Nifedipine Does Not Impede Clenbuterol-Stimulated Muscle Hypertrophy (44402)

RENÉ J. L. MURPHY,*‡ LOUISE BÉLIVEAU,*‡[1] PHILLIP F. GARDINER,* AND ANGELINO CALDERONE†‡

Département de Kinésiologie, Département de Physiologie,† Groupe de Recherche sur le Système Nerveux Autonome,‡ Université de Montréal, Montreal, Canada H3C 3J7*

Abstract. **The mechanism(s) responsible for β_2-adrenergic receptor-mediated skeletal muscle and cardiac hypertrophy remains undefined. This study examined whether calcium influx through L-type calcium channels contributed to the development of cardiac and skeletal muscle (plantaris; gastrocnemius; soleus) hypertrophy during an 8-day treatment with the β_2-adrenergic receptor agonist clenbuterol. Concurrent blockade of L-type calcium channels with nifedipine did not reverse the hypertrophic action of clenbuterol. Moreover, nifedipine treatment alone resulted in both cardiac and soleus muscle hypertrophy (6% and 7%, respectively), and this effect was additive to the clenbuterol-mediated hypertrophy in the heart and soleus muscles. The hypertrophic effects of nifedipine were not associated with increases in total β-adrenergic receptor density, nor did nifedipine reverse clenbuterol-mediated β-adrenergic receptor downregulation in either the left ventricle or soleus muscle. Both nifedipine and clenbuterol-induced hypertrophy increased total protein content of the soleus and left ventricle, with no change in protein concentration. In conclusion, our results support the hypothesis that β_2-adrenergic receptor agonist-induced muscle hypertrophy is mediated by mechanisms other than calcium influx through L-type calcium channels.**

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The *in vivo* administration of β_2-adrenergic receptor agonists has been shown to promote skeletal muscle hypertrophy and cardiac hypertrophy (1–5). The mechanism(s) responsible for this growth response remains undefined. Elevation of intracellular cAMP levels and calcium influx through L-type calcium channels are two signaling events coupled to β_2-adrenergic receptor activation. In various cell types, elevated cAMP levels have been shown to exert a growth inhibitory action (6). Furthermore, β_2-receptor stimulation in adult rat ventricular myocytes has been reported to evoke only a minor increase in intracellular cAMP accumulation (7). By contrast, calcium-dependent mechanisms are a prerequisite for the growth-promoting action of several growth factors (8, 9) and β_2-adrenergic receptor agonists have been reported to promote calcium influx through L-type calcium channels *via* a cAMP-independent mechanism (10). Thus, the following study tested whether calcium influx through L-type calcium channels contributed to the development of hypertrophy induced by clenbuterol, a β_2-adrenergic receptor agonist.

Materials and Methods

All experiments were performed in accordance with the guidelines of the Canadian Council on Animal Care (11) and the University's Ethics and Research Committee. Thirty-seven female Sprague-Dawley rats (Charles River, St. Constant, Quebec) with an initial body weight of ≈ 160 g were studied. Rats were housed individually in standard cages in an environmentally controlled facility (12:12-hr light:dark cycle, ~21°C) and had free access to food and water. After an acclimatization period in the animal care

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[1]To whom requests for reprints should be addressed at Département de Kinésiologie, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montreal, Quebec H3C 3J7, Canada. E-mail: louise.beliveau@umontreal.ca

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facility, 34 animals were randomly divided into four groups: control, nifedipine (L-type calcium channel blocker), clenbuterol (selective β_2-adrenergic receptor agonist), and nifedipine plus clenbuterol. Animals treated with nifedipine received the drug by intraperitoneal injections twice daily at a dose of 5 mg/kg (12) whereas untreated rats received the corresponding volume of the vehicle (1% Tween 80) for 9 days. Animals treated with clenbuterol received the drug intermittently (3 days on, 2 days off, 3 days on) in their food (ProLab RMH 4018; ground to a fine powder) at a dose of 4 mg/kg diet (4). Animals treated with nifedipine plus clenbuterol received the nifedipine treatment for 24 hr before and throughout the clenbuterol treatment to block L-type calcium channels prior to β-adrenergic agonist administration.

