Effects of $N^G$-Monomethyl-L-arginine on Ca$^{2+}$ Current and Nitric-Oxide Synthase in Rat Ventricular Myocytes

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ABSTRACT

The effects of $N^G$-monomethyl-L-arginine (L-NMMA), a nitric-oxide synthase (NOS) inhibitor, on the L-type Ca$^{2+}$ current (ICa) and NO effects on NOS were determined in rat ventricular myocytes. L-NMMA (10 and 100 μM) had no significant effect on basal ICa, but in a cAMP-stimulated condition due to forskolin (1 μM) or milrinone (10 μM), a cGMP-inhibited cAMP-phosphodiesterase (PDE), L-NMMA (10 and 100 μM) concentration dependently augmented ICa. The enhancing effects of L-NMMA (10 and 100 μM) on ICa were not seen in the presence of either a nonselective inhibitor of PDE, 3-isobutyl-1-methylxanthine (IBMX), or a cGMP-dependent protein kinase activator, 8-bromo-cGMP (200 μM). 8-Bromo-cGMP (200 μM) inhibited 100 μM L-NMMA-induced ICa increase in the simultaneous application of forskolin (1 μM). Acetylcholine (ACH; 1 and 3 μM) inhibited 1 μM forskolin-stimulated ICa in a concentration-dependent manner, but this inhibitory action of ACh was significantly attenuated by the additional application of L-NMMA (100 μM). In the continuing presence of both L-NMMA (100 μM) and forskolin (1 μM), ACh (6 μM) had no inhibitory effect on ICa. In another series of experiments with isolated ventricular myocytes, we obtained both the positive staining of NADPH-diaphorase activity and the expression of the endothelial isoform of NOS. These data suggest that the effect of L-NMMA on ICa in a cAMP-stimulated condition with or without cholinergic inhibition is due to inhibition (acute effects) of a cGMP-stimulated cAMP-PDE via inhibition of the endothelial isoform of NOS.

The conversion of L-arginine to L-citrulline plus nitric oxide (NO) is produced by constitutive NO synthase (cNOS), or after cytokine stimulation, by inducible NOS (Moncada et al., 1991; Nathan and Xie, 1994). cNOS in saline-treated rats and inducible NOS in rats after pretreatment with endotoxin or cytokine are found in ventricular tissue slices as well as in isolated cardiac myocytes (Schultz et al., 1992). Furthermore, two isoforms of cNOS have been cloned from rat vascular endothelium (ecNOS) and brain (Nathan and Xie, 1994). The ecNOS protein expression also occurs in rat cardiac myocytes (Balligand et al., 1995). Administration of the NOS inhibitor $N^G$-monomethyl-L-arginine (L-NMMA) causes increases in mean blood pressure and systemic vascular resistance suppresses cardiac output in anesthetized dogs pretreated with either saline or endotoxin (Klabunde and Ritger, 1991). Perfusion with 1-methyl-L-arginine in an isolated rat heart inhibits cardiac contractility in isoproterenol-stimulated hearts and this inhibitory action is accompanied by a decrease in both myocardial cGMP and cAMP concentrations (Klabunde et al., 1992). In contrast, $N^G$-nitro-L-arginine, one of the NOS inhibitors, potentiates positive inotropic action of isoproterenol on electrically stimulated rat cardiac myocytes but does not significantly alter basal contractility (Balligand et al., 1993). In addition, Balligand et al. (1995) reported that L-NMMA attenuated the inhibitory effects of carbachol on the increase in contractility induced by isoproterenol. Nawrath et al. (1995), however, demonstrated that L-NMMA failed to influence the muscarinic effect on the force of contraction or frequency in rat and guinea pig hearts. Therefore, contradictory opinions remain as to how NOS inhibitors influence myocardial contractility in the β-adrenergically stimulated condition with or without the activation of muscarinic receptors.

cGMP is known to regulate both cGMP-dependent protein kinase (PKG; Lincoln and Corbin, 1983) and phosphodiesterase (PDE; Whalin et al., 1988). The physiological action of NO donors generally occurs as a result of the activation of soluble guanylyl cyclase (Katsuki et al., 1977). In mammalian myocardium, a PKG plays a major role in the cGMP-
induced decrease in calcium current (ICa; Levi et al., 1989; Mery et al., 1991, 1993). Indeed, the inhibitory effect of NO donor molsidomine is thought to be related to the activation of PKG in rat ventricular myocytes (Matsumoto, 1997). In frog ventricular myocytes, 3-morpholine-syndnonimine (SIN-1), one of the metabolic products of molsidomine, inhibits ICa by accumulating intracellular cGMP to activate a cGMP-stimulated cAMP-PDE (Mery et al., 1993). The resulting reduction in the cAMP level due to the activation of a cGMP-stimulated cAMP-PDE is responsible for NO-mediated cholinergic inhibition of ICa in isolated primary pacemaker cells from the rabbit sinoatrial node (Han et al., 1995). The question arises whether the effect of NOS inhibitors on ICa is the result of inhibition of PKG or cGMP-stimulated cAMP-PDE.

