Effects of Calcium Antagonists on Insulin-Mediated Glucose Metabolism in Skeletal Muscle

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The effect of three calcium antagonists (verapamil, diltiazem, and nifedipine) on insulin effects was investigated in isolated rat soleus muscles. Soleus muscles were incubated in the presence of insulin (100 μU/ml), a concentration that stimulates the rates of lactate formation and glycogen synthesis half-maximally and with and without a calcium antagonist. A decrease (48%; $P < 0.001$) was noted in the insulin-mediated rate of glycogen synthesis by verapamil at 100 μM; no effect was observed at lower concentrations of verapamil. Diltiazem decreased the insulin-mediated rates of glycogen synthesis by 36 ($P < 0.001$), 64 ($P < 0.001$), and 73% ($P < 0.001$) at 1, 10, and 100 μM, respectively. Nifedipine decreased the insulin-mediated rates of glycogen synthesis by 37% at 0.1 μM ($P < 0.001$), 36% at 1 μM ($P < 0.001$), 21% at 10 μM ($P < 0.05$), and 72% at 100 μM ($P < 0.001$). Verapamil at 100 μM decreased lactate formation by 48% ($P < 0.001$). However, diltiazem increased the rate of lactate formation by 22 ($P < 0.01$), 43 ($P < 0.001$), and 61% ($P < 0.001$) at 1, 10, and 100 μM, respectively. In contrast, nifedipine increased the insulin-mediated rate of lactate formation by 45% only at 100 μM ($P < 0.01$). The increased rate of lactate formation was probably caused by an increased rate of glycogenolysis, because high concentrations of all the calcium antagonists significantly decreased muscle glycogen content. The insulin-stimulated rate of 3-*O*-methyl-D-glucose transport or cAMP content was not affected by diltiazem at 1 or 10 μM. The results suggest that the calcium antagonists work by a mechanism, possibly by activating a calcium channel or an extracellular receptor, to influence markedly insulin-mediated intracellular glucose metabolism in skeletal muscle. *Diabetes* 43:73–79, 1994

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Received for publication 23 April 1991 and accepted in revised form 17 August 1993.

NIDDM, non-insulin-dependent diabetes mellitus; BSA, bovine serum albumin.

Calcium channel antagonists are used as major therapeutic agents in the treatment of systemic hypertension and ischemic heart disease (1). Calcium antagonists also are used to treat individuals with non-insulin-dependent diabetes mellitus (NIDDM), mainly because calcium antagonists have an insignificant effect on carbohydrate metabolism in vivo (2–5). However, in some cases, calcium antagonists are reported to cause diabetes mellitus (6,7) or insulin resistance (8). Therefore, we investigated whether calcium antagonists have direct effects on glucose metabolism in skeletal muscle, which is the major site for insulin-mediated glucose disposal in rats and humans (9,10). Consequently, we have measured the concentration-dependent effects of various calcium antagonists on insulin-stimulated (one concentration of insulin, 100 μU/ml, was used) processes in isolated incubated rat soleus muscle preparations, namely glycolysis (lactate formation), radiochemical glucose incorporation into glycogen (glycogen synthesis), and glucose oxidation. The calcium antagonists verapamil (a phenylalkamine), diltiazem (a benzothiazepine), and nifedipine (a dihydropyridine) were used. We used these three drugs because studies examining alteration in glucose tolerance in humans have normally used nifedipine or verapamil or diltiazem (2–7). Caution is important with regard to extrapolating effects observed in skeletal muscle preparations in vitro to skeletal muscle in vivo. However, the effects of drugs can be investigated in a precisely controlled manner in isolated incubated skeletal muscle preparations. Furthermore, considering the enormous differences in the condition of soleus muscle in vitro compared with in vivo, the concentration of insulin that is required to stimulate glucose utilization half-maximally is identical in the isolated incubated stripped soleus muscle preparation to that for the rat soleus muscle in vivo (i.e., ~100 μU/ml) (11,12).