Following treatment, the animals were injected intraperitoneally with sodium pentobarbital at a dose of 50 mg/kg, and the soleus, plantaris, gastrocnemius, and heart muscles were removed, weighed , frozen in liquid nitrogen, and stored at −80°C until analysis. Total protein concentration (Bradford protein assay) was measured in homogenates of the left ventricle and soleus muscles. β-adrenergic receptor density of the left ventricles and the soleus was measured in triplicate using a radioligand binding technique (3). A saturating [^{125}I]-iodocyanopindolol concentration and (−)-alprenolol (10 μM) were used to determine total and nonspecific binding. Statistical significance ($P < 0.05$) was assessed using a two-way analysis of variance followed by a Tukey HSD *posthoc* test when necessary.

To determine the effectiveness of the nifedipine treatment, systolic blood pressure was measured in three other animals using the tail cuff method. Rats were treated with nifedipine (5 mg/kg) twice (1-day treatment), and blood pressure responses were monitored over the next 24 hr. These results were analyzed using a repeated measures ANOVA followed by a Tukey HSD *posthoc* test.

Results

To ascertain the effectiveness of the nifedipine treatment, blood pressure responses were monitored in three control animals who received a 1-day treatment. There was a significant decrease in blood pressure, which was maintained for several hours following the last nifedipine injection (Fig. 1).

Clenbuterol administration resulted in a significant increase of hindlimb skeletal muscle (plantaris 24%; gastrocnemius 17%; soleus 12%) and heart (16%) weight (Table I) and total protein content. All the increases in muscle weight reported in this study were paralleled by increases in protein content, such that protein concentrations were unchanged (Table II).

In nifedipine-treated animals there was a modest but significant increase in soleus (7%) and heart (6%) weight. The administration of clenbuterol to nifedipine-treated rats did not reverse the growth-promoting action of clenbuterol on skeletal and cardiac muscle (Table I). Moreover, in the soleus and heart, nifedipine and clenbuterol exerted an additive effect on muscle growth (Table I). This phenomenon was not observed in the gastrocnemius and plantaris muscles.

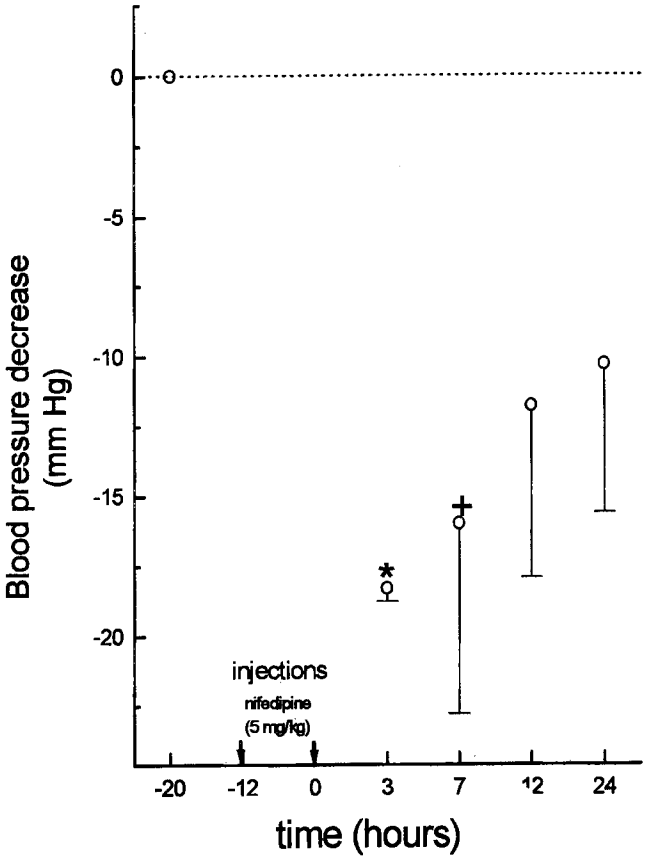

Figure 1. Blood pressure responses of animals treated one day with nifedipine. *Indicates significant nifedipine effect, $P < 0.05$, and + indicates $P = 0.08$.

Chronic treatment with clenbuterol significantly reduced total β-adrenergic receptor density in the soleus muscle (26%) and left ventricle (23%) whereas nifedipine had no effect (Fig. 2). The co-administration of nifedipine to the β_2-agonist treated animals did not reverse the clenbuterol-induced β-adrenergic receptor downregulation.

