Delta Opioid Receptors Mediate Glucose Uptake in Skeletal Muscles of Lean and Obese-Diabetic (ob/ob) Mice

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Specific binding sites for [^{125}I]β-endorphin and the ∂_1-opioid [^{3}H][D-pen^2, D-pen^5]enkephalin (DPDPE) were quantified using autoradiography in soleus and extensor digitorum longus (EDL) muscles of lean and obese-diabetic (ob/ob) mice. The density of binding was significantly higher in obese-diabetic than lean mice. The uptake of 2-deoxy-D-[1-^{3}H]deoxyglucose, a nonmetabolized glucose analogue, into isolated soleus and EDL muscles was stimulated by β-endorphin, β-endorphin 1-27, and DPDPE, but not by the ∂_2-opioid deltorphin II. Both β-endorphin and DPDPE stimulated deoxyglucose uptake in obese-diabetic mice. Thus, glucose transport in skeletal muscle may be partly mediated via ∂_1-opioid receptors. The increased receptor density in obese-diabetic mice may be an adaptive response.

IT HAS BEEN suggested that glucose uptake into skeletal muscle during exercise is mediated, at least in part, by a non–insulin-dependent effect.[1] β-Endorphin increases the uptake of glucose in isolated diaphragm muscle of normal mice and since this peptide is released during exercise, it might have a role in glucose utilization in contracting skeletal muscle.[2-4] Certain C-terminal derivatives of β-endorphin, which lack the opioid sequence, are as effective as the parent molecule in stimulating glucose uptake,[2,5] and the effect was therefore ascribed to a nonopioid action of β-endorphin. However, it has long been known that opioids influence glucose homeostasis,[6] and opioid binding sites have been demonstrated on the surface membranes of skeletal muscles.[7-9] It is important, therefore, to quantify and characterize the opioid receptors present and to investigate whether opioid agonists, which act via specific opioid receptor subtypes, can influence glucose uptake in muscles. In the present study, we used the slow soleus and the fast extensor digitorum longus (EDL) muscles of the mouse to further characterize the opioid binding sites on the muscle membranes using quantitative autoradiography. We also investigated the effect of opioid agonists on glucose uptake in these muscles. In view of previous reports of differential effects in males and females of β-endorphin derivatives in mice[5] and of opioids in humans,[10] both male and female animals were used in this study.

Genetically obese-diabetic mice with the ob/ob mutation, which do not produce functional leptin,[11] inherit a syndrome of severe insulin-resistance, many features of which resemble those of type 2 diabetes mellitus in humans.[12] The number of β-endorphin binding sites has been shown to be higher in muscles from obese-diabetic mice than those from their lean littermates.[8,13] It seemed important, therefore, to study the opioid effects of β-endorphin on glucose uptake in muscles from obese-diabetic mice. Part of this work has been reported in abstract form.[14]

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MATERIALS AND METHODS

Animals

Male and female lean mice and obese-diabetic female mice of the Aston ob/ob (C57BL/6J) derived strain[15] were used at 12 to 14 weeks of age. At this age, the mice are known to be hyperglycemic and to manifest severe insulin resistance. The plasma glucose levels in the mice were determined using an automated glucose oxidase procedure,[16] and the plasma insulin levels were determined by a double-antibody radioimmunoassay.[17] The plasma nonesterified free fatty acids (NEFA) were determined as indicated previously.[18] The mice were killed by cervical dislocation, a Schedule 1 method of the Home Office Scientific Procedures (Animals Act), and the soleus and EDL muscles were rapidly dissected out.

Autoradiography

After removal from the animal, the muscles were washed thoroughly in 0.1 mol/L phosphate-buffered saline containing phenylmethylsulphonyl fluoride (0.1 mmol/L), pH 7.4, and cycloheximide (0.1 mmol/L), and mounted on cork discs using OCT (optimum cutting temperature) mounting medium. They were then quickly frozen in isopentane cooled in liquid nitrogen and stored at −80°C until required.