To elucidate the mechanism of the NOS inhibitor effect on rat cardiac myocytes, l-NMMA that does not inhibit muscarinic receptors (Buxton et al., 1993) and that is not necessary for de-esterification (Han et al., 1994) was used. We therefore investigated the effects of l-NMMA on the L-type ICa responses to the PKG activator 8-bromo-cGMP (8-Br-cGMP; Geiger et al., 1992; Lincoln and Cornwell, 1993) and a cAMP-stimulated condition due to forskolin, 3-isobutyl-1-methylxanthine (IBMX, a potent inhibitor of cGMP-stimulated PDE; Levi et al., 1989), or milrinone (a specific inhibitor of the cGMP-inhibited PDE; Nicholson et al., 1991) as well as the acetylcholine (ACh)-induced ICa inhibition in the presence of forskolin with or without l-NMMA application. We also examined the effects of 8-Br-cGMP on ICa responses to simultaneous administration of l-NMMA and forskolin. In another series of experiments with NADPH-diaphorase assay and Western blotting analysis, we examined whether the induction of a protein corresponding to ecNOS is occurring in isolated rat ventricular myocytes.

Materials and Methods

Animal Preparations and Electrophysiological Experiments. Single cardiac cells were isolated from the ventricles of rat hearts as described in Matsumoto (1997). Briefly, rats weighing 200 to 300 g were anesthetized by inhalation of ether vapor in a chamber or with i.p. administration of pentobarbital sodium. Then the heart was rapidly removed and perfused via the aorta with Langendorf apparatus. To obtain single ventricular cells the heart was perfused with Ca"-free Tyrode’s solution containing collagenase (5–10 mg/50 ml; Wako Pure Chemical, Tokyo, Japan) for 20 min.

The whole-cell patch-clamp method is basically the same as reported by Hamill et al. (1981). Current was measured with an amplifier (Nihon Koden CEZ-2300). A seal was formed with an internal solution in a pipette (1.2–2.3 MΩ). Then a higher negative pressure was applied inside the pipette to rupture the patch membrane to establish the whole-cell mode. The current-voltage (I-V) relationship was first monitored by using step pulses (200 ms) from the holding potential (–60 mV) to +60 mV at a frequency of 0.1 Hz. The holding potential level was adjusted to ~40 mV to avoid contamination of the Na" channel current. In most experiments, the cells were depolarized every 10 s from a ~40-mV holding potential to 0 mV for 200 ms, and K" currents were blocked by replacing all K" with intracellular and extracellular Ca". The data were acquired on-line with an NEC PC-980/RX computer. Experiments were performed at 33–37°C.

Solutions and Drugs. The normal Tyrode’s solution contained 140 mM NaCl, 5.4 mM KCl, 1 mM MgCl2, 0.33 mM NaH2PO4, 5.5 mM glucose, 5 mM HEPES (Wako Pure Chemicals), and 4 mM CaCl2. The pH was adjusted to 7.4 with NaOH. The KB solution consisted of 70 mM KOH, 50 mM glutamic acid (Wako Pure Chemicals), 40 mM KCl, 3 mM MgCl2, 20 mM taurine (Wako Pure Chemicals), 20 mM KH2PO4, 10 mM glucose, 5 mM EGTA (Wako Pure Chemicals), and 10 mM HEPES. The pH was adjusted to 7.4 with KOH. The internal solution contained 20 mM NaCl, 90 mM CsOH (Sigma Chemical Co., St. Louis, MO), 40 mM aspartic acid (Wako Pure Chemicals), 5 mM ATP magnesium salt (Sigma Chemical Co.), 3 mM MgCl2, 5 mM potassium creatine phosphate (Funakoshi, Tokyo, Japan), 20 mM HEPES, 0.3 mM Na2GTP (Sigma Chemical Co.), and 20 mM 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (Wako Pure Chemicals). The pH was adjusted to 7.2 with CsOH. CaCl2 (6 mM) was added.

l-NMMA, 8-Br-cGMP, forskolin, IBMX, and milrinone were purchased from Sigma Chemical Co. ACh was obtained from Daiichi-seiyaku (Tokyo, Japan). These drugs were dissolved in either normal Tyrode’s solution or ethanol as a 10 mM stock solution. All the stock solutions were kept in glass containers.