Discussion

The dose regimen of nifedipine used in the present study resulted in a significant decrease in blood pressure that was maintained for several hours (Fig. 1), thus demonstrating the efficacy of this treatment to block L-type calcium channels. The decrease in systolic blood pressure observed was consistent with the previously reported effects of this drug in normotensive animals (13).

The increases in cardiac and skeletal muscle wet weight and protein content with β_2-adrenergic agonist treatment were consistent with previous results (1–5). The nifedipine treatment did not inhibit the effects of clenbuterol suggesting that calcium influx through L-type calcium channels may not contribute to muscle hypertrophy induced by β_2-adrenergic receptor stimulation. However, the possibility of

Table I. Effects of Nifedipine and Clenbuterol on Body and Muscle Mass

Group	*n*	Mass				
		Body (g)[a]	Gastrocnemius (mg)[a]	Plantaris (mg)[a]	Soleus (mg)[a,b]	Heart (mg)[a,b]
Control	9	198 (10)	1022 (72)	198 (20)	85 (9)	585 (52)
Nifedipine	9	199 (12)	1054 (84)	210 (23)	90 (8)	626 (54)
Clenbuterol	8	214 (6)	1204 (46)	253 (16)	95 (11)	684 (47)
Nifedipine and Clenbuterol	8	214 (9)	1226 (46)	250 (19)	103 (9)	716 (16)

Note. Means (Standard Deviations). [a] significant clenbuterol main effect $P < 0.05$ and [b] significant nifedipine main effect $P < 0.05$.

Table II. Effects of Nifedipine and Clenbuterol on Muscle Protein Concentration

Group	*n*	Soleus (mg/g)	Left ventricle (mg/g)
Control	9	242 (91)	128 (30)
Nifedipine	9	217 (59)	135 (41)
Clenbuterol	7	216 (54)	118 (46)
Nifedipine and Clenbuterol	8	218 (68)	139 (28)

Note. Means (Standard Deviations).

a partial blockade by nifedipine, temporally or spatially, resulting in a residual amount of available channels sufficient to maintain the hypertrophic response, or the possibility of the presence of a redundant pathway cannot be totally excluded.

Surprisingly, nifedipine treatment was associated with an increase in soleus (7%) and heart (6%) weight and total protein. Moreover, the co-administration of nifedipine and clenbuterol was additive in promoting cardiac and soleus muscle hypertrophy. This was not observed in the plantaris and gastrocnemius muscles, which could be due to differences in calcium channels of fast- and slow-twitch muscles. For instance, cardiac isoforms of calcium channels have been observed in the slow soleus muscle (14).

The mechanism(s) implicated in this effect of nifedipine on the heart and soleus muscles remains unknown. One potential mechanism could be a reflex activation of the sympathetic system due to the decrease in blood pressure, resulting in norepinephrine release (15, 16). Furthermore, regulation of the calcium-dependent proteinases (calpains I and II) and their specific inhibitor calpastatin, has been suggested to be involved in protein turnover (17), and could contribute to this response.

An increased blood volume in the muscles due to the vasodilatory effects of the drugs could also contribute to the weight changes observed in this study. However, if this were the primary mechanism involved, similar changes would have been observed in the plantaris and gastrocnemius muscles. Furthermore, clenbuterol-induced increases in muscle mass have been reported to parallel changes in contractile force (18), and clenbuterol has been shown to increase protein content in cultures established from rat muscle cells (19), suggesting a direct effect on muscles. As for the nifedipine treatment, tissue was collected between 7 and 14 hr after the last injection, at a time when the acute effect was low (Fig. 1) and thus unlikely to cause a 6%–7% increase in the soleus and heart weights.

Previous studies have demonstrated that chronic treatment with a β_2-adrenergic agonist causes β-adrenergic receptor downregulation (2). By contrast, in cardiac myocytes, calcium channel antagonist therapy has been shown to promote an increase in β-adrenergic receptor density (20). Therefore, the additive effect of the L-type calcium channel blocker and the β_2-adrenergic receptor agonist on muscle hypertrophy could be due to an antagonistic effect of nifedipine on clenbuterol-induced β-adrenoceptor downregulation. However, whereas chronic treatment with clenbuterol significantly reduced the β-adrenergic receptor density in the soleus and the left ventricle, nifedipine treatment had no effect. Moreover, the administration of nifedipine to clenbuterol treated rats did not reverse clenbuterol-mediated β-adrenergic receptor downregulation (Fig. 2). These results strongly suggest that a change in β-adrenergic receptor density is not responsible for the additive effect of nifedipine and clenbuterol on muscle growth.