Longitudinal (20 μm) cryostat sections of the muscles were prepared, and binding sites for β-endorphin and the ∂_1-opioid agonist [D-pen^2, D-pen^5]enkephalin (DPDPE) were revealed using an autoradiographic method based on that of Herkenham and Pert.[19] Sections to be incubated with [^{125}I]β-endorphin were subjected to 3 (P 10-minute) washes in ice-cold Tris citrate buffer (50 mmol/L Tris citrate, 2 mmol/L EDTA, 2 mmol/L EGTA, adjusted to pH 7.4 at 0°C with citric acid). They were then incubated in Tris-buffer (0.17 mol/L, pH 7.4) containing [^{125}I]β-endorphin (2,000 Ci/mmol; Amersham International PLC, Little Chalfont, UK), for 1 hour at 4°C. In saturation binding experiments with β-endorphin, 100-fold excess of the μ-opioid agonist [D-Ala^2, N-Me-Phe^4, Gly^5-ol]enkephalin (DAGO, Sigma Chemical, Poole, UK) and the κ-opioid agonist (5α, 7α, 8β)-+-N-methyl-N-(7-[pyrrolidinyl]-1-oxaspiro[4.5]-dec-8-yl)-benzeneacetamide (U69593, Sigma Chemical) were included in the incubation mixture. Nonspecific binding was assessed by incubation of serial sections with 100-fold excess of unlabeled β-endorphin (Sigma Chemical) or DPDPE (Peninsula Laboratories, Liverpool, UK). In some experiments β-endorphin (β-endorphin 1-31, Sigma Chemical), β-endorphin 1-27 (Sigma Chemical), DPDPE, the ∂_2-opioid agonist [D-Ala^2]deltorphin II (deltorphin II, Peninsula Laboratories), the ∂_1-opioid antagonist 7-benzylidenenaltrexone (BNTX), or the ∂_2-opioid antagonist naltriben (NTB) were added in 100-fold excess. Following incubation, the test sections were washed immediately (3 times for 10 seconds) in ice-cold Tris-citrate

buffer and then dipped in ice-cold distilled water to remove the buffer. Sections to be incubated with [^{3}H]DPDPE (DuPont, NEN, Hounslow, UK) were treated similarly except that the buffer used was Tris-HCl. The specific radioactivity of radiolabeled DPDPE was 46 Ci/mmol.

The sections were dried rapidly in a dessicator and fixed over paraformaldehyde vapor at 80°C for 2 hours to cross-link the ligand-receptor complexes and left overnight at room temperature to remove excess formaldehyde. The slides were dipped in K2 nuclear emulsion (Ilford, Cheshire, UK) and developed in D19 developer (Kodak) after exposure at −20°C in the dark for 7 days for sections incubated with [^{125}I]β-endorphin and for 13 weeks for sections incubated with [^{3}H]-DPDPE. The muscle sections were counterstained with Mayer's hemalum (BDH).

The specific binding sites were quantified using an Olympus (London, UK) light microscope linked to a JVC (London, UK) camera with input to National Institutes of Health (NIH) image analysis software on an Apple Macintosh (Middlesex, UK) computer. The results were expressed as the number of specific binding sites/mm^2 tissue scanned. Four sections (6 to 8 random quadrats from each) were analyzed for each muscle at each ligand concentration. Levels of significance were determined using analysis of variance (ANOVA) with the Bonferroni-Dunn post hoc test or Student's *t* test, where appropriate.

Iterative curve fitting techniques (Kaleidograph 3.8; Apelbeck software, Middlesex, UK) were used to produce the saturation binding curves. The values of Bmax and Kd were obtained from the binding by fitting the untransformed data to a single-site binding model. The nonlinear plots were analyzed using the Hill equation.

Glucose Uptake

Immediately after removal from the animal, each muscle was weighed and incubated for 30 minutes at 37°C in 1 mL modified Krebs-Henseleit buffer containing NaCl (118 mmol/L), KCl (4.7 mmol/L), $CaCl_2.2H_2O$ (8.7 mmol/L), $Mg_2SO_4.7H_2O$ (1.17 mmol/L), KH_2PO_4 (1.2 mmol/L), $NaHCO_3$ (25 mmol/L), sodium pyruvate (2 mmol/L), bovine serum albumin (2%), L-[1-^{14}C] glucose (0.17 mmol/L, 0.06 mCi/mmol, Amersham International), and 2-deoxy-D-[1-^{3}H] glucose (2-DOG, Amersham International, 1 mmol/L, 0.1 mCi/mmol). The effects of β-endorphin 1-31, β-endorphin 1-27, DPDPE, deltorphin II, insulin (Sigma Chemical) or buffer (controls) were examined in the presence of 100-fold excess of DAGO and U 69593. L-[1-^{14}C]glucose which is not transported into muscle cells via carrier-mediated transport, was included in the incubation medium to determine the extracellular space. At the end of the incubation, the muscles were removed and blotted to remove excess fluid. The tissues were then digested in 1 mol/L NaOH (0.5 mL each) at 90°C. Hisafe II Scintillant (5 mL, Wallac, Milton Keynes, UK) was added, and the samples were counted on a Packard (Pangborne, UK) 1800 TR liquid scintillation analyzer. The uptake of glucose into the muscle cells was then determined after correction for the extracellular space. The results were analyzed using ANOVA with the concentration of peptide as the grouping factor, followed by the Bonferroni-Dunn post hoc test, or Student's *t* test where appropriate.

