM, pH 7), centrifuged and the supernatant was frozen until analyzed for FFA by the enzymatic method of Shimizu et al. [8] as modified by Kiens [9]. Duplicate 100 μl aliquots of the perfusate plasma were mixed with liquid scintillation fluid (Maxifluor, J.T. Baker, Deventer, The Netherlands) and counted in a Packard liquid scintillation counter model 2000 CA (Packard Instrument Co., Downers Grove, IL). Since FFA concentration was very low in the absence of added palmitate and since palmitate was the only fatty acid added, measured FFA concentrations were taken to equal palmitate concentrations.

To ascertain that the radioactivity in the plasma was due solely to FFA, lipid extraction [10] and separation [11] were performed on a subsample of perfusate samples ($n = 5$). The recovered palmitate fraction contained 98% of the total radioactivity on the plate and that amount corresponded to more than 92% of the total radioactivity present in the plasma samples. Thus, aliquots of the plasma were counted directly and the total radioactivity was used to calculate the specific activity of palmitate in the perfusate.

Perfusate samples for the determination of pO_2, pCO_2, pH and haemoglobin were collected anaerobically, placed on ice and measured with an ABL 30 acid base laboratory and an OSM2 hemoximeter (Radiometer, Copenhagen, Denmark), respectively.

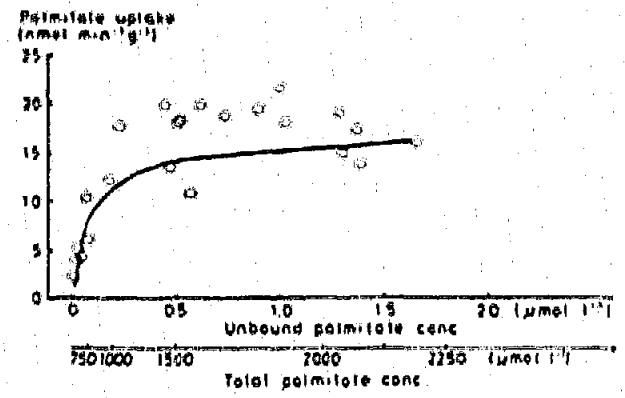

Fig. 1. Palmitate uptake of perfused hindquarters as a function of unbound palmitate concentration. Abscissa also indicates the corresponding total palmitate concentrations from which the unbound fraction was calculated by the stepwise equilibrium method (albumin concentration = 510 μM). Each point represents the average uptake value of one rat ($n = 23$).

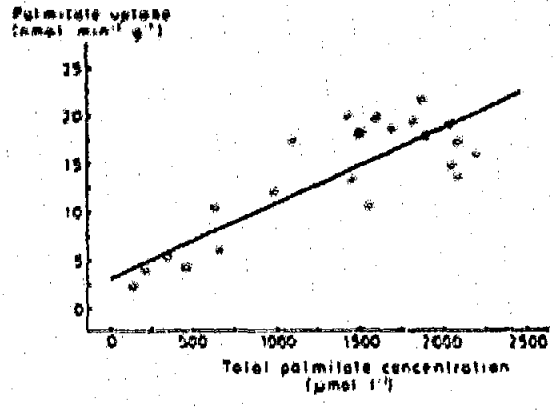

Fig. 3. Correlation between total palmitate concentration and the palmitate uptake ($n = 23$). The regression equation and correlation coefficient are: $y = 3.8x + 0.007$; $r = 0.83$, $P < 0.05$.

The equilibrium concentration of unbound palmitate was calculated by the stepwise equilibrium method as described by Spector et al. [12] and Wosilait and Nagy [13]. Palmitate uptake was calculated by multiplying the fractional uptake of palmitate with the perfusate plasma flow and the palmitate concentration in arterial perfusate plasma. Oxygen uptake was calculated by multiplying the arteriovenous difference with the perfusate flow. Uptake values were expressed per gram of perfused muscle which was assumed to be 1/6 of body weight [7].