RESEARCH DESIGN AND METHODS

Male Wistar rats (Harlan-Olac, Bicester, UK) were purchased at 5 weeks of age and were kept in the animal quarters at the department until experimentation. Rats were fed on a standard chow diet (supplied by SDS, Whitham, UK) consisting of 52% digestible carbohydrate, 16% protein, 2% fat, and 30% nondigestible residue (all by weight). The animals were housed in controlled conditions (23 ± 1°C) with a 12-h light-dark cycle and received standard laboratory chow and water ad libitum except for the 14-h period before isolation of muscles when only food was withdrawn. Stripped soleus muscles were routinely prepared between 0900 and 1000.

Isolation and incubation procedure. Soleus muscles were isolated and prepared as originally described by Crettaz et al. (13). Muscles were tied at resting tension to stainless steel clips and placed in Erlenmeyer flasks that contained Krebs-Ringer bicarbonate buffer (pH 7.4) containing 5.5 mM D-glucose, 1.35% (wt/vol) defatted bovine serum albumin (BSA), and 1.1 mM $CaCl_2$. After 30 min, preincubation muscle preparations were transferred to flasks that contained the identical medium plus 0.3 μCi of [U-^{14}C]glucose/ml (Amersham PLC, Amersham, UK) and bovine insulin (Sigma, Poole, UK) in the absence or presence of the calcium antagonists (verapamil, diltiazem, and nifedipine [Sigma]). The flasks were gassed with 5% O_2-95% CO_2 for the whole of the preincubation and for the first 15 min of the incubation. After 60 min of incubation, muscles were removed, blotted, and immediately frozen in liquid N_2.

Analytical procedures. The concentration of lactate in the incubation medium (spectrophotometric or net) (14) and the rates of incorporation of ^{14}C-labeled glucose into glycogen (15) or conversion to radiochemical lactate (16) were measured. Note that the net rate of lactate formation, which is measured by a spectrophotometric assay, yields a measure of the rate of glycolysis from glucose potentially supplied from muscle glycogen and/or glucose in the incubation medium. Radiochemical lactate formation is probably solely from glucose in the incubation medium. The methods for the measurement of glucose oxidation to $^{14}CO_2$ were those described previously (12); measured $^{14}CO_2$ production rates in this study were not corrected for any decrease in specific radioactivity of pyruvate owing to any glycogenolysis. Glycogen content was measured as described previously (17). cAMP content (18) and rates of ^{14}C-labeled 3-*O*-methyl-D-glucose transport (19) were measured by methods that were described previously.

Statistical analysis. All data are presented as means ± SE. Statistically significant differences between groups were determined with the Student's *t* test for paired or unpaired results as appropriate.

RESULTS

The effects of various concentrations of verapamil, diltiazem, and nifedipine on the insulin-stimulated rate of glycogen synthesis are given in Figs. 1–3. Rat soleus muscles were incubated with 100 μU/ml insulin, a concentration that stimulates the rates of lactate formation and glycogen synthesis half-maximally (16–18). A 48% decrease was noted in the insulin-mediated rate of glycogen synthesis by verapamil at 100 μM (Fig. 1). No effect was observed at lower concentrations of verapamil (Fig. 1). Diltiazem decreased the insulin-mediated rate of glycogen synthesis by 36, 64, and 73% at 1, 10, and 100 μM, respectively (Fig. 2). Nifedipine decreased the insulin-mediated rate of glycogen synthesis by 37% at 0.1 μM, 36% at 1 μM, 21% at 10 μM, and 72% at 100 μM (Fig. 3).