Identification of Oxidative Muscle Fibers

Transverse cryostat sections (20 μm thick) were prepared from the central regions of EDL muscles. Oxidative muscle fibers (slow type 1 and fast type IIA) were identified by staining for succinate dehydrogenase reaction product according to the method of Naclas et al.[20]

RESULTS

Animals

Table 1 shows the values for body weight, plasma glucose, plasma insulin, and plasma NEFA for lean and obese-diabetic mice. The mean body weight of the obese-diabetic mice was over twice that of the lean mice. The mean glucose level in the obese mice was over twice as high, and the mean insulin level was approximately 17-fold higher, while the mean NEFA level was twice as high as that in the lean mice.

Table 1. Body Weights and Plasma Glucose, NEFA, and Insulin Concentrations in Lean and Obese-Diabetic Mice

	Lean	Obese
Body weight (g)	38.2 ± 1.4	86.5 ± 2.1*
Glucose (mmol/L)	6.9 ± 0.4	15.0 ± 1.8*
Insulin (ng/mL)	1.8 ± 0.4	31.0 ± 7.1*
NEFA (mmol/L)	0.38 ± 0.05	0.76 ± 0.08*

NOTE. The values are given as means ± SEM for 12 animals in each group.

Abbreviation: NEFA, nonesterified fatty acids.

*$P < .001$ (Student's *t* test).

Autoradiography

Figure 1 shows photographs of typical sections of soleus muscle, which had been incubated with [^{125}I]β-endorphin or [^{3}H]DPDPE in the presence of excess DAGO and U69593. Binding sites for the labeled ligands can be seen along the membranes of the muscle fibres. This distribution is typical of the numerous binding studies described in this report. Figure 1 also shows adjacent control sections from the same muscles, which had been incubated with an excess of unlabeled ligand, to indicate nonspecific binding. The lack of membrane binding sites in the control sections excludes the possibility that the membrane binding is nonspecific.

[^{125}I]β-Endorphin Binding

Figure 2 shows the binding profiles for [^{125}I]β-endorphin in sections of soleus muscles from lean and obese-diabetic mice. The nonspecific binding was less than 20% of the total. The Kd and Bmax values are indicated in Fig 1. The Bmax value for [^{125}I]β-endorphin binding was over 2-fold higher in the obese-diabetic mice than in the lean mice. The mean Kd value was higher for the obese mice ($P < .001$), but it was not significantly different from the value for lean mice.

Effect of Opioid Ligands on [^{125}I]β-Endorphin Binding

To determine whether the specific binding sites were opioid receptors, sections from soleus and EDL muscles of lean or obese-diabetic mice were incubated with [^{125}I]β-endorphin and 100-fold excess of either unlabeled β-endorphin, the μ-opioid agonist DAGO, the κ-agonist U69593, the ∂_1-opioid agonist DPDPE, or the ∂_2-opioid agonist deltorphin II. The density of binding was compared with controls incubated with β-endorphin alone. Table 2 shows that the binding was blocked by both the ∂_1 and ∂_2-opioid agonists, but was not blocked to any significant extent by the specific μ- or κ-opioid ligands. The results also indicate a higher binding density on EDL muscles compared with soleus muscles in obese mice in the control sections incubated with [^{125}I]β-endorphin alone ($P < .05$). Mean binding density on EDL muscles was higher than on soleus, but the difference was not statistically significant except

