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Short communication

Does nitric oxide contribute to the negative chronotropic and inotropic effects of endothelin-1 in the heart?

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Abstract

N^{ω}-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, was used to examine whether nitric oxide was involved in the negative chronotropic and inotropic effects of endothelin-1 in the presence of isoprenaline in mammalian heart. In isolated rabbit right atria, endothelin-1 elicited a negative chronotropic effect in the presence of isoprenaline, which was associated with a decrease in the isoprenaline-induced accumulation of cyclic AMP. On the other hand, in the dog ventricular trabeculae, the negative inotropic effect of endothelin-1 was not accompanied by a significant reduction in the isoprenaline-induced accumulation of cyclic AMP. N^{ω}-nitro-L-arginine methyl ester affected neither the negative chronotropic effect nor the negative inotropic effect of endothelin-1. The effects of endothelin-1 on the isoprenaline-induced cyclic AMP accumulation were not influenced by N^{ω}-nitro-L-arginine methyl ester either. These results indicate that the negative chronotropic and inotropic effects of endothelin-1 in the presence of isoprenaline in mammalian cardiac muscle do not involve the nitric oxide-mediated signaling pathway. © 1997 Elsevier Science B.V.

Keywords: Nitric oxide (NO); Endothelin-1; cAMP

1. Introduction

Endothelin is a potent vasoactive peptide (Yanagisawa et al., 1988), which is produced and secreted mainly by vascular endothelial and endocardial cells (Haynes and Webb, 1993; Mebazaa et al., 1993; Rubanyi and Polokoff, 1994). It has been proposed that endothelin is involved in the regulation of cellular activity under various pathophysiological conditions (Rubanyi and Polokoff, 1994; Sakai et al., 1996). Endothelin has a pronounced positive inotropic effect in cardiac tissue from many species, such as the rat (Moravec et al., 1989; Reid et al., 1991), guinea pig, ferret, rabbit (Takanashi and Endoh, 1991, 1992; Watanabe et al., 1989) and human (Moravec et al., 1989). In isolated dog ventricular trabeculae, neither endothelin-1 nor endothelin-3 has a positive inotropic effect (Takanashi and Endoh, 1991; Yang et al., 1997), while it has been reported that endothelin-1 elicited a positive inotropic effect in the dog in vivo (Kitayoshi et al., 1989).

The chronotropic effect of endothelin shows also a wide range of species-dependent variation. In isolated guinea-pig atria, endothelin-1 elicits a positive chronotropic effect by itself (Ishikawa et al., 1988), but it has a negative chronotropic effect in the presence of β-adrenoceptor stimulation, which is mediated by an inhibition of adenylyl cyclase by activation of endothelin ET_A receptor through coupling to a pertussis toxin-sensitive G protein (James et al., 1994; Ono et al., 1994, 1995).

Endothelin-1 has also been shown to cause the release of a potent vasodilator, namely, endothelium-derived nitric oxide. Recently, Brady et al. (1993) showed that the contractility of guinea-pig cardiac myocytes was attenuated by nitric oxide released from endothelium and this attenuation of contractility could be abolished by nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, indicating that nitric oxide may have an important effect on myocardial contractility in guinea-pig heart. The mRNA of endothelial type nitric oxide synthase has been shown to be expressed abundantly in rat atrial as well as ventricular cardiomyocytes (Seki et al., 1996), supporting the role of nitric oxide in the regulation of cardiac functions. Han et

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al. (1994) found that nitro-L-arginine methyl ester could block the carbachol-induced inhibition of L-type Ca^{2+} current that had been augmented by β-adrenoceptor stimulation in isolated spontaneously beating rabbit sinoatrial node cells. Thus, it has been suggested that an nitric oxide-mediated signaling pathway might be involved in the cholinergic inhibition of cardiac function (Balligand et al., 1993).

The present study was carried out to examine the mode of interaction of endothelin-1 with the chronotropic or inotropic response to β-adrenoceptor stimulation and the role of nitric oxide in the interaction in the mammalian heart.