The effects of various concentrations of verapamil, diltiazem, and nifedipine on the insulin-stimulated rate of lactate formation are given in Figs. 1–3. Decreases of 17 and 48% were noted in the rate of lactate formation by verapamil at 10 and 100 μM, respectively (Fig. 1). Diltiazem increased the insulin-stimulated rate of lactate formation by 22, 43, and 61% at 1, 10, and 100 μM, respectively (Fig. 2). In one experiment, the rates of radiochemical lactate formation were not different in isolated soleus muscle preparations incubated with 1 μM (16.63 ± 1.42 $\mu mol \cdot h^{-1} \cdot g^{-1}$), 10 μM (15.88 ± 0.52 $\mu mol \cdot h^{-1} \cdot g^{-1}$), and 100 μM (18.89 ± 2.85 $\mu mol \cdot h^{-1} \cdot g^{-1}$) diltiazem compared with rates in muscles incubated in the absence of diltiazem (18.89 ± 2.89 $\mu mol \cdot h^{-1} \cdot g^{-1}$). In marked contrast, nifedipine increased the insulin-mediated rate of lactate formation by 45% but only at 100 μM (Fig. 3).

The effects of various concentrations of verapamil, diltiazem, and nifedipine on glycogen content in isolated, incubated rat soleus muscle preparations are given in Figs 1–3. Verapamil (100 μM) decreased the content of glycogen by 19% (Fig. 1). Diltiazem decreased glycogen content by 17% at 1 μM, 16% at 10 μM, and 27% at 100 μM, respectively (Fig. 2). Nifedipine decreased glycogen content by 17 and 37% at 10 and 100 μM (Fig. 3).

Soleus muscles were incubated with insulin at 1 and 10, 100, and 1000 μU/ml, which, respectively, represent basal, submaximal, and maximal concentrations of insulin, and the rates of glucose oxidation, lactate formation, and glycogen synthesis were measured (Table 1). The results of the effects of 10 μM diltiazem on these rates are given in Table 1. At basal levels of insulin, a significant inhibition of glycogen synthesis and marked elevation of the rates of net lactate formation occurred. At 100 μU/ml of insulin, diltiazem decreased the rate of glycogen synthesis. Insulin, at 1000 μU/ml, did not overcome the stimulation of the rate of lactate formation or inhibition of glycogen synthesis elicited by diltiazem. No effect of diltiazem on the rate of glucose oxidation was noted at any insulin concentration (Table 1).

The effects of incubation of soleus muscle preparations with a combination of nifedipine and diltiazem on insulin-stimulated rates of glycogen synthesis, lactate formation, and glycogen content were measured (Table 2). Incubation of soleus muscle preparations with 100 μU/ml insulin and either 100 μM nifedipine or 10 μM diltiazem caused a marked decrease in the rate of glycogen synthesis, increased rate of lactate formation, and decreased glycogen content (Table 2). However, incubation of soleus muscles with 100 μU/ml of insulin and a combination of diltiazem (10 μM) and nifedipine (100 μM) did not