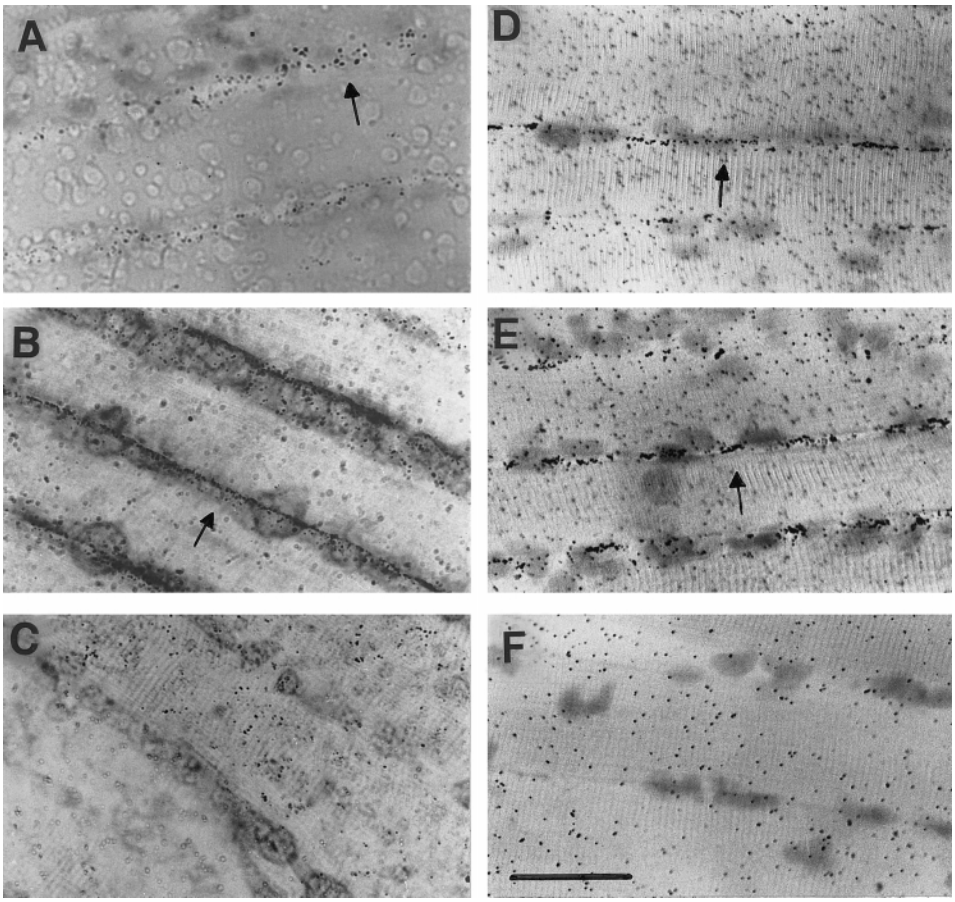

Fig 1. Autoradiographs of muscle sections incubated with [^{125}I]β-endorphin (A and B) or [^{3}H]DPDPE (D and E). Putative receptors on the surface membranes of muscle fibers are indicated by the arrows. (A and D) Sections from lean mice clearly showing binding sites distributed along the muscle fiber membranes. (B and E) Sections from obese-diabetic mice showing a higher density of binding sites distributed along the membranes. (C and F) Control sections incubated in the presence of [^{125}I]β-endorphin and [^{3}H]DPDPE, respectively, but with 100-fold excess of unlabeled DPDPE to show nonspecific binding. Bar = 40 μm.

with sections incubated in the presence of excess DAGO ($P < .05$).

Table 3 shows the effects of β-endorphin 1-27, which lacks the C-terminal tetrapeptide of the parent peptide, but contains the N-terminal opioid sequence, BNTX, a specific ∂_1-opioid antagonist, and NTB, a specific ∂_2-opioid antagonist, on the binding of [^{125}I]β-endorphin. These compounds all caused a significant blockade of the binding in both lean and obese-diabetic mice.

[^{3}H]DPDPE Binding

To demonstrate the presence of specific ∂-opioid binding sites directly, sections of soleus muscles from obese mice were incubated with different concentrations of [^{3}H]DPDPE. The distribution of a high density of DPDPE binding sites along the muscle fiber membranes is evident from Fig 1. Figure 3 shows the averaged binding profile. The Bmax and Kd values are also shown in Fig 3. These results indicate the presence of a specific high-affinity ∂-opioid binding site on the muscle membranes.

Glucose Uptake

Basal uptake of DOG. The basal rates of uptake of DOG were compared in soleus and EDL muscles in lean male and female mice. The values for basal uptake in the soleus were 0.45 ± 0.25 (SEM, n = 4) nmol/mg/h in the males and 2.52 ± 0.14 (SEM, n = 12) nmol/mg/h in the females. The values for the EDL muscles were 0.27 ± 0.20 (SEM, n = 4) nmol/mg/h in the males and 0.48 ± 0.16 (SEM, n = 12) nmoles/mg/h in the females. The basal uptake in the soleus of females was significantly higher than in either the soleus or the EDL muscles of male mice ($P < .05$ in each case). Furthermore, in the female mice, the basal uptake was significantly (over 5-fold) higher in soleus muscles than EDL muscles ($P < .05$).

Effect of β-Endorphin on DOG Uptake

Figure 4 compares the effect of β-endorphin and insulin on DOG uptake in soleus and EDL muscles of lean female mice. β-Endorphin stimulated DOG uptake in both soleus and EDL muscles. At the higher concentrations used (10^{-8} mol/L and 10^{-7} mol/L), the effects of β-endorphin in EDL muscles were similar to those seen with insulin at the same concentrations. β-Endorphin also increased the uptake of DOG in soleus muscles of lean male mice. In the male mice, the uptake was increased from 0.45 ± 0.25 nmol/mg/h (SEM, n = 4) to 2.36 ± 0.14 nmol/mg/h (SEM, n = 5) and 2.44 ± 0.25 nmol/mg/h (SEM, n = 3) by β-endorphin and insulin, respectively, at a concentration of 10^{-9} mol/L ($P < .01$ in each case). Both peptides caused an approximately 5-fold increase in uptake.