In conclusion, our studies provide evidence that mechanisms other than calcium influx through L-type calcium channels are involved in β_2-adrenergic receptor–mediated cardiac and skeletal muscle hypertrophy. By contrast, nifedipine was capable of promoting muscle hypertrophy, and this growth-promoting action occurred in the absence of any change in β-adrenergic receptor density. The hypertrophic effect was greatest when nifedipine and clenbuterol were co-administered as compared to either drug alone. The combined effect of nifedipine and clenbuterol was also found to be independent of any change in β-adrenergic receptor density. Thus, additional studies are required to elucidate the mechanism(s) implicated in β_2-adrenergic receptor–stimulated muscle hypertrophy.

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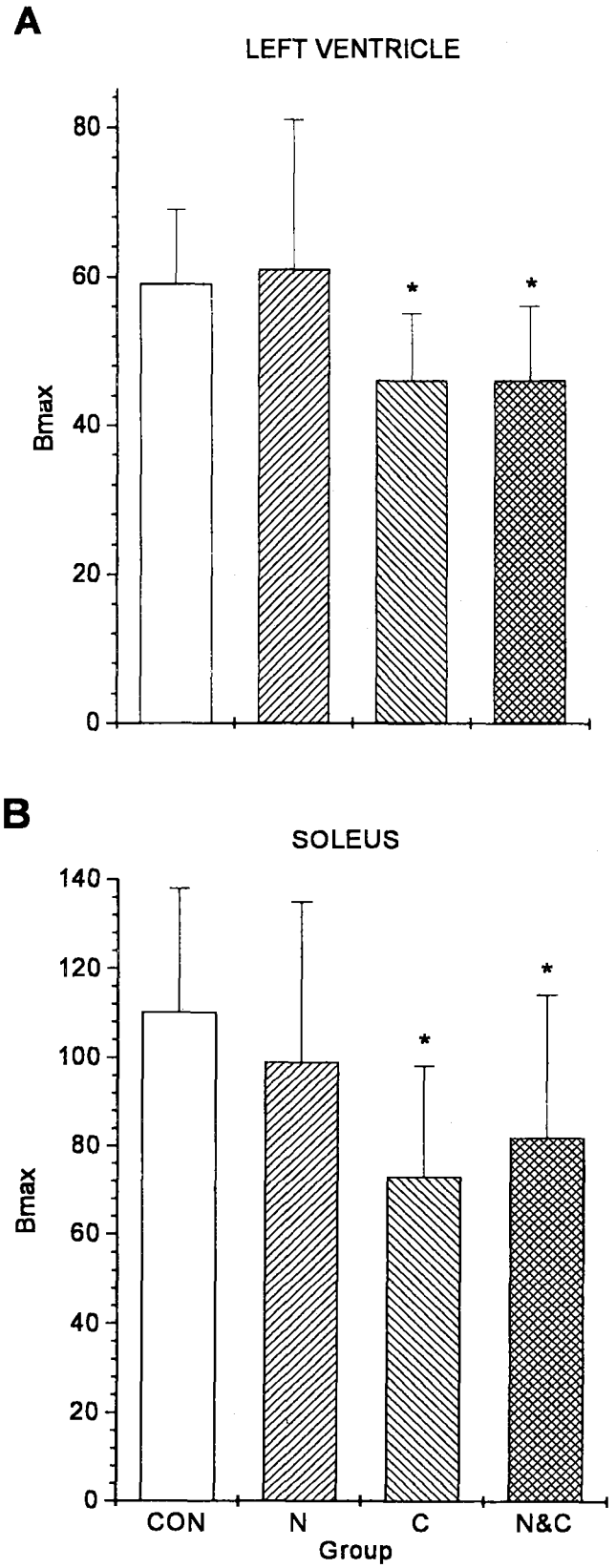

Figure 2. β-Adrenergic receptor density (fmol/mg protein) of (A) the left ventricles and (B) the soleus muscles of control (CON), nifedipine (N), clenbuterol (C), and nifedipine and clenbuterol (N&C) treated animals. Group means and standard deviations. *Indicates significant clenbuterol main effect $P < 0.05$.

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