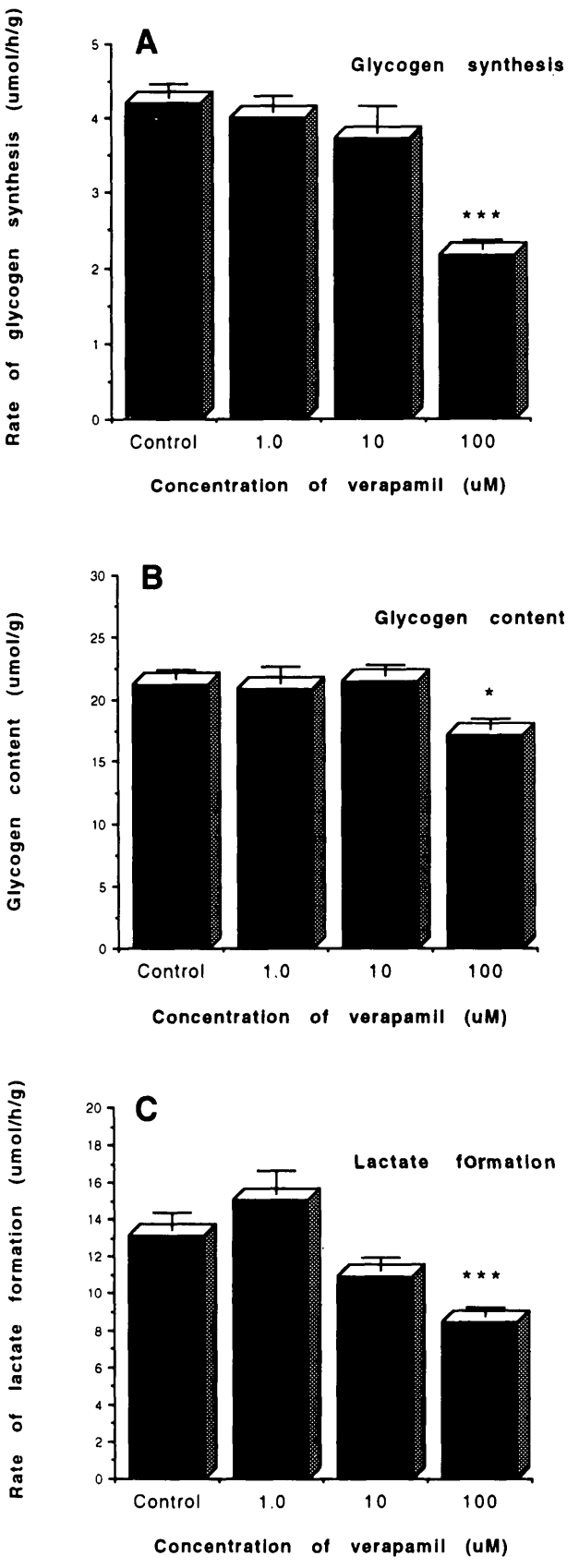

FIG. 1. Effect of verapamil on insulin-stimulated rates of glycogen synthesis (*A*), content (*B*), and net lactate formation (*C*). Data are means ± SE for at least 4 separate experiments. $^{*}P < 0.05$ vs. control, $^{***}P < 0.001$ vs. control.