Effect of β-Endorphin 1-27

The effect of β-endorphin 1-27 (10^{-7} mol/L) on the uptake of glucose was studied in soleus and EDL muscles of lean

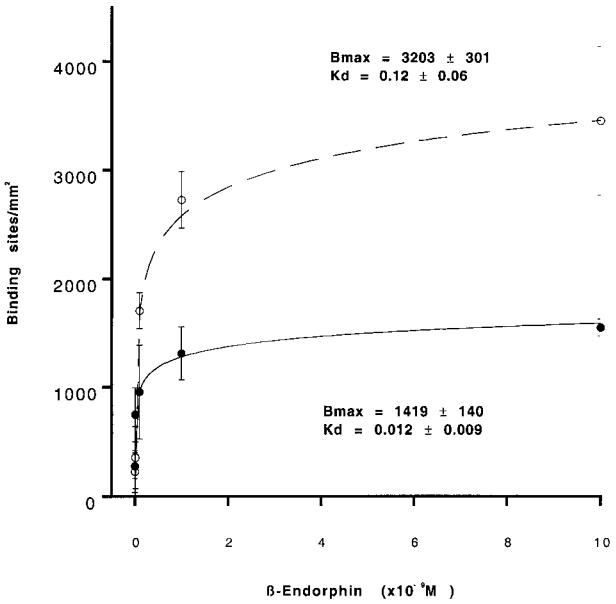

Fig 2. Binding profile for [^{125}I]β-endorphin in soleus muscles of lean (——) and obese-diabetic (— — —) mice. The muscle sections were incubated in the presence of 100-fold excess of DAGO and U69593. The binding curves are averaged from 4 experiments. The results are given as the means ± SEM for 4 animals at each concentration. The Bmax values are given as number of binding sites/mm^2 and the Kd values as nmol/L.

female mice. In the soleus muscles, the values were 2.52 ± 0.13 (SEM, n = 12) nmol/mg/h and 3.15 ± 0.11 (SEM, n = 4) nmol/mg/h for control and β-endorphin 1-27–treated muscles, respectively. In the EDL muscles, the values were 0.48 ± 0.16 (SEM, n = 12) nmol/mg/h and 1.63 ± 0.68 (SEM, n = 4) nmol/mg/h for control and β-endorphin 1-27–treated muscles, respectively. Thus, the peptide caused a significant increase in both soleus and EDL muscles ($P < .05$ in both cases). In the case of the EDL, the increase was over 3-fold.

Table 2. Effect of Opioid Ligands on the Binding of [^{125}I]β-Endorphin to Soleus and EDL Muscles of Lean and Obese-Diabetic Mice

		Binding Sites/mm^2	
		Lean	Obese
Control	Sol	394 ± 45	3,939 ± 498
	EDL	546 ± 142	6,142 ± 1,056
β-End	Sol	80 ± 21*	990 ± 674*
	EDL	87 ± 34†	1,223 ± 392*
DAGO	Sol	325 ± 80	4,735 ± 286
	EDL	719 ± 162	5,932 ± 925
U69593	Sol	515 ± 67	3,730 ± 1,527
	EDL	618 ± 125	6,806 ± 711
DPDPE	Sol	226 ± 47‡	1,747 ± 585†
	EDL	146 ± 13‡	1,962 ± 926†
Delt II	Sol	176 ± 25†	305 ± 84†
	EDL	126 ± 21†	827 ± 427†

NOTE. Radiolabeled β-endorphin was present at a concentration of 0.05 nmol/L. The unlabeled ligands were present at 5 nmol/L. The results are given as the means ± SEM for 4 animals in each case. Significance compared with the corresponding control values (ANOVA, Bonferroni-Dunn post hoc test).

*$P < .005$.

†$P < .01$.

‡$P < .05$.