2. Materials and methods

2.1. Isolated right atria and ventricular trabeculae

Male albino rabbits (1.8–2.2 kg) and mongrel dogs (7–10 kg) were anesthetized with pentobarbital sodium (50 mg/kg in rabbit and 30 mg/kg in dog, i.v.). Then the heart of each was removed and the right atrium was excised from the rabbit heart and the ventricular trabeculae were excised from the right ventricle of the dog. Atria and ventricular trabeculae were mounted in 20 ml organ baths that contained Krebs–Henseleit solution bubbled with 95% O_2 and 5% CO_2 at 37°C (pH 7.4). The ventricular trabeculae were electrically stimulated at a frequency of 0.5 Hz with a pulse duration of 5 ms and a voltage 20% above the threshold (approx. 0.4 V). The spontaneous beating rate of atria of the rabbit was determined from the number of contractions in actual tracings at a high paper speed. The preparations of dog ventricular trabeculae had the average dimension of 4.67 ± 0.22 mm in length and 1.26 ± 0.06 mm^2 in cross-sectional area ($n = 15$).

Prazosin (300 nM) was allowed to act for 30 min before the addition of isoprenaline and it was present in the organ bath throughout the experiments. After the response to a single concentration of isoprenaline became stable, isoprenaline was added again and allowed to act until a steady response had been achieved, namely, for 5 min in the case of atria and for 15 min in the case of ventricular trabeculae, before the addition of endothelin-1. Endothelin-1 was administered at a single concentration of 10 nM to each preparation. N^{ω}-nitro-L-arginine methyl ester, an inhibitor of nitric oxide synthase, was used to examine whether nitric oxide was involved in the negative chronotropic and inotropic effects of endothelin-1 in the presence of isoprenaline. N^{ω}-nitro-L-arginine methyl ester (100 μM) was administered 20 min before the addition of isoprenaline and it was present in the organ bath throughout the experiments. The chronotropic and inotropic responses to isoprenaline and endothelin-1 were expressed as percentages of the maximal response to isoprenaline in each preparation.

2.2. Quantitation of cyclic AMP

The levels of cyclic AMP in rabbit right atria and dog ventricular trabeculae were determined as described previously (Kawabata and Endoh, 1995). In brief, atria and ventricular trabeculae were treated under the same experimental conditions as described above. The preparations were removed from the organ bath 10 min (in atria) or 15 min (in ventricular trabeculae) after the administration of 10 nM endothelin-1, when the chronotropic or inotropic response had reached a steady level. They were frozen rapidly in liquid nitrogen, weighed immediately and stored overnight at −80°C. After addition of 0.5 ml of ice-cold 6% trichloroacetic acid, each frozen preparation was homogenized mechanically by means of microdismembrator (B. Braun, Melsungen, Germany). The homogenate was thawed and centrifuged at $6000 \times g$ for 10 min and after addition of 10 μl of 1 M HCl, 100 μl aliquots of the supernatant were extracted five times with 1 ml of water-saturated ether and dried with air at 40°C overnight. The each dried sample was resuspended in 100 μl of distilled water. The level of cyclic AMP was determined by a sensitive radioimmunoassay method (cyclic AMP kit, Yamasa Shoyu, Choshi, Japan).

2.3. Statistics

Experimental values are presented as means ± S.E.M. Significant differences between mean values were estimated by a repeated measures analysis or one-way analysis of variance by using analytical software STATVIEW J-4.5 (Abacus Concepts, Berkeley, CA, USA). A P value smaller than 0.05 was considered to be significant.

2.4. Drugs

The drugs used were endothelin-1 (Peptide Institute, Osaka, Japan), (−)-isoprenaline hydrochloride and N^{ω}-nitro-L-arginine methyl ester (Sigma, St. Louis, MO, USA), prazosin hydrochloride (Pfizer Taito, Tokyo, Japan) and pentobarbital sodium (Tokyo Kasei, Tokyo, Japan).