3. RESULTS

Since values measured at 10, 25 and 40 min of rest did not differ, mean values of the measurements are given in the figures. Oxygen uptake was 24 ± 1 $\mu mol \cdot g^{-1} \cdot h^{-1}$ ($n = 5$) which is a normal value for perfused rat hindquarter at rest [14]. When uptake of palmitate was plotted as a function of the calculated unbound palmitate concentration, uptake followed saturation kinetics and could consequently be fitted to a Michaelis-Menten relationship (Fig. 1). By plotting the inverse of the uptake velocity as a function of the inverse of the palmitate concentration, a straight line was obtained ($y = 0.0035x + 0.06$; $r = 0.96$, $P < 0.05$) which yielded a V_{max} of 16.3 $nmol \cdot min^{-1} \cdot g^{-1}$ and an apparent K_m of 0.06 $\mu mol \cdot l^{-1}$ (Fig. 2). Similar values for the V_{max} (19 $nmol \cdot min^{-1} \cdot g^{-1}$) and K_m (0.08 $\mu mol \cdot l^{-1}$) were obtained when the data were fitted to a rectangular hyperbolic function as assessed by the computer program ALL-FIT [15]. The unbound palmitate concentration increases exponentially when total palmitate increases and the albumin concentration is constant [12]. This explains why uptake plotted versus total palmitate concentration follows different kinetics than when plotted against the unbound palmitate concentration. When plotted against the total palmitate concentration, uptake could be fitted to a straight line relationship, although levelling off as apparent at high concentrations (Fig. 3).

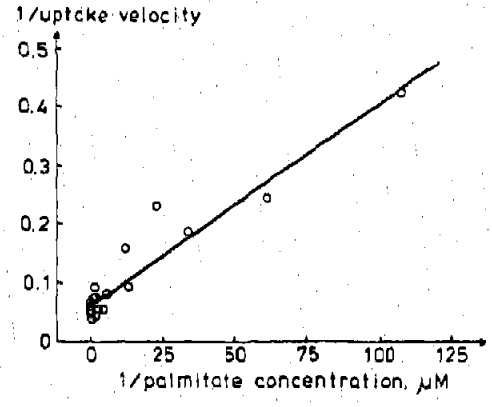

Fig. 2. Lineweaver plot of the uptake velocity as a function of the palmitate concentration. The V_{max} and K_m of the relationship were 16.3 $nmol \cdot min^{-1} \cdot g^{-1}$ and 0.06 $\mu mol \cdot l^{-1}$, respectively ($n = 23$). The regression equation and correlation coefficient are: $y = 0.0035x + 0.06$; $r = 0.96$, $P < 0.05$.

4. DISCUSSION

For the first time, the present study shows that in intact perfused skeletal muscle, uptake of free fatty acids (FFA) follows saturation kinetics when plotted against the unbound FFA concentration. The existence of saturation kinetics between FFA uptake and unbound FFA concentration has recently been documented in isolated cell culture systems. Thus, Sorrentino et al. [3] showed that [^{3}H]oleate uptake into isolated hepatocytes, adipocytes and cardiac myocytes followed saturation kinetics when the concentration of albumin was in the physiological range. These data as well as the data in the present study agree with the conventional theory for cellular uptake of protein bound ligand which states that ligand uptake is a function of the un-

bound ligand concentration and that protein-bound ligand serves as a reservoir for uptake by remaining in constant equilibrium with the unbound fraction. FFA are bound to albumin and under most physiological conditions less than 0.1% of the total FFA in plasma is unbound.