Table 3. Effect of Endorphin 1-27 and Specific ∂-Opioid Ligands on the Binding of [^{125}]β-Endorphin to Soleus Muscles of Obese-Diabetic Mice

	Binding Sites/mm^2	
	Lean	Obese
Control	818 ± 343	3,203 ± 385
β-EP 1-27	232 ± 136*	432 ± 171†
BNTX	143 ± 129*	286 ± 86†
NTB	203 ± 114*	635 ± 314†

NOTE. Radiolabeled β-endorphin was present in a concentration of 0.05 nmol/L. DAGO and U69593 were present in 100-fold excess. β-Endorphin 1-27 or ∂-opioid ligands were added to give a concentration of 5 nmol/L. The results are given as the means ± SEM for 4 animals in each case. Significance compared with the corresponding control values (ANOVA, Bonferroni-Dunn post hoc test).

*$P < .05$.

†$P < .005$.

Effect of ∂-Opioid Agonists

Figure 5 shows the effect of the DPDPE on glucose uptake in soleus and EDL muscles of lean male mice. The ∂_1-opioid agonist caused a significant increase in DOG uptake in both muscles. The concentration profiles were bell-shaped. DPDPE (10^{-8}mol/L) also increased DOG uptake in EDL muscles of

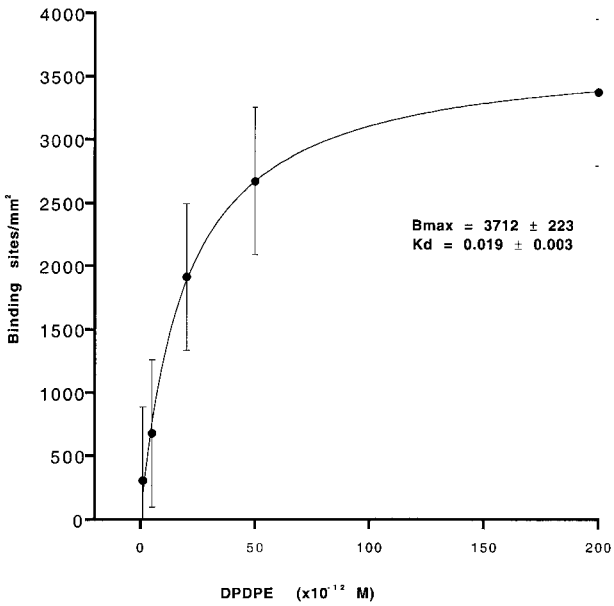

Fig 3. Binding profile for [^{3}H]DPDPE in soleus muscles of lean mice. The muscle sections were incubated in the presence of 100-fold excess of DAGO and U69593. The binding curves are averaged from 4 experiments. The results are given as the means ± SEM for 4 animals at each concentration. The Bmax value is given as number of binding sites/mm^2 and the Kd value as nmol/L.

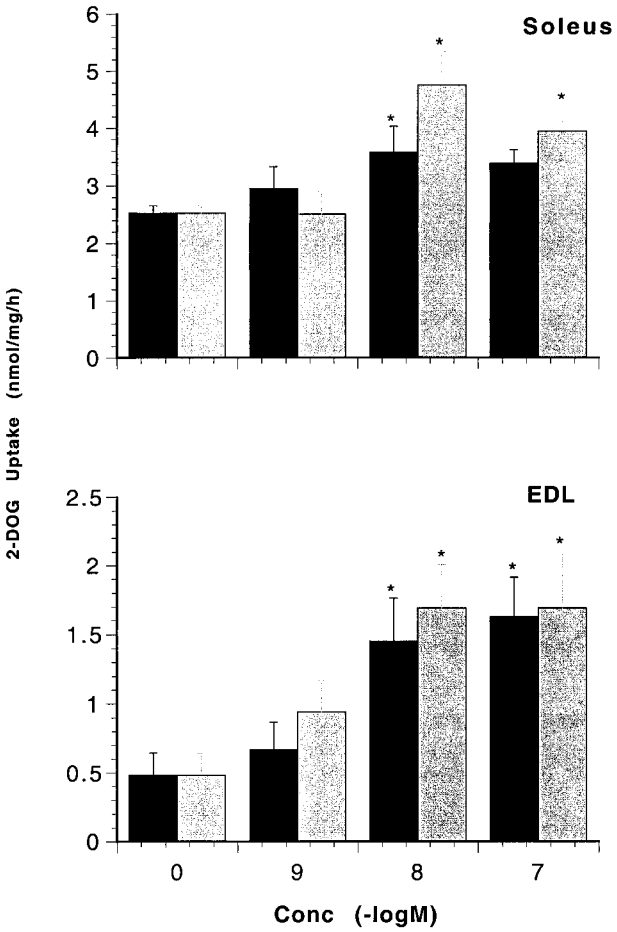

Fig 4. Effect of β-endorphin (■) and insulin (▨) on DOG uptake in soleus and EDL muscles of lean female mice. The values are given as the means ± SEM for 5 to 12 animals. *$P < .05$, significantly different from controls.