3. Results

3.1. Influence of the nitric oxide synthase inhibitor on the negative chronotropic effect of endothelin-1

In spontaneously beating right atria from rabbits, after an equilibration period of 1 h, the basal spontaneously beating rate was 156.6 ± 2.9 beats/min ($n = 15$) and remained stable for 1 h and endothelin-1 at 10 nM did not affect significantly the beating rate (data not shown). Isoprenaline at 30 nM rapidly increased the beating rate. The response reached a peak level of 196.8 ± 5.0% of the basal

rate 5 min after the administration of isoprenaline, and it was sustained for 1 h in the presence of isoprenaline. The extent of the spontaneous reduction in rate 1 h after the addition of isoprenaline was not significant ($3.9 \pm 1.8\%$ of the maximum response; $n = 5$). Endothelin-1 at 10 nM elicited a negative chronotropic effect in the presence of isoprenaline (Fig. 1A). The extent of the negative chronotropic effect 3 min after the addition of endothelin-1 was $16.8 \pm 3.6\%$ of the maximum response ($n = 4$ each). At 10–20 min, the negative chronotropic effect of endothelin-1 reached the maximum level ($25.6 \pm 4.0\%$). N^{ω}-nitro-L-arginine methyl ester (100 μM) itself did not have any direct chronotropic effect and it did not significantly alter the negative chronotropic effect of endothelin-1 (Fig. 1A).

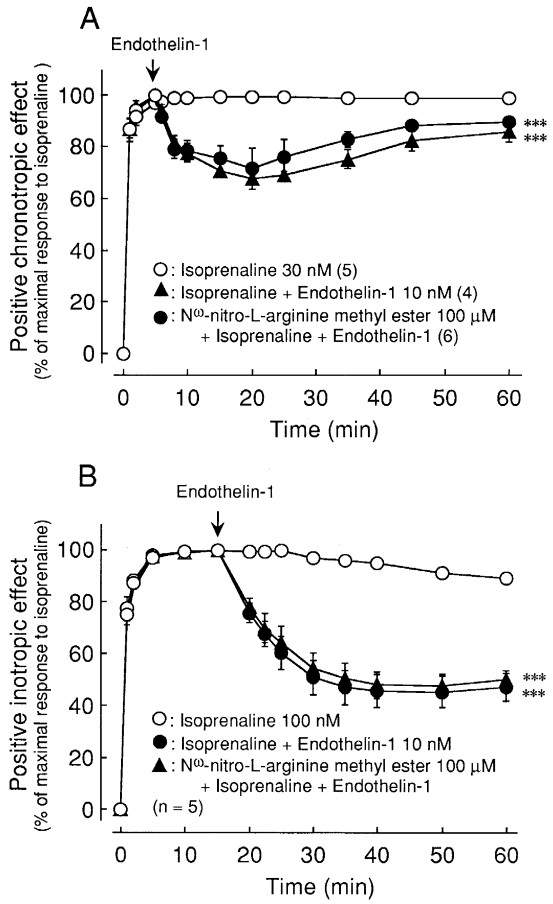

Fig. 1. Influence of N^{ω}-nitro-L-arginine methyl ester on the negative chronotropic effect of endothelin-1 in right atria isolated from rabbits (A) and on the negative inotropic effect of endothelin-1 in ventricular trabeculae from dogs (B), both in the presence of prazosin and isoprenaline. In Fig. 1A, the basal heart rate (HR) was 156.6 ± 2.9 beats/min ($n = 15$). The maximum increase in the heart rate after application of isoprenaline (30 nM) was 153.8 ± 3.7 beats/min ($n = 15$). The maximum increase in each preparation was taken as 100%. In Fig. 1B, the baseline force of contraction and the maximum response to 100 nM isoprenaline were 3.75 ± 0.67 and 14.09 ± 2.10 mN/mm^2, respectively. The maximum response to 100 nM isoprenaline before the addition of endothelin-1 (10 nM) was taken as 100% for each preparation, and the changes in the force of contraction are expressed as percentages of the control response. (***) $P < 0.001$ vs. the control group.