Data Analysis. Before external application of l-NMMA the amplitude of basal ICa was measured, averaged, and normalized as the control. The maximal changes in the amplitude of whole-cell ICa were measured after external application of l-NMMA (10 and 100 μM) and expressed as percentage of changes in the control. The statistical difference in the concentration-dependent effects of l-NMMA on the changes in basal ICa was calculated by a one-way ANOVA for repeated measurements. The maximal changes in ICa were measured after external application of l-NMMA (10 and 100 μM) in the absence or presence of 8-Br-cGMP (200 μM), forskolin (1 μM), IBMX (10 μM), and milrinone (10 μM). Similarly, maximal changes in ICa seen after external application of ACh (1 and 3 μM) were compared in the presence of forskolin (1 μM) and forskolin (1 μM) plus l-NMMA (100 μM). Furthermore, maximal changes in ICa induced by simultaneous application of both l-NMMA (100 μM) and forskolin (1 μM) were compared in the absence or presence of 8-Br-cGMP (200 μM) or ACh (6 μM). The maximal changes in basal ICa induced by l-NMMA before or during the application of 8-Br-cGMP, forskolin, IBMX, or milrinone; the ACh-induced maximal decreases in a forskolin-stimulated ICa before and after l-NMMA application; and the l-NMMA-induced maximal increases in a forskolin-stimulated ICa before and after the application of 8-Br-cGMP or ACh were analyzed by a paired t-test. All values were expressed as the mean ± S.E. A value of P < .05 was statistically significant.

NADPH-Diaphorase Assay. The NADPH-diaphorase assay technique, as described in Prabhakar et al. (1993), was used to determine whether rat ventricular myocytes express NOS activity. After isolation, myocytes were collected into 0.5-mI Eppendorf tubes that contained Tyrode’s solution in the absence or presence of 100 μM l-NMMA, and they were incubated at 36°C for 10 to 30 min. After suction of the solution, the cells were then fixed with 4% paraformaldehyde and washed in PBS (pH 7.4). Fixed cells were incubated for 2 h in PBS containing 0.3% Triton X-100 and 0.2 mM nitroblue tetrazolium, either in the absence (control) or presence of 1 mM β-NADPH. In the presence of β-NADPH, NOS reduced tetrazolium to formazan, which appeared as a dark blue stain.

Western Blotting. Cardiac cells were studied in Tyrode’s solution. The cells were prepared and treated for 10 to 30 min. At the end of the treatments, the medium was aspirated, and cells were lysed in ice-cold Laemmli buffer [50 mM Tris-HCl, 2% dithiothreitol, 2% SDS, 0.1% bromophenol blue, and 10% glycerol (pH 6.8)] as described in Bradford (1976). Before electrophoresis, samples were boiled for 5 min at 100°C and then centrifuged for 10 min at 10,000g at 4°C to remove insoluble materials. Protein concentrations at each group were determined by Bio-Rad protein assay (Laemmli, 1970) and varied <10%. Aliquots of samples (50 μg of protein) were applied to SDS-polyacrylamide gel electrophoresis (8% linear-gel; Laemmli, 1970), and separated proteins were transferred onto a nitrocellulose membrane (0.4-μm pore size; Amersham, Buckinghamshire, UK) by electrophblotting on ice at 100 V for 70 min (Towbin et al., 1979). The uniformity and completeness of protein transfer was established by...
staining the membrane with Ponceau S (Sigma Chemical Co.). Immunoblotting was performed by incubating blots for 1 h at room temperature with blocking buffer [10 mM Tris-HCl, 150 mM NaCl, 1% Tween 20, and 3% BSA (pH 7.6, fraction v; Sigma Chemical Co.)] and subsequently probing with a monoclonal antibody to anti-ecNOS (1:6000; Transduction Laboratories, Lexington, KY) in blocking buffer overnight or for 1 h at 4°C. Antibody bound to ecNOS was detected with horseradish peroxidase–protein A (1:8000; Zymad Laboratory, South San Francisco, CA) in blocking buffer and visualized by the Amersham enhanced chemiluminescence system.