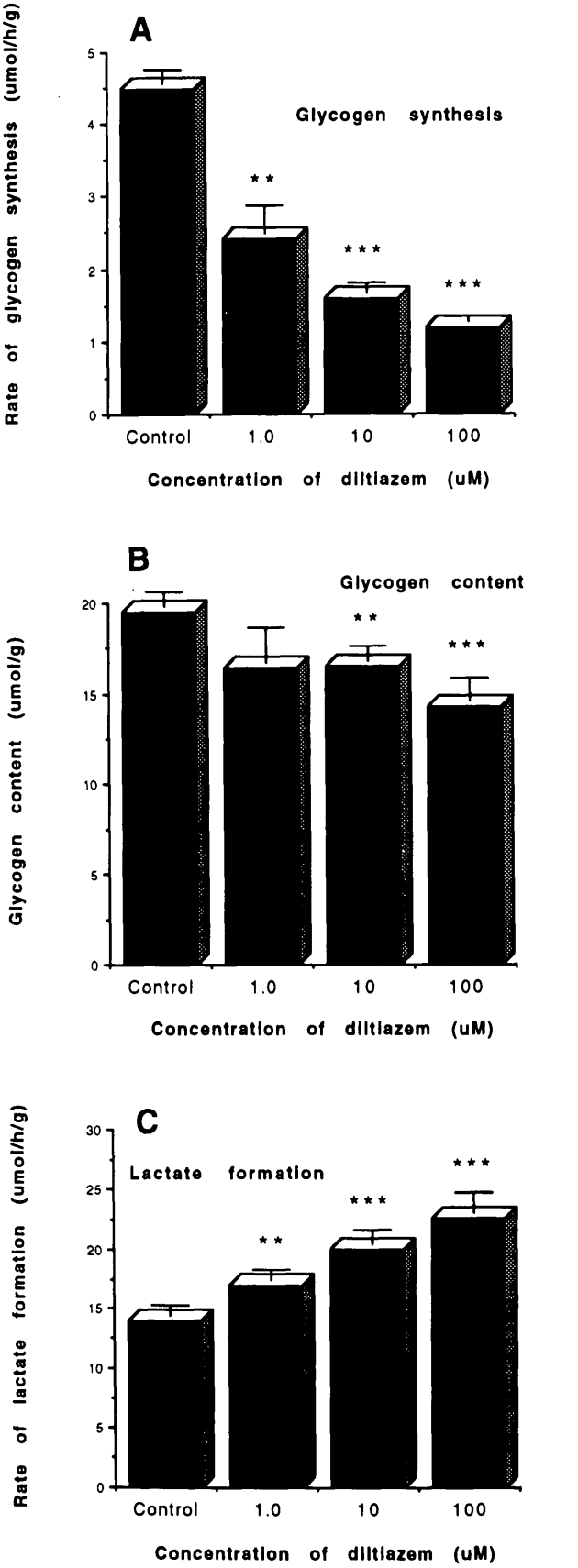

FIG. 2. Effect of diltiazem on insulin-stimulated rates of glycogen synthesis (*A*), glycogen content (*B*), and net lactate formation (*C*). Data are means ± SE for at least 4 separate experiments. $^{**}P < 0.01$ vs. control, $^{***}P < 0.001$ vs. control.