3.2. Influence of the nitric oxide synthase inhibitor on the negative inotropic effect of endothelin-1

The basal contractile force was stable for 1 h after the equilibration period of 1 h and endothelin-1 by itself did not significantly affect the contractile force in isolated dog right ventricular trabeculae as reported previously (Takanashi and Endoh, 1991). However, endothelin-1 had a pronounced negative inotropic effect in the presence of isoprenaline. Isoprenaline (100 nM) elicited a positive inotropic effect that reached a peak level 10 to 15 min after the administration and was sustained with a slight but significant decrease over a 1 hour period: the positive inotropic effect of isoprenaline declined spontaneously by $4.7 \pm 1.4\%$ ($P > 0.05$, $n = 5$) at 45 min, and at 60 min it declined by $10.3 \pm 1.7\%$ of the maximum level in control preparations ($P < 0.01$). Endothelin-1 (10 nM), administered 15 min after the application of isoprenaline, induced a significant negative inotropic effect (Fig. 1B). The extent of the reduction at 5 min after the addition of endothelin-1 was $24.0 \pm 3.6\%$ ($P < 0.001$), and at 20 min it was $52.8 \pm 6.4\%$ ($P < 0.001$). N^{ω}-nitro-L-arginine methyl ester (100 μM) did not have any effect on the negative inotropic effect of endothelin-1 in the presence of isoprenaline (Fig. 1B).

3.3. Influence of the nitric oxide synthase inhibitor on the actions of endothelin-1 on the cyclic AMP

Table 1 shows the influence of N^{ω}-nitro-L-arginine methyl ester on the action of endothelin-1 on the isoprenaline-induced increase in the level of cyclic AMP in right atria from rabbits. Isoprenaline (30 nM) increased the level of cyclic AMP to 2.9 × the control level, 15 min after the administration. Endothelin-1 (10 nM) significantly reduced the isoprenaline-induced increase in the level of cyclic AMP at 10 min after the addition. N^{ω}-nitro-L-arginine methyl ester by itself did not significantly affect the basal level or the isoprenaline-stimulated accumulation of cyclic AMP either (data not shown). Pretreatment with 100 μM N^{ω}-nitro-L-arginine methyl ester did not alter the inhibitory action of endothelin-1 on the isoprenaline-stimulated accumulation of cyclic AMP.

In ventricular trabeculae from dogs, the level of cyclic AMP was also markedly increased by isoprenaline (100 nM) and remained elevated for at least 30 min. Endothelin-1 (10 nM) was added 15 min after the application of isoprenaline and the level of cyclic AMP was measured 15 min after the administration of endothelin-1. Endothelin-1 did not significantly affect the level of cyclic AMP that had previously been elevated by isoprenaline. N^{ω}-nitro-L-

Table 1
Effects of endothelin-1 on the isoprenaline-induced increase in cyclic AMP, and the influence of N^{ω}-nitro-L-arginine methyl ester on the action of endothelin-1 in isolated right atria from rabbits and ventricular trabeculae from dogs

	n	Cyclic AMP (pmol/mg wet wt)
Right atria from rabbits		
Basal	4	3.21 ± 0.11
Isoprenaline (30 nM)	5	7.92 ± 0.28 [a]
Isoprenaline + endothelin-1 (10 nM)	6	5.56 ± 0.57 [a,b]
N^{ω}-nitro-L-arginine methyl ester (100 μM) + isoprenaline + endothelin-1 (10 nM)	4	5.63 ± 0.54 [a,b]
Ventricular trabeculae from dogs		
Basal	6	3.05 ± 0.58
Isoprenaline (100 nM)	5	7.48 ± 0.67 [a]
Isoprenaline + endothelin-1 (10 nM)	6	8.48 ± 1.29 [a]
N^{ω}-nitro-L-arginine methyl ester (100 μM) + isoprenaline + endothelin-1	4	7.49 ± 0.89 [a]

Values are means ± SE.; *n* numbers of muscle preparations.
[a] $P < 0.001$ vs. the respective basal values.
[b] $P < 0.001$ vs. the corresponding value with isoprenaline (30 nM) alone.

arginine methyl ester did not affect the level of cyclic AMP in the presence of isoprenaline and endothelin-1 either (Table 1).