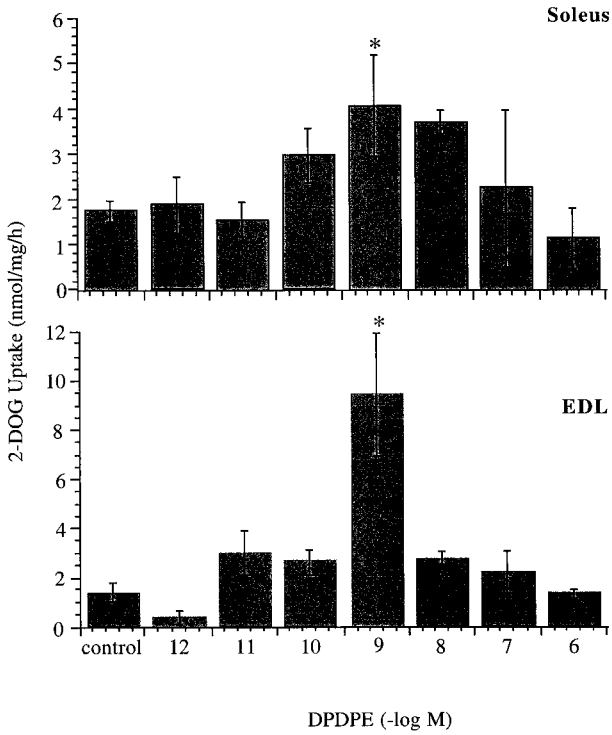

Fig 5. Effect of DPDPE on glucose uptake in soleus and EDL muscles of lean male mice. The values are given as the means ± SEM for 4 animals at each concentration. *$P < .05$, significantly different from controls.

female mice, where the uptake was 0.48 ± 0.16 (SEM) nmol/mg/h and 2.05 ± 0.56 (SEM) nmol/mg/h, respectively, in the absence and presence of DPDPE at a concentration of 10^{-8} mol/L ($P < .01$).

The ∂_2-opioid agonist, deltorphin II, in a concentration range of 10^{-9} mol/L to 10^{-7} mol/L, had no significant effect on DOG uptake in soleus or EDL muscles (results not shown).

Effect in Obese-Diabetic Mice

The basal rates of DOG uptake in muscles of obese-diabetic female mice (see Table 4) were significantly higher than in the corresponding muscles of lean female mice (quoted above, $P < .05$). The difference was approximately 2-fold in the soleus muscle and over 5-fold in the EDL muscle. As the basal uptake was higher in slow muscles than in fast muscles in the lean mice, an attempt was made to see whether the difference could be related to an alteration in the proportion of oxidative fibers in the obese-diabetic mice. As the EDL muscles exhibited a redder appearance in the obese-diabetic mouse than the lean mouse and normal EDL muscles consist of a relatively low proportion of oxidative fibers compared with soleus muscles, the composition of the EDL was examined. The proportion of oxidative (type 1 + type 2A) fibres was significantly higher in the obese-diabetic mice. The percentage of oxidative fibers was 48.6 ± 1.9 (SEM, n = 4) in lean mice and 61.5 ± 0.9 (SEM, n = 4) in obese-diabetic mice ($P < .001$, using Student's t test).

Table 4 shows the effects of β-endorphin and DPDPE on DOG uptake in soleus and EDL muscles of obese-diabetic mice. Both β-endorphin and DPDPE increased the mean uptake of DOG in the 2 muscles, although only the effect of DPDPE in soleus muscle was statistically significant.

Table 4. Effect of β-Endorphin and DPDPE on DOG Uptake in Muscles of Obese Female Mice

	2-DOG Uptake (nmol/mg/h)		
	Control	β-Endorphin	DPDPE
Soleus	5.16 ± 0.54 (7)	7.49 ± 1.12 (7)	9.86 ± 1.36 (5)*
EDL	2.82 ± 0.30 (7)	3.89 ± 0.58 (7)	4.49 ± 0.76 (5)

NOTE. The radiolabeled ligands were present at a concentration of 10^{-7} mol/L. DAGO and U69593 were present in 100-fold excess. The results are given as the means ± SEM. The number of animals is given in parentheses.

* $P < .05$ compared with the controls.

DISCUSSION

In previous work, it was shown that C-terminal derivatives of β-endorphin can increase glucose uptake in isolated muscles.[5] In the present study, β-endorphin 1-27, which lacks the C-terminal sequence, and the ∂-opioid ligand DPDPE stimulated glucose uptake in isolated muscles. As there is no homology between the C-terminal amino acid sequence and the N-terminal opioid region, it is possible that β-endorphin itself acts via 2 different mechanisms on skeletal muscle to promote glucose uptake.