**Results**

Effects of l-NMMA on NADPH-Diaphorase Activity and ecNOS Protein Expression of Isolated Ventricular Myocytes. As shown in Fig. 1, the NADPH-diaphorase assay technique demonstrated the presence of NOS activity in isolated rat ventricular myocytes. The cells prepared in the absence of β-NADPH (Fig. 1A) failed to exhibit a positive staining reaction, and the cells treated in the presence of β-NADPH stained positively (Fig. 1B). Similar results were obtained in 10 cells isolated from 3 hearts. However, cardiac myocytes constitutively expressed in ecNOS contained a 130-kDa protein (Fig. 1C). Similar results were obtained in ventricular myocytes from four hearts.

Effect of l-NMMA on Basal ICₐ. Typical examples of l-NMMA (100 μM) effects on the ICₐ seen in step pulses are shown in Fig. 2A. Activation of ICₐ usually started at −40 mV and reached a maximum at 0 mV. Figure 2B presents the changes in I-V relationships of ICₐ from five cardiac myocytes in the control solution and after l-NMMA (10 and 100 μM) application. l-NMMA did not shift either to the right or the left on the step pulse-induced I-V curves. Figure 2B shows the changes in the basal ICₐ seen in step pulses in response to external application of l-NMMA at two concentrations (10 and 100 μM). l-NMMA had a weak stimulatory effect on the basal ICₐ. The changes in ICₐ induced by l-NMMA at 10 and 100 μM are summarized in Fig. 2C. The mean value for basal ICₐ was 672 ± 49 pA (n = 8). l-NMMA (10 and 100 μM) had no significant effect on basal ICₐ.

Effects of l-NMMA on ICₐ before and after 8-Br-cGMP. External application of 8-Br-cGMP (200 μM) that strongly and selectively activates PKG (Geiger et al., 1992; Lincoln and Cornwell, 1993) inhibited basal ICₐ by approximately 75%. In the continuing presence of both l-NMMA and forskolin, 8-Br-cGMP (200 μM) inhibited l-NMMA-induced ICₐ stimulation (Fig. 4A). The applications of l-NMMA (100 μM) and forskolin (1 μM) increased basal ICₐ, and under these conditions, 8-Br-cGMP significantly attenuated the increase in ICₐ induced by l-NMMA application in a cAMP-stimulated condition due to forskolin (Fig. 4B).

Effects of l-NMMA on ICₐ before and after Forskolin. Figure 5A shows the changes in basal ICₐ in response to external application of l-NMMA (100 μM) before and after forskolin application (1 μM). l-NMMA had a weak stimulatory effect on the basal ICₐ. In the presence of forskolin (1 μM) that potentiated the amplitude of basal ICₐ by approximately 53%, additional application of l-NMMA (100 μM) noticeably potentiated forskolin-stimulated ICₐ. Figure 5B summarizes the effects of two concentrations of l-NMMA (10 and 100 μM) before and after external application of forskolin (1 μM). l-NMMA concentration dependently augmented forskolin-stimulated ICₐ.

Effects of l-NMMA on ICₐ before and after IBMX. IBMX (10 μM), a nonselective PDE inhibitor, potentiated basal ICₐ by approximately 59%. In the continuing presence of IBMX, l-NMMA (100 μM) did not significantly alter IBMX-induced ICₐ stimulation (Fig. 6A). Although l-NMMA (10 and 100 μM) had a weak stimulatory effect on basal ICₐ before IBMX application, such an effect was not observed in the presence of IBMX (Fig. 6B).

Effects of l-NMMA on ICₐ before and after Milrinone. Milrinone (10 μM) potentiated basal ICₐ by approximately 71%, and a weak stimulatory action of l-NMMA (100 μM) in the absence of milrinone was significantly augmented by external application of milrinone (Fig. 7A). As shown in Fig. 7B, the stimulatory effect of l-NMMA on ICₐ occurred in a cAMP-stimulated condition closely related to the inhibition of cGMP-inhibited cAMP-PDE.

Effect of l-NMMA on ACh-Induced ICₐ Inhibition in Forskolin-Stimulated ICₐ. When cardiomyocytes were exposed to 1 μM forskolin, forskolin increased basal ICₐ by approximately 64%, and under these conditions, additional application of ACh (3 μM) greatly reduced forskolin-stimulated ICₐ. External application of l-NMMA (100 μM) that further increased the ICₐ stimulated by 1 μM forskolin significantly attenuated the inhibitory action of ACh (1 μM; Fig. 8A). As shown in Fig. 8B, the changes in ACh (1 and 3 μM)-induced ICₐ decreases in the forskolin (1 μM)-stimulated condition before and after external application of l-NMMA.