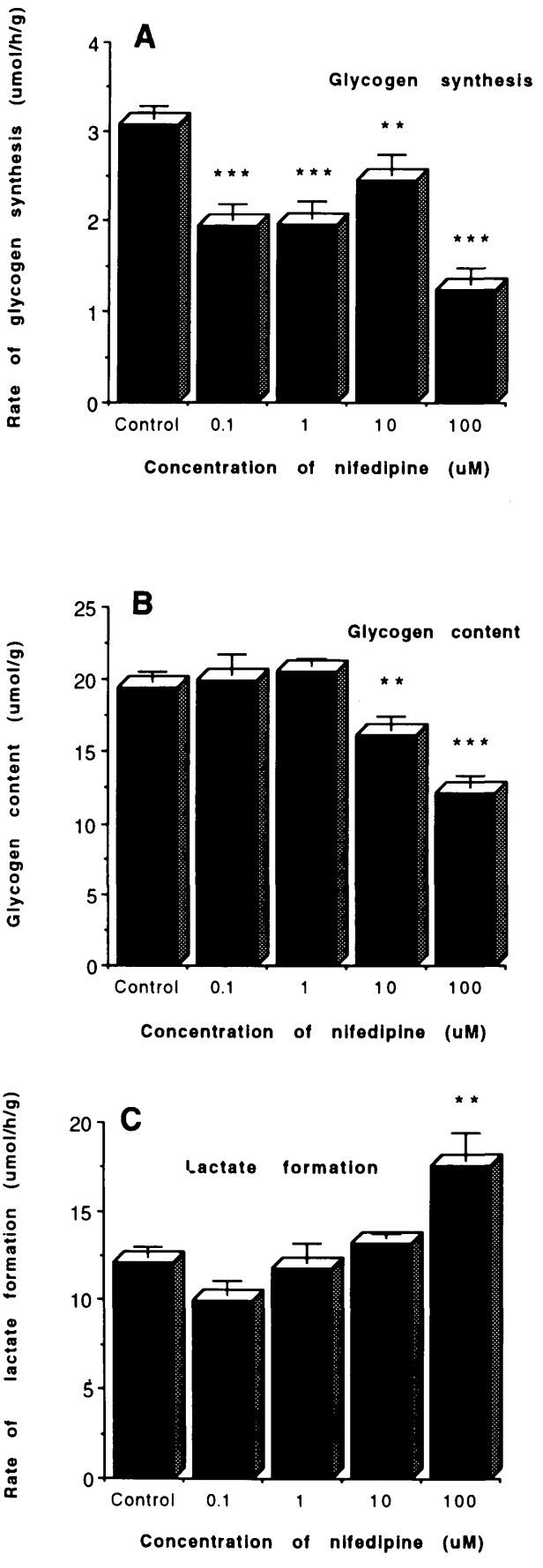

TABLE 1
Effects of diltiazem (10 μM) on insulin effects in soleus muscle

Insulin concentration	Rates (μmol · h^{-1} · g wet wt^{-1}) or content (μmol/g)		
	$^{14}CO_2$ formation	[^{14}C]glycogen synthesis	Net lactate formation
1 μU/ml	0.43 ± 0.12	2.08 ± 0.20	8.76 ± 1.36
Plus diltiazem	0.61 ± 0.13	1.75 ± 0.11	15.16 ± 1.16*
10 μU/ml	0.65 ± 0.20	3.33 ± 0.90	12.45 ± 3.05
Plus diltiazem	0.59 ± 0.10	1.06 ± 0.45†	15.55 ± 1.44
100 μU/ml	0.58 ± 0.03	5.51 ± 1.31	14.48 ± 1.48
Plus diltiazem	0.80 ± 0.22	2.21 ± 0.29†	22.99 ± 2.58‡
1,000 μU/ml	0.85 ± 0.02	5.24 ± 1.19	19.48 ± 0.68
Plus diltiazem	0.80 ± 0.24	2.86 ± 0.16†	26.02 ± 0.71*

Data are means ± SE for 4 separate experiments.
*$P < 0.01$ vs. control.
†$P < 0.05$ vs. control.
‡$P < 0.01$ vs. control.

significantly alter the rates of glycogen synthesis, lactate formation, or glycogen content from those rates inhibited by either drug alone (Table 2).

The effect of diltiazem, at 1 and 10 μM, on the maximally insulin-stimulated rate of transport of 3-*O*-methyl-D-glucose, the nonmetabolizable glucose analogue, into soleus muscle preparations was measured (Table 3). Insulin, at 1,000 μU/ml, stimulated the rate of 3-*O*-methyl-D-glucose uptake almost twofold. No significant effect of diltiazem on the insulin-mediated 3-*O*-methyl-D-glucose transport was observed. Diltiazem at 1 and 10 μM did not alter the content of cAMP in isolated incubated soleus muscle preparation (Table 4). However, isoprenaline, a β-adrenoceptor agonist, at 100 μM significantly stimulated the content of cAMP by ~2.5-fold.

DISCUSSION

Calcium channel antagonists are reportedly associated with the development of diabetes, impaired glucose tolerance, and insulin resistance (6–8,20,21). However, a large body of evidence also suggests that calcium antagonists do not alter glucose handling in nondiabetic and diabetic subjects (5,22). Therefore, we investigated whether calcium antagonists, commonly used in glucose tolerance studies, cause insulin resistance in skeletal muscle. To delineate which, if any, insulin-mediated processes might be resistant to the effects of insulin, we used the isolated soleus muscle preparation. Insulin facilitates the transport of glucose into muscle fibers, and once glucose enters the muscle cells, it may undergo several metabolic fates: conversion to glycogen (glycogen synthesis) or oxidative (glucose oxidation) and nonoxidative (mainly lactate formation) metabolism or glycolysis. The in vitro soleus muscle preparation was used because it is a biochemically viable preparation

FIG. 3. Effect of nifedipine on insulin-stimulated rates of glycogen synthesis (*A*), glycogen content (*B*), and net lactate formation (*C*). Data are means ± SE for at least 4 separate experiments. **$P < 0.01$ vs. control, ***$P < 0.001$ vs. control.

TABLE 2
Effects of the combination of diltiazem and nifedipine on glucose metabolism

Treatment	Rates (μmol glucosyl units · h^{-1} · g wet wt^{-1})		
	[^{14}C]glycogen synthesis	Net lactate formation	Glycogen content
Control	4.48 ± 0.20 (20)	14.62 ± 0.20 (12)	19.49 ± 0.83 (12)
Diltiazem (10 μM)	1.60 ± 0.14 (12)*	20.00 ± 1.16 (11)*	16.46 ± 0.82 (12)†
Nifedipine (100 μM)	1.25 ± 0.10 (4)*	17.59 ± 1.57 (4)†	12.13 ± 0.84 (4)*
Diltiazem (10 μM) plus nifedipine (100 μM)	1.21 ± 0.10 (4)*	18.81 ± 0.72 (4)*	11.51 ± 0.35 (4)†

Data are means ± SE for the number of observations in parentheses. All muscles were incubated in the presence of 100 μU insulin/ml.
*$P < 0.01$ vs. control.
†$P < 0.05$ vs. control.

