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Nifedipine enhances cGMP production through the activation of soluble guanylyl cyclase in rat ventricular papillary muscle

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Abstract

It is known that nifedipine, an L-type calcium channel blocker, increases cGMP production, which partially contributes to the relaxation of vascular smooth muscle. The aim of our investigation was to clarify whether or not nifedipine regulates cGMP production, which has a physiological role in cardiac muscle. To measure contractile responses and tissue cGMP levels, left ventricular papillary muscles prepared from male Wistar rats (350–400 g) were mounted in the isolated organ chamber under isometric conditions and electrically paced by means of platinum punctate electrodes (1 Hz, 1 ms duration). In papillary muscle preparation, the negative inotropic effect induced by nifedipine (30 to 300 nM) was significantly inhibited in the presence of ODQ (1H-[1,2,4]oxidazolo[4,3-a]quinoxaline-1-one; 10 μM), a soluble guanylyl cyclase inhibitor. Furthermore, nifedipine (100 nM) strongly increased the tissue cGMP level, which was significantly decreased in the presence of ODQ. On the other hand, N^G-monomethyl-L-arginine (100 μM), a nitric oxide synthase inhibitor, did not inhibit either the negative inotropic effect or cGMP production induced by nifedipine. These results indicate that in rat left ventricular papillary muscle, nifedipine augments its negative inotropic effect at least partly through direct activation of cardiac soluble guanylyl cyclase but not nitric oxide synthase.

Introduction

Calcium channel blockers are important drugs in the treatment of hypertension and coronary heart diseases. Nifedipine is known as an L-type calcium channel blocker, which results in a reduced calcium influx in vascular smooth muscle cells and cardiomyocytes (Triggle & Janis 1984; Tsien et al 1991).

Recently, Kishi et al (1995) demonstrated that nifedipine raises the tissue cGMP level in vascular smooth muscle. Furthermore, Berkels et al (2001) reported that nifedipine enhances the bioavailability of endothelial nitric oxide (NO) via its antioxidative protection. On the other hand, Ding & Vaziri (2000) reported that nifedipine up-regulated both eNOS expression and activity in cultured endothelial cells. These reports suggest that nifedipine activates the NO–cGMP pathway in the vascular system, inducing important pharmacological effects, such as vasodilatation.

In cardiac muscle, there is no report of whether or not nifedipine stimulates cGMP production. It has been demonstrated that cGMP in cardiac muscle inhibits the L-type calcium channel, resulting in the induction of a negative inotropic effect (Hartzell & Fischmeister 1986; Shah et al 1994). If nifedipine increases the cardiac cGMP level, its negative inotropic effect as an L-type calcium channel blocker may be augmented by enhancing cGMP production induced by nifedipine itself. In this study, we investigated whether or not nifedipine regulates cGMP production in cardiac muscle.

Materials and Methods

This study was approved by the Animal Research Committee, Hirosaki University and carried out in accordance with the Guidelines for Animal Experimentation, Hirosaki University.

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Reagents

Nifedipine, 1H-[1,2,4]oxidazolo[4,3-a]quinoxaline-1-one (ODQ), N^G-monomethyl-L-arginine (L-NMMA) monoacetate, isoproterenol and DMSO were purchased from Wako Pure Chemical Industries (Japan). All chemicals used were of the highest purity commercially available. All solutions were made fresh in sufficiently high concentrations so that only very small aliquots were added to the assay tubes. Nifedipine and ODQ were freshly dissolved in DMSO, whose amounts had no effect on the assays.

Left ventricular papillary muscle preparation and mounting

Left ventricular papillary muscle strips were prepared according to methods previously described (Motomura et al 1990; Seya et al 1999). The strips were isolated from previously anaesthetized male Wistar rats, 350–400 g, and mounted in 25 mL organ baths containing modified Krebs–Henseleit solution (composition in mM: NaCl, 120; KCl, 4.7; $CaCl_2$, 1.3; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; D-(+)-glucose, 11.7) maintained at 30°C and continuously bubbled with 95% O_2/5% CO_2 mixture. The resting tension of left ventricular papillary muscle strips was adjusted to 1 g. Tension was measured with an isometric force transducer. Left ventricular papillary muscle strips were electrically paced by means of platinum punctuate electrodes (1 Hz, 1 ms duration, at twice the threshold voltage).

After an equilibration period of 1 h, the strips were twice contracted with isoproterenol (1 μM) in order to test their contractile capacity. The strips were then washed three times during a 1 h period. After ascertaining strips, nifedipine and another drug were added. The negative inotropic effect evoked by nifedipine was expressed as a percentage in basal tension. The response of strips was measured in the presence of ODQ (10 μM) or L-NMMA (100 μM), and each drug was added 10 min before the addition of nifedipine.