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**Fig. 1.** NADPH-diaphorase activity of isolated rat ventricular myocytes. Cells exposed to nitroblue tetrazolium in the absence (A) or presence (B) of β-NADPH. The presence of β-NADPH caused a positive staining (dark blue) for NOS. Cells shown in A and B were isolated from the same heart. C, identification of ecNOS protein by Western blotting analysis in rat ventricular myocytes. The specific 130-kDa (ecNOS) protein is found in the myocytes.
NMMA at two concentrations (10 and 100 μM) were compared. L-NMMA attenuated the decrease in ICa induced by ACh application in a cAMP-stimulated condition due to forskolin.

**Effect of ACh on ICa Response to Simultaneous Application of Both L-NMMA and Forskolin.** Simultaneous application of both L-NMMA (100 μM) and forskolin (1 μM) potentiated basal ICa by approximately 73%, and additional application of ACh (6 μM) no longer attenuated ICa (Fig. 9A). No inhibitory action of ACh (6 μM) on ICa was observed in the presence of both L-NMMA and forskolin (Fig. 9B).

**Discussion**

This study provided evidence that L-NMMA, an NOS inhibitor, attenuated the ACh-induced ICa decrease in a
cAMP-stimulated condition due to forskolin, and that L-NMMA did not potentiate ICa when IBMX, a nonselective inhibitor in several PDEs, raised the cAMP level. Because the presence of NADPH-diaphorase assay and the expression of ecNOS protein (130 kDa) were identified in isolated rat cardiac myocytes, it is possible that inhibition of NOS blocks the cholinergic attenuation of ICa in a cAMP-stimulated condition and that this blocking action is mediated by inhibition of cGMP-stimulated cAMP-PDE, which can hydrolyze cAMP. This hypothesis was further confirmed by evidence demonstrating that both forskolin and milrinone potentiated the effect of L-NMMA on ICa, whereas the ICa response to
L-NMMA was not significantly altered by application of 8-Br-cGMP.

NADPH-diaphorase and NOS activities are thought to reflect different properties of the same enzyme (Hope et al., 1991), but NADPH-diaphorase assay has been used as a marker for NOS (Klimaschewski et al., 1992). In this study, the presence of NADPH-diaphorase activity was found in isolated rat cardiac myocytes. The expression of ecNOS protein (130 kDa) also was identified. The results are in agreement with a report showing that single myocytes obtained from rat ventricles can express the endothelial isofrm of NOS (Balligand et al., 1995).

Because the NOS inhibitor L-NMMA does not block muscarinic receptors commonly expressed in mammalian cardiovascular tissues (Buxton et al., 1993), one can expect that the appearance of L-NMMA effects on both the basal ICa and the ACh-induced ICa inhibition in a cAMP-stimulated condition is mediated by the blocking action of NOS. Because NOS influences the NO-cGMP pathway, the effect of L-NMMA on basal ICa would show opposite reactions compared with that from rat ventricles.
observed after application of NO donors. In isolated frog ventricular myocytes, the NO donor SIN-1 has no effect on basal ICa (Méry et al., 1993). In addition, Balligand et al. (1993) reported that carbachol, one of the muscarinic receptor agonists, elicited NO generation from cardiac myocytes, whereas molsidomine and nitroprusside failed to elicit NO generation. Matsumoto (1997), however, demonstrated that molsidomine, a precursor of SIN-1, concentration dependently inhibited the basal ICa amplitude. Furthermore, two NO-generating agents, isosorbide dinitrate and nitroprusside, in the absence of a cAMP-stimulated condition can reduce basal ICa in single myocytes from guinea pig ventricles (Yoshinaga, 1994). This difference may imply that products of endogenous NO are different from NO that is liberated by exogenous vasodilator drugs (Myers et al., 1990). Gallo et al. (1998) reported that the two NOS inhibitors, L-NMMA (1 mM) and N³-nitro-L-arginine (1 mM), caused a rapid increase in the ICa obtained from guinea pig ventricular myocytes. Presumably, conflicting data also are involved in the species differences. In this study, L-NMMA (10 and 100 µM) tended to increase ICa but this increase was not significant compared with the control ICa amplitude. When external application of forskolin increased intracellular cAMP levels, L-NMMA application (10 and 100 µM) resulted in a significant increase. This finding suggests that the stimulating effect of L-NMMA on basal ICa appears only when the level of cAMP is already high. Indeed, we found that L-NMMA (10 and 100 µM) caused a further stimulation of milrinone-enhanced ICa. Milrinone that is a specific inhibitor of the cGMP-inhibited cAMP-PDE (Nicholson et al., 1991) is expected to increase cAMP levels. These findings are basically consistent with a report demonstrating that inhibition of endogenous NO with NOS inhibitors potentiates the amplitude of shortening, in response to β-receptor agonists, of freshly isolated ventricular myocytes from normal rats (Balligand et al., 1993).