(23) and responds to physiological concentrations of insulin (24,25), and the direct effects of drugs on insulin-stimulated metabolism or glucose transport can be monitored (26,27).

This study demonstrates that calcium antagonists decreased basal and insulin-mediated rates of glycogen synthesis, increased rates of lactate formation, and did not alter the rates of glucose oxidation in skeletal muscle in vitro (Figs. 1–3 and Tables 1 and 2). However, the three calcium antagonists (verapamil, nifedipine, and diltiazem) had different concentration-dependent effects on the various intracellular processes. The rank order for potency of inhibition of glycogen synthesis was nifedipine > diltiazem > verapamil, with nifedipine being ~1,000 times more potent than verapamil. The rank order for potency for stimulation of lactate formation was diltiazem > nifedipine, with a high concentration (100 μM) of verapamil causing a decreased rate of lactate formation. The effect of verapamil to decrease the rate of release of lactate into the incubation medium might indicate that verapamil affects other processes in skeletal muscle. Additional studies are required to determine whether a high verapamil concentration affects, for example, the rate of lactate efflux in skeletal muscle, thereby, perhaps increasing the intracellular lactate concentration. However, note that nifedipine, at the lowest concentration (i.e., 0.1 μM), inhibited the insulin-stimulated rate of glycogen synthesis but did not stimulate lactate formation. Therefore, for nifedipine it was possible to dissociate its effects on the various insulin-stimulated processes.

The calcium antagonist diltiazem did not affect the rate of radiochemical lactate formation (see RESULTS). The rate of net lactate formation was increased by diltiazem (Fig. 2) indicating that diltiazem increased the rate of glycogenolysis. This indication is supported by the fact that the decrease in the content of glycogen, caused by calcium antagonists, is more than can be accounted for by an inhibition of the rate of glycogen synthesis (Figs. 2 and 3). The rate of radiochemical lactate formation appears to be a reasonable index of the rate of glucose transport into muscle fibers (15,24). Indeed, no effect of diltiazem on the insulin-stimulated rate of 3-*O*-methyl-D-glucose transport into soleus muscle was observed. Although this might indicate no effect of calcium antagonists on the rate of glucose transport, further studies are required, and it is important to be cautious about drawing conclusions of this nature. Measurement of the accumulation of this glucose analogue will only reflect the balance between the rates of glucose influx and efflux from skeletal muscle fibers. Therefore, additional studies are necessary before any definite conclusion can be drawn on the effects of calcium antagonists on the rate of glucose transport into skeletal muscle cells. Diltiazem, nifedipine, or verapamil at 100 μM do not affect phospholipase C–stimulated rates of 3-*O*-methyl-D-glucose transport in isolated incubated epitrochlearis muscle preparations (28).

Because nifedipine and diltiazem markedly inhibited glycogen synthesis in soleus muscle preparations in vitro, we examined the effects of a combination of both these antagonists at maximally effective concentrations on insulin-mediated glucose metabolism (Table 2). Addition of 100 μM nifedipine or 10 μM diltiazem significantly decreased the content of glycogen and the rate of glycogen synthesis and increased the rate of lactate formation. These rates or content were not altered by incubation of soleus muscle with a combination of diltiazem and nifedipine. This suggests that, for glycogen synthesis, nifedipine and diltiazem are operating via the same biochemical mechanism to produce their effects.

Skeletal muscle has the highest density of calcium channel antagonist binding sites (29). However, calcium currents in skeletal muscle are relatively insensitive, compared with cardiac and smooth muscle, to blockade by calcium antagonists (29). In addition, skeletal muscle L-type calcium channels resemble but are not identical to the calcium channels found in smooth and cardiac muscle (29).