Measurement of cGMP

Rat left ventricular papillary muscle strips for measurement of cGMP were incubated at 30°C in gassed (95% O_2 and 5% CO_2) Krebs–Henseleit solution. When the contractile responses of strips were stable, strips were exposed either to nifedipine in the absence or in the presence of ODQ or L-NMMA. After nifedipine exposure for over 30 min, the reaction was stopped by rapidly exchanging the Krebs–Henseleit solution in the bath for ice-cold 10% trichloroacetic acid. Following homogenization of preparations and centrifugation, the supernatant was collected for cGMP determination using an enzymatic assay developed by our laboratory (Seya et al 1999). The protein content was determined by the Bradford method.

Statistical analysis

To determine the statistical significance between paired groups, the paired Student's *t*-test was used for normally distributed values. Group comparisons were performed by ANOVA with the Student–Newman–Keuls post hoc correction procedure. Values were presented as the mean ± s.e.m. $P < 0.05$ was considered statistically significant.

Results

Effect of ODQ on the negative inotropic effect and cGMP production induced by nifedipine in rat left ventricular papillary muscle

The negative inotropic effect was measured after 30 min of administration of nifedipine when the contractile response reached plateau. Nifedipine dose-dependently augmented its negative inotropic effect in left ventricular papillary muscle (Figure 1A). The negative inotropic effect induced by 100 nM nifedipine was 41.0 ± 1.5% (n = 6).

The basal level of tissue cGMP in left ventricular papillary muscle was 2.8 ± 1.8 fmol mg^{-1} protein (n = 4). After 30 min of nifedipine (100 nM) administration, the cGMP level was increased to 34.3 ± 5.6 fmol mg^{-1} protein ($P < 0.05$, n = 4) (Figure 1B).

To investigate the effect of cGMP on the negative inotropic effect of nifedipine, 10 μM ODQ, an inhibitor of soluble guanylyl cyclase, was added 10 min before nifedipine administration. ODQ significantly reduced the tissue cGMP level in the left ventricular papillary muscle to 10.5 ± 7.3 fmol mg^{-1} protein ($P < 0.05$, n = 4) (Figure 1B). Furthermore, the negative inotropic effect of nifedipine (30 to 300 nM) significantly declined in the presence of ODQ (10 μM). The negative

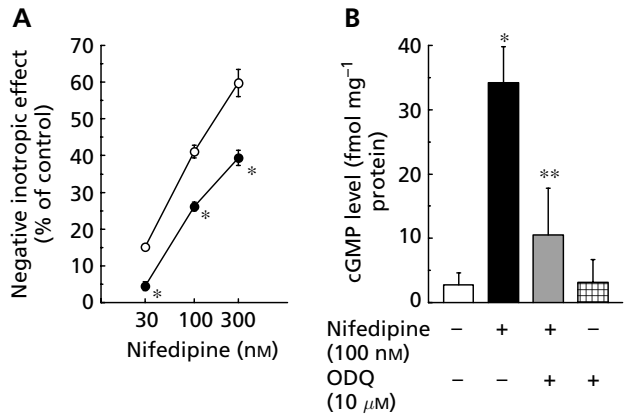

Figure 1 Effect of ODQ on the negative inotropic effect and cGMP production induced by nifedipine in rat left ventricular papillary muscle. (A) Dose–response curves for nifedipine (30 to 300 nM)-induced negative inotropic effect in the presence (closed circles, n = 4) or absence (open circles, n = 6) of ODQ (10 μM) in rat ventricular papillary muscle strips. Values are mean ± s.e.m. Significant differences: *$P < 0.05$ compared with the value of nifedipine. (B) Tissue cGMP levels were measured in the presence of 100 nM nifedipine (closed bar), 100 nM nifedipine + 10 μM ODQ (grey bar) and 10 μM ODQ (hatched bar) or absence of nifedipine and ODQ (open bar) in rat ventricular papillary muscle strips. Values are the mean of four experiments; mean ± s.e.m. Significant differences: *$P < 0.01$ compared with the control value; **$P < 0.01$ compared with cGMP level in the presence of nifedipine.

inotropic effect of 100 nM nifedipine in the presence of ODQ was 26.0 ± 1.2% ($P < 0.05$, n = 4) (Figure 1A). ODQ (10 μM) alone did not affect either basal tension or the tissue cGMP level.

Effect of L-NMMA on the negative inotropic effect and cGMP production induced by nifedipine in rat left ventricular papillary muscle

To confirm whether or not nifedipine regulates the activity of the NO–cGMP pathway, we investigated the effect of L-NMMA (100 μM), an NO synthase inhibitor, on the negative inotropic effect and the cardiac cGMP production induced by nifedipine in rat ventricular papillary muscle. L-NMMA did not inhibit either the negative inotropic effect (36.0 ± 2.1%, n = 5, Figure 2A) or cardiac cGMP production (25.7 ± 7.0 fmol mg^{-1} protein, n = 7, Figure 2B) induced by nifedipine. L-NMMA (100 μM) alone did not affect either basal tension or the tissue cGMP level.

Discussion

In this study, first we demonstrated that nifedipine augments cGMP production in rat cardiac muscle. Although ODQ, a soluble guanylyl cyclase inhibitor, inhibited not only its negative inotropic effect but also cGMP production induced by nifedipine, L-NMMA, a nitric oxide (NO) synthase inhibitor, did not affect either of these responses in the left ventricular papillary muscle. These results demonstrate that nifedipine augments its negative inotropic effect at least partly through activating the cardiac soluble guanylyl cyclase in rat cardiac ventricular muscle.