It is not known, where in the steps from the dissociation of free fatty acids from albumin to oxidative metabolism, the saturation occurs. Until recently, transport across the plasma membrane was believed to be achieved by a simple process of passive diffusion because of the lipid nature of the fatty acid molecule. However, accumulated evidence suggests that the saturation may occur in the transport process itself. Saturation kinetics are characteristic for carrier-mediated transport processes. Fatty acid binding proteins (FABP) have been isolated from the plasma membrane of cells that have a high demand for fatty acid uptake [4-6]. The 40-kDa FABP proteins that have been isolated from the plasma membrane of cultured hepatocytes, adipocytes and cardiac myocytes are remarkably similar [4-6]. They possess a high affinity for long chain fatty acids and antibodies raised to this class of membrane proteins inhibit both binding to the plasma membrane and cellular influx of free fatty acids in a dose-dependent fashion [5,6]. Thus, it appears that at least part of the free fatty acid uptake is mediated by a specific membrane protein. Evidence for the existence of a FABP in the plasma membrane of skeletal muscle cells is lacking. However, as in other cell types, functionally similar FABP have been located in the cytoplasm of muscle cells [16]. Since the kinetics of free fatty acid uptake are similar between cell types, it is likely that FABP are also located in the plasma membrane of skeletal muscle cells. If so, these FABP may constitute functional FFA transporters and consequently be the structural basis behind the apparent saturation type of kinetics for FFA uptake.

Previous studies of uptake and/or turnover of FFA in humans and dogs have suggested a linear relationship between total FFA concentration and FFA uptake or turnover at rest and during exercise [1,2]. If our data on palmitate uptake are plotted as a function of total FFA then a linear relationship can also be found (Fig. 3). However, since uptake occurs from the small pool of unbound FFA it is physiologically more relevant to plot uptake versus the unbound concentration of FFA than versus the total FFA concentration. Furthermore, plotting against the total FFA concentration obscures the saturable nature of the uptake, since the unbound concentration increases exponentially with an increase in total concentration.

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REFERENCES

[1] Armstrong, D.T., Steele, R., Altszuler, N., Dunn, A., Bishop, J.S. and De Bodo, R.C. (1961) Am. J. Physiol. 201, 9-15.
[2] Issekutz, B., Bortz, W.M., Miller, H.I. and Paul, P. (1967) Metabolism 16, 1001-1009.
[3] Sorrentino, D., Robinson, R.B., Kiang, C. and Berk, P.D. (1989) J. Clin. Invest. 84, 1325-1333.
[4] Stremmel, W., Strohmeyer, G., Borchard, F., Kochwa, B. and Berk, P.D. (1985) Proc. Natl. Acad. Sci. USA 82, 4-8.
[5] Schwieterman, W., Sorrentino, D., Potter, B.J., Rand, J., Kiang, C.L., Stump, D. and Berk, P.D. (1988) Proc. Natl. Acad. Sci. USA 85, 359-363.
[6] Sorrentino, D., Stump, D., Potter, B.J., Robinnson, R.B., White, R., Kiang, C. and Berk, P.D. (1988) J. Clin. Invest. 82, 928-935.
[7] Ruderman, N.B., Houghton, C.R.S. and Hems, R. (1971) Biochem. J. 124, 639-651.
[8] Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1979) Anal. Biochem. 98, 341-345.
[9] Kiens, B. (1984) Thesis, University of Copenhagen, Denmark.
[10] Dole, V.P. (1956) J. Clin. Invest. 35, 150-154.
[11] Kelly, T.F. (1966) J. Chromatogr. 22, 456-457.
[12] Spector, A.A., Fletcher, J.E. and Ashbrook, J.D. (1971) Biochemistry 10, 3229-3232.
[13] Wosilait, W.D. and Nagy, P. (1976) Computer Programs in Biomedicine 6, 142-148.
[14] Richter, E.A., Hansen, S.A. and Hansen, B.F. (1988) Am. J. Physiol. 255, E621-E628.
[15] DeLean, A., Munson, P.J. and Rodbard, D. (1978) Am. J. Physiol. 235, E97-E102.
[16] Miller, W.C., Hickson, R.C. and Bass, N.M. (1988) Proc. Soc. Exp. Biol. Med. 189, 183-188.