4. Discussion

In isolated rabbit right atria, endothelin-1 had a negative chronotropic effect in the presence of isoprenaline, which was associated with a decrease in the isoprenaline-induced accumulation of cyclic AMP. These results are essentially consistent with the previous findings in guinea-pig atria, indicating that a pertussis toxin-sensitive G protein/adenylyl cyclase inhibitory pathway is partly responsible for the negative chronotropic effect of endothelin-1 (Ono et al., 1995). The nitric oxide-mediated signaling pathway has been shown to be involved in cholinergic inhibition of the spontaneous beating of rat (Balligand et al., 1993) and rabbit (Han et al., 1994) cardiac myocytes. N^{ω}-monomethyl-L-arginine, an inhibitor of nitric oxide synthase and methylene blue, an inhibitor of guanylyl cyclase, both blocked the negative chronotropic effect of carbachol (Balligand et al., 1993). N^{ω}-monomethyl-L-arginine and N^{ω}-nitro-L-arginine methyl ester have also been shown to block the carbachol-induced inhibition of L-type calcium current that had been enhanced by β-adrenoceptor stimulation (Han et al., 1994). In the present study, N^{ω}-nitro-L-arginine methyl ester (100 μM) did not influence the negative chronotropic effect of endothelin in the presence of isoprenaline, an indication that the nitric oxide-mediated signaling pathway is not involved in the negative chronotropic effect of endothelin in rabbit right atria. These findings are essentially consistent with those of Nawrath et al. (1995) that nitric oxide has no direct effects on force of contraction in atrial myocardium isolated from rats, rabbits, guinea pigs, frogs and humans. It appears to be evident that in mammalian atrial muscle nitric oxide does not play a role in regulation of the function (Nawrath et al., 1995).

It is noteworthy that, in dog ventricular trabeculae, the negative inotropic effect of endothelin-1 was not accompanied by a significant reduction in the accumulation of cyclic AMP. These results imply that the inhibitory effect of endothelin was exerted independently of the accumulation of cyclic AMP.

It has been reported that endothelin-1 causes the release of endothelium-derived nitric oxide, a potent vasodilator. Nitric oxide stimulates soluble guanylyl cyclase, thereby increasing intracellular levels of cyclic GMP, with a subsequent reduction in intracellular levels of Ca^{2+} ions in vascular smooth muscle cells. Recently, Brady and coworkers showed that the contractility of guinea-pig isolated cardiac myocytes could be attenuated by generation of nitric oxide from endothelium in normal (Brady et al., 1993) and from myocytes themselves in endotoxin-induced shock (Brady et al., 1992) animals. The attenuation of contractility of cardiac myocytes by endogenously produced nitric oxide could be abolished by inhibitors of either nitric oxide synthase or guanylyl cyclase (Brady et al., 1992, 1993). In the present study, N^{ω}-nitro-L-arginine methyl ester (100 μM) had no effect on the negative inotropic effect of endothelin-1, an indication that the nitric oxide-mediated inhibitory pathway is not involved in the negative inotropic effect of endothelin-1 in the dog ventricular myocardium. These observations imply that the mechanism underlying the inhibitory actions of endothelin-1 on cardiac function shows a wide range of species-dependent variation among the mammalian heart.

In conclusion, the following observations were made in the present study: (1) endothelin-1 had a negative chronotropic effect in rabbit atria and a negative inotropic effect of isoprenaline in dog ventricular trabeculae both in the presence of isoprenaline; (2) nitric oxide is not considered to be involved in the negative chronotropic and inotropic effect of endothelin-1 in the heart of these species.

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