In the vascular system, it is well known that the NO–cGMP pathway has a physiologically important role for inducing relaxation of vascular smooth muscle cells. Various mechanisms for the activation of the NO–cGMP pathway by nifedipine have been proposed, for example, NO synthase stimulation inducing NO release in the endothelial cells (Dhein et al 1995; Ding & Vaziri 1998), inhibition of the production of reactive oxygen species (Berkels et al 2001) and so on. In cardiac muscle, several reports have demonstrated that the tissue cGMP level is increased by NO donors (Lohmann et al 1991; Kirstein et al 1995). We hypothesized that nifedipine may also activate the NO–cGMP pathway in cardiomyocytes. In our experiments, ODQ significantly reduced the cardiac cGMP production by nifedipine in left ventricular papillary muscle (Figure 1). On the other hand, L-NMMA did not inhibit either the negative inotropic effect or cardiac cGMP production induced by nifedipine (Figure 2). These results indicate that nifedipine might activate the soluble guanylyl cyclase itself. However, there is no report describing the activation of soluble guanylyl cyclase by nifedipine. Although YC-1 and arachidonic acid are known activators of soluble guanylyl cyclase, the activation mechanism of these substances is still unclear (Zhuo et al 1994; Wu et al 1995). The precise mechanism by which nifedipine activates soluble guanylyl cyclase therefore awaits further investigation.

In left ventricular papillary muscle, the negative inotropic effect of nifedipine was significantly reduced by ODQ (Figure 1A). Many researchers have demonstrated that stimulation of cGMP-dependent protein kinase by cGMP and/or cGMP analogues results in the inhibition of L-type calcium channels (Hartzell & Fischmeister 1986; Shah et al 1994; Wegener et al 2002). Hartzell et al reported that when cardiac intracellular cGMP concentration is 30 μM, the Ca^{2+} current is significantly decreased through the activation of cGMP-dependent protein kinase. This effective concentration of intracellular cGMP is near to the tissue level of cGMP increased by nifedipine (100 nM, Figure 1B). Our data suggest that the negative inotropic effect by nifedipine has at least dual effects on the L-type calcium channel: direct inhibition of this channel plus indirect inhibition via activation of cGMP-dependent protein kinase by cGMP production. As our findings indicate that cGMP produced by nifedipine in the left ventricle is responsible for the reduction in cardiac loading, we think the development of drugs that accelerate cardiac cGMP production by the activation of soluble and/or receptor guanylyl cyclase is medically important.

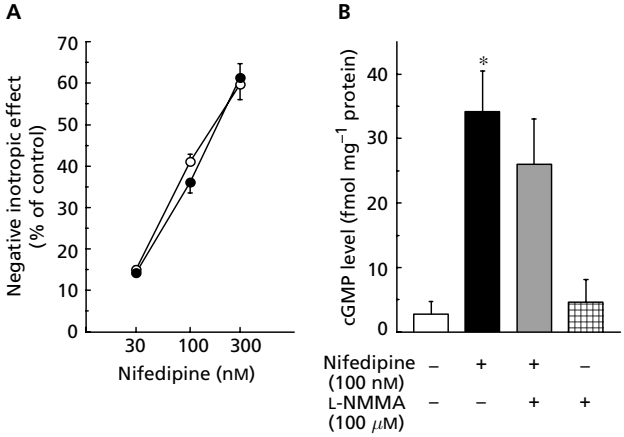

Figure 2 Effect of L-NMMA on the negative inotropic effect and cGMP production induced by nifedipine in rat left ventricular papillary muscle. (A) Dose–response curves for nifedipine (30 to 300 nM)-induced negative inotropic effect in the presence (closed circles, n = 5) or absence (open circles, n = 6) of L-NMMA (100 μM) in rat ventricular papillary muscle strips. Values are mean ± s.e.m. (B) Tissue cGMP levels were measured in the presence of 100 nM nifedipine (closed bar, n = 4), 100 nM nifedipine + 100 μM L-NMMA (grey bar, n = 7), and 100 μM L-NMMA (hatched bar, n = 4) or absence of nifedipine and L-NMMA (open bar, n = 4) in rat ventricular papillary muscle strips. Values are mean ± s.e.m. Significant differences: *$P < 0.01$ compared with the control value.

Conclusion

An L-type calcium channel blocker, nifedipine, demonstrated the acceleration of cGMP production in rat left ventricular papillary muscle. ODQ, a soluble guanylyl cyclase inhibitor, but not L-NMMA, a nitric oxide synthase inhibitor, significantly decreased not only the negative

inotropic effect but also cGMP production induced by nifedipine. These results indicate that in rat left ventricular papillary muscle, nifedipine augments its negative inotropic effect at least partly through the activation of cardiac soluble guanylyl cyclase but not NO synthase. The more detailed mechanism by which nifedipine activates soluble guanylyl cyclase awaits further investigation.

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