TABLE 3
Diltiazem and insulin-stimulated 3-*O*-methyl-D-glucose transport in soleus muscle

Additions	Rates (dpm · 15 min^{-1} · mg wet wt^{-1})
Control	339 ± 18
Insulin (1,000 μU/ml)	594 ± 12
Insulin plus diltiazem (1 μM)	612 ± 28
Insulin plus diltiazem (10 μM)	579 ± 14

Data are means ± SE for 4 separate experiments.

TABLE 4
Effect of diltiazem and isoprenaline on the content of cAMP in soleus muscle

Additions	Content (pmol/g wet wt)
Control	162 ± 13
Diltiazem (1 μM)	136 ± 12
Diltiazem (10 μM)	173 ± 17
Isoprenaline (100 μM)	504 ± 10

Data are means ± SE for 4 separate experiments. All muscles were incubated in the presence of 100 μU insulin/ml.

In skeletal muscle, glycogen synthesis is inhibited and glycogenolysis is stimulated by mechanisms that depend on increases in the cytoplasmic concentration of cAMP and/or calcium (30). For example, a rise in the concentration of cytoplasmic calcium results in inactivation of glycogen synthase activity and an increase in phosphorylase activity, the flux-generating enzymes for glycogen synthesis and glycogenolysis, respectively. Diltiazem did not change the intracellular concentration of cAMP (Fig. 4), which suggests that the effects of calcium channel antagonists were not mediated by cAMP-dependent protein kinase (30). Therefore, the effects of calcium antagonists on glycogen metabolism might be attributable to alterations in intracellular calcium homeostasis. It is not possible to measure changes in intracellular calcium levels in intact skeletal muscle preparations (28). Nevertheless, it is possible that some of the calcium channel antagonists used in this study (i.e., nifedipine and diltiazem) were acting as agonists on a calcium channel in nondepolarized muscle fibers. Indeed, 4 μM nifedipine does stimulate radiochemical calcium uptake into rat soleus muscle preparations, which would lead to an elevated concentration of cytoplasmic Ca^+ (31). This latter effect might also be caused by calcium antagonists entering muscle fibers to cause release of calcium from the sarcoplasmic reticulum (32). However, this seems unlikely because nifedipine and diltiazem do not permeate readily into skeletal muscle fibers (33).

Our results provide a clear demonstration that calcium channel antagonists exert marked effects on insulin-mediated glycogen synthesis in the isolated skeletal muscle preparation. Nifedipine or diltiazem caused a dramatic decrease in the rate of glycogen synthesis. In individuals with NIDDM, it is well-established that insulin resistance is a major determinant of the decline in glucose tolerance (10,34). Defective insulin-mediated glycogen synthesis makes a large contribution to this insulin resistance in humans (10,34). We believe that the concentration of calcium antagonists used in this in vitro study were within the therapeutic range. For example, 3 h after a 90 mg dose of diltiazem, the peak plasma total concentration of the drug is 0.25 μM. However, diltiazem is 78–87% bound to plasma proteins, largely albumin. In this study, the calcium antagonists were incubated in the presence of BSA, and during these conditions, we observed significant effects with 0.1 μM nifedipine and 1 μM diltiazem. Thus, in cases where it is suggested that calcium antagonists cause either insulin resistance or impaired glucose tolerance, it will be important to determine if the calcium antagonists are being displaced from the plasma-binding proteins. If subsequent clinical studies demonstrate that treatment of some individuals with calcium antagonists, either alone or in combination with other drugs, causes either insulin resistance (8), impaired glucose tolerance (21,22), or diabetes (6,7), then this study may play a role in the initiation of additional biochemical studies of the action of calcium antagonists on insulin-mediated responses in skeletal muscle.

ACKNOWLEDGMENTS

Some of these results were presented in abstract form at the 27th Annual European Association for the Study of Diabetes meeting held September 1992 in Dublin, Ireland.

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