In the quantitative binding studies, binding of β-endorphin to the opioid receptors present in muscle membranes could be displaced by an excess of various ∂-opioid ligands, but not by a μ-opioid or a κ-opioid ligand. Moreover, specific, high-affinity binding sites for the ∂_9-opioid [^{3}H]DPDPE were revealed, suggesting the presence of a ∂-subtype of opioid receptor. There is considerable pharmacologic evidence for the existence of 2 distinct ∂-opioid receptor subtypes in the central nervous system (for a review, see Zaki et al[21]T), although only 1 ∂-opioid receptor has been cloned.[22,23] In the present study, the ∂_1-opioid agonist DPDPE increased glucose uptake, whereas the ∂_2-opioid agonist deltorphin II did not. The greater efficacy of DPDPE compared with deltorphin II, indicates that the opioid receptor present resembles the ∂_1-subtype that has been characterized pharmacologically in the nervous system.[24-26] However, further studies are required to confirm the nature of the opioid receptor in skeletal muscle.

There was a significantly higher density of β-endorphin binding sites in EDL muscles compared with soleus muscles. Furthermore, there was a greater stimulation of glucose uptake by β-endorphin in EDL compared with soleus muscles, although the basal uptake in the absence of added peptide was higher in soleus muscles, the difference being especially marked in the female mice. The differences between the muscle types may be related to fiber type composition, capillary density, or the proportions and density of glucose transporter isoforms. Another possibility is that β-endorphin may alter the relative rates of utilization of glucose through different metabolic pathways in the 2 muscle types. Interestingly in this respect, it has been shown that red slow muscles are more sensitive than white fast muscles in their response to insulin (for a review, see Zorano et al[27]).

It has been reported that in the human, males and females exhibit differences in their sensitivities to κ-opioid drugs.[10] Furthermore, in previous work in this laboratory, it was shown that male and female mice exhibit differences in sensitivity to an analogue of the β-endorphin C-terminal tetrapeptide.[5] In the present study, β-endorphin and DPDPE strongly stimulated glucose uptake in muscles from either sex, but a detailed study of differences between the sexes was not undertaken. However, the basal uptake was higher in females than males. The reason for this difference is not known.

The concentration-response curves for the effect of DPDPE on glucose uptake were bell-shaped, as noted previously for the effect of β-endorphin in the diaphragm.[2] Although the significance of this is unclear, possible explanations are that agonist-bound receptors are desensitized or internalized at high concentrations of the ligand or that there are physical interactions between molecules at high concentrations. Alternatively, the ligand may be toxic to some extent at the higher concentrations. Another possibility is that binding of 1 molecule of the agonist to more than 1 site is necessary for the physiologic effect to be triggered, and that at high concentrations, each site binds a different molecule.

The high plasma glucose, insulin, and NEFA levels of the obese mice used in this study indicate their diabetic (type 2) status at the age used in this study. Higher levels of all 3 of the main types of opioid receptors have been reported in the brain of the obese-diabetic ob/ob mouse compared with the lean mouse.[28] In the present study, a significantly higher density of specific binding sites for β-endorphin was shown in skeletal muscles of obese-diabetic ob/ob mice compared with lean mice. As the tissues are insulin-resistant in the obese-diabetic mouse, the higher density in muscle may represent an adaptive response that contributes to the maintenance of glucose homeostasis. Interestingly, the basal uptake of glucose was higher in obese-diabetic mice than lean mice. It has been shown that the levels of pro-opiomelanocortin (POMC) peptides, including β-endorphin, are also higher in blood[28] and in both soleus and EDL muscles[30] and intramuscular nerves[13] of obese-diabetic ob/ob mice than in their lean littermates. The relatively higher basal uptake of glucose in the obese-diabetic mice may, in part, reflect a mass action effect of chronic exposure to higher glucose concentrations.[31] However, it could also be consequent to residual β-endorphin or its derivatives in the tissues, in the presence of a high density of β-endorphin receptors. Fast and slow muscles handle glucose differently,[27] and it is also possible that the difference is partly related to a change in the proportions of oxidative and glycolytic fibers in the obese-diabetic mice, as has been observed in some other insulin-resistant states.[32] Indeed, in this study, a higher proportion of oxidative fibers was demonstrated in EDL muscles of obese-diabetic mice than lean mice. Nevertheless, the presence of either β-endorphin or DPDPE in the incubation medium resulted in an additional uptake of glucose in the obese-diabetic mice, indicating that any endogenous stimulation was suboptimum. The therapeutic potential of these peptides in the treatment of type 2 diabetes remains to be explored.

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