In the study to measure the rat cardiac ICa, external application of L-NMMA (1 mM) or internal dialysis with this NOS inhibitor at the same concentration attenuated the ACh-induced inhibition in the isoproterenol-stimulated ICa (Balligand et al., 1995). With guinea pig ventricular myocytes, Stein et al. (1993), however, reported that the increase in cGMP content and the contractile response to carbachol were not mediated by endogenous NO formation from L-arginine. In the same species, Zakharov et al. (1996) found that NOS inhibition did not result in any detectable change in the response of cAMP-regulated Cl⁻ current to ACh in a β-adrenergically stimulated Cl⁻ current. From these observations, the different types of ion channels and species differences may contribute to the conflicting data. Furthermore, there are conflicting data on the muscarinic regulation of β-adrenergically stimulated ICa in ecNOS knockout mice (Han et al., 1998; Vandecasteele et al., 1999). In this study, additional application of L-NMMA (100 µM) to the forskolin-stimulated ICa could attenuate ACh-induced inhibition, and simultaneous application of both forskolin (1 µM) and L-NMMA (100 µM) abolished the ICa response to ACh (6 µM). The cholinergic inhibition would occur as a result of the L-NMMA-induced ICa increase in a cAMP-stimulated condition. The cholinergic regulation of ICa in a cAMP-stimulated condition is thought to be mediated by two different biochemical pathways. In one pathway, PKG can reduce ICa in marmalian cardiac cells (Levi et al., 1989; Méry et al., 1991) and in the other pathway, activation of a cGMP-stimulated CAMP-PDE selectively breaks down cAMP (Méry et al., 1993). We found that the nonselective PDE inhibitor IBMX preventing the resulting reduction in cAMP levels had an inhibitory effect on L-NMMA-induced ICa change. This finding suggested that the cholinergic inhibition of ICa in a CAMP-stimulated condition is due to cGMP-stimulated CAMP-PDE, which can hydrolyze cAMP and inhibit cAMP-dependent phosphorylation of ICa channels. This suggestion was further confirmed because 8-Br-cGMP did not significantly alter the changes of ICa in response to L-NMMA application (10 and 100 µM). We also found that external application of 8-Br-cGMP could inhibit the L-NMMA-induced ICa increase in the simultaneous application of forskolin. Because 8-Br-cGMP selectively activates PKG and is insensitive to breakdown by PDE (Geiger et al., 1992; Lincoln and Cornwell, 1993), this finding probably implies that the inhibitory effect of 8-Br-cGMP on ICa in the presence of both L-NMMA and forskolin is independent of the cGMP-stimulated CAMP-PDE pathway. Accordingly, the data from this study lead us to suggest that the modification of both cholinergic and β-adrenergic influences caused by L-NMMA would not be mediated by the biochemical events subsequent to inhibition of PKG.

In the myocardium, a pertussis toxin-sensitive G protein, Gi, that is coupled to M₂ receptors may mediate the indirect action of ACh (Hescheler et al., 1986; Fischmeister and Shrier, 1989; Nakajima et al., 1990). Although high concentrations of the α subunit of Gi directly inhibit adenylyl cyclase, this inhibitory action is only partial and depends on the subtype of this enzyme (Taussig et al., 1993). The βγ-subunits from Gi directly inhibit the type I brain adenylyl cyclase, but these isoforms have not been identified in the heart (Tang and Gilman, 1991). The exact mechanism by which Gi inhibits cardiac calcium current remains to be determined.

In conclusion, our results demonstrate that the application of L-NMMA in a cAMP-stimulated condition could modify both basal ICa and ACh-induced ICa inhibition and that the induction of ecNOS protein expression occurs in isolated rat cardiac myocytes. These results suggest that ecNOSs in rat cardiac myocytes may mediate NO production.

References


