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Increase in human skeletal muscle lactate produced by fenfluramine

OUR recent hypothesis[1] that "anorectic agents" such as fenfluramine and mazindol owe at least part of their anti-obesity properties to a peripheral action on glucose metabolism by causing an increase in glucose uptake into skeletal muscle and a subsequent "wasting of calories" has been criticised[2] on the grounds that there is no information on the fate of the glucose so taken up, nor convincing evidence for an increase in metabolic rate with these drugs. We have now studied the effect of fenfluramine on lactate production in isolated human skeletal muscle, our previous work having shown no increase in glycogen levels[3] nor in carbon dioxide production[4]. An increase in lactate production would indicate metabolism of the glucose entering the cell under the influence of fenfluramine.

Human skeletal muscle was obtained at surgery for total hip joint replacement. The preparation and incubation of the tissue (in Krebs–bicarbonate buffer with added bovine serum albumin 2 mg ml^{-1} and glucose 16.67×10^{-3} M) was carried out using the method of Kirby, Leighton and Turner[5]. Muscle strips, wet weight 80–140 mg, were prepared from each sample and incubated in 2 ml of media containing (1) 100 μU ml^{-1} insulin, (2) 100 μU ml^{-1} insulin plus 100 ng ml^{-1} fenfluramine (previously shown to cause a maximal increase in glucose uptake[6]), (3) 100 μU ml^{-1} insulin plus 100 ng ml^{-1} amphetamine (amphetamine has no significant effect on glucose uptake into isolated muscle[3]). For each muscle sample two strips were prepared and used for control lactate determinations; they were not incubated. Flasks were flushed with a mixture of 95% oxygen and 5% carbon dioxide for 10 s before incubation at 37 °C for 90 min. The lactate content of the muscle and the residual media were then determined.

The method for measurement of muscle and residual media lactate was based on that for blood lactate using NAD/LDH enzymes[7] and the Boehringer Mannheim Biochemica Test Combination for lactate. Muscle strips were removed from the media and homogenised for 5 min at 25,000 r.p.m. with a blade homogeniser in 2 ml of 0.6 M perchloric acid. The homogenate was centrifuged for 10 min at 3,000 r.p.m. and 0.2 ml of the supernatant used for the lactate determination. One millilitre of the residual medium was added to 1 ml of 0.6 M perchloric acid and centrifuged as before, 0.2 ml of the supernatant being used for the subsequent analysis, with the Biochemica Test Combination. A lactate standard (0.5 mM) was included as a blank. Preliminary work had demonstrated a linear relationship between absorbance at 340 nm and lactate concentration over the range 0–1.0 mM. Good reproducibility was obtained with the method, the mean of six muscle lactate determinations in strips from the same sample being 6.91 ± 0.21 mmol per kg wet weight of muscle.

Table 1 Lactate formed by isolated human skeletal muscle incubated with insulin, fenfluramine or amphetamine

Insulin*	Fenfluramine plus insulin: change compared with insulin alone*	Amphetamine plus insulin: change compared with insulin alone*
13.85	+1.04	−0.10
14.11	−2.09	+1.82
9.79	+2.33	+0.84
9.12	+0.23	−3.00
10.97	+0.10	−0.53
8.78	+1.67	+2.07
7.68	+1.07	−1.67
8.58	+1.53	−1.57
7.48	+2.53	−0.08
8.79	+1.07	−0.70
Means ± s.e.m.	+0.95 ± 0.42	−0.29 ± 0.50
t	2.26	0.58
Significance	$P = 0.05$	NS

All values are means of two incubations.
*mmol lactate per kg wet weight skeletal muscle per 90 min; 100 μU ml^{-1} insulin, 100 ng ml^{-1} fenfluramine and 100 ng ml^{-1} amphetamine.

The results (Table 1) demonstrate that fenfluramine produced an increase in muscle lactate concentrations. Amphetamine, which has no effect on glucose uptake, has no significant effect on lactate concentration. Further studies are needed to determine in more detail the metabolic fate of the glucose taken up under the influence of fenfluramine, but these results are further evidence for a peripheral metabolic mechanism in its anti-obesity activity.

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Ribosome slowed by mutation to streptomycin resistance

ALTHOUGH the basic mechanisms of protein synthesis are now rather well understood, particularly in *Escherichia coli*, it is less clear how the high accuracy of this process is achieved. (The error rate in protein synthesis in *E. coli* seems to be about one in 10^4 amino acid misincorporations (Edelman and Gallant, unpublished results, and our own unpublished data)). A major site determining accuracy in protein synthesis is the ribosome, and in addition to the ribosome-mediated effects on accuracy (*in vivo* and *in vitro*) of various metal ions, aminoglycoside antibiotics and organic solvents, there are ribosomal mutations which increase or decrease accuracy[2]. Certain mutations of the 30S ribosomal protein S12 conferring streptomycin resistance (the *strA* locus) enhance accuracy[3–5], whereas certain mutations in the S4 protein (the *ram* locus) are known to decrease accuracy[6]. The *strA* mutations are restrictive (reducing) in their effect on both missense and nonsense suppression *in vivo* and on miscoding *in vitro*[3], the *ram* mutation, on the other hand, has the opposite effect in both cases[6]. It is possible that it is the kinetics of polypeptide synthesis at the ribosome that determines accuracy and that the kinetics in turn are affected by the *strA* and *ram* mutations. Because the same transfer RNA (tRNA) discrimination kinetics that may determine accuracy may also contribute to the elongation speed, we have investigated the effect of mutation to streptomycin resistance on the speed of polypeptide elongation.

A simple kinetic model for discrimination of charged