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Synergism between low-dose nicorandil and neuropeptides on adenosine-induced vasodepression in rats

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Abstract

We hypothesized that the effect of nicorandil may be enhanced by interaction with naturally occurring vasodilators. To clarify this hypothesis, the effects of low-dose nicorandil alone and in combination with low doses of vasoactive intestinal polypeptide (VIP) or calcitonin gene-related peptide (CGRP) on adenosine-induced vasodepression were studied in rats. Intravenous (i.v.) bolus injections of adenosine (3–100 μg kg^{-1}) elicited dose-dependent decreases in blood pressure, accompanied by slight decreases (except for 100 μg kg^{-1}) in heart rate. Simultaneous i.v. infusion of either nicorandil (1 μg kg^{-1} min^{-1}) and VIP (0.003 μg kg^{-1} min^{-1}) or CGRP (0.1 ng kg^{-1} min^{-1}) significantly enhanced the adenosine-induced vasodepression, although each agent alone in the dose used had no effects on vasodepressor responses to adenosine. The potentiation of the effect of adenosine was not observed in the presence of 3,7-dimethyl-1-propargylxanthine (DMPX) (1 mg kg^{-1}, i.v.) or glibenclamide (20 mg kg^{-1}, i.v.). The present results suggest that low-dose nicorandil modifies the response to adenosine in interaction with low levels of endogenous neuropeptides such as VIP and CGRP, and that the reciprocal interaction is mediated partly through K_{ATP} channel activation in vascular smooth muscle.

Keywords: Nicorandil; VIP (vasoactive intestinal polypeptide); CGRP (calcitonin gene-related peptide); Adenosine-induced vasodepression; K_{ATP} channel

1. Introduction

Nicorandil [*N*-(2-hydroxyethyl)nicotinamide nitrate ester] is an orally effective anti-anginal drug (Sakai, 1989; Kinoshita and Sakai, 1990; Krumenacker and Roland, 1992) which possesses a dual mechanism of action, combining K_{ATP} channel activating properties and stimulation of guanylate cyclase (Taira, 1989). Recently, we have demonstrated that this drug enhances the vasodepression induced by several naturally occurring vasodilators such as adenosine, vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP), at least partly through K_{ATP} channel activation (Sakai et al., 1998a; Saito and Sakai, 1998a). It is well known that adenosine, a metabolite of adenine nucleotides, exerts a variety of functions in various organs, especially in ischemic states (Hori and Kitakaze, 1991; Mubagwa et al., 1996), and also that neuropeptides such as VIP and CGRP seem to regulate the physiological control of vascular tone and blood flow, through activation of a network of signal transduction pathways (Ignarro et al., 1987; Rubino and Burnstock, 1996). Even though we found evidence of synergistic effects between either nicorandil and VIP, CGRP (Sakai et al., 1998a) or adenosine (Saito and Sakai, 1998a) on vasodepression in rats, it has not yet been investigated whether the effect of nicorandil can be amplified by an interaction with endogenous vasodilators such as VIP, CGRP or adenosine. To examine this possibility, the present study was undertaken to compare the effectiveness of low to high doses of nicorandil alone with that of low doses of nicorandil combined with low doses of VIP or CGRP on adenosine-induced vasodepression in rats.

2. Materials and methods

2.1. Chemicals

The chemicals used were: nicorandil [*N*-(2-hydroxyethyl)nicotinamide nitrate ester] (Chugai, Tokyo, Japan), vasoactive intestinal polypeptide (VIP) (human, porcine), calcitonin gene-related peptide (CGRP) (human) (both

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Peptide Institute, Osaka, Japan), adenosine free base (Sigma, St. Louis, MO, USA), acetylcholine chloride, 3,7-dimethyl-1-propargylxanthine (DMPX) (both RBI, Natick, MA, USA), and glibenclamide (Wako Junyaku, Osaka, Japan). CGRP was dissolved in distilled water and diluted with 0.45% saline solution, according to the report of Elhawary et al. (1995). Glibenclamide was dissolved in 1 ml of 0.1 M NaOH, followed by slow addition of 4 ml of 5% glucose solution under sonication to reach a final concentration of 5 mg ml^{-1} (Furukawa et al., 1993). Other compounds were dissolved in and diluted with 0.9% saline solution.

2.2. Animal preparations

All experiments were carried out according to the Regulations of the Animal Research Committee of the Chugai Pharmaceutical, Tokyo, Japan. Male Sprague–Dawley rats (Charles River Japan, Atsugi, Kanagawa) weighing 405.0 $\pm$ 4.1 g ($n = 90$) were allowed free access to food and water. The rats were anesthetized initially with pentobarbital-Na (55 mg kg^{-1}, i.p.) and an additional dose of pentobarbital (40 mg kg^{-1}) was injected subcutaneously (s.c.), as required. Polyethylene tubes (PE 10) were inserted into peripheral veins, the left jugular and femoral veins for intravenous (i.v.) drug infusions, and the right femoral vein for i.v. bolus drug injections. For i.v. bolus injection, 0.2 ml kg^{-1} of the drug solutions was given over a period of approximately 10 s and then flushed in with 0.9% saline solution. For i.v. infusion, drug solutions were given at a rate of 0.1 ml kg^{-1} min^{-1} by means of a Terumo syringe pump (STC-525, Tokyo, Japan). Arterial blood pressure was measured from the right femoral artery by means of a Nihon Kohden pressure transducer (DX-360, Tokyo, Japan). Heart rate was measured by means of a heart rate counter (Nihon Kohden, AT-601G). All recordings were made on a chart by using a Graphtec Linearcorder (WR-3101, Tokyo, Japan). Following surgery, a period of at least 30 min was allowed for stabilization of preparations.

2.3. Experimental protocols

The experiments were performed in seven sets, as shown in Fig. 1. In the 1st set of the experiment, five animals were used to record the dose–response curve for adenosine (3–100 μg kg^{-1}, i.v.) on blood pressure before and during i.v. infusion of 0.9% saline solution (0.1 ml kg^{-1} min^{-1}). Thereafter, glibenclamide (20 mg kg^{-1}, 4 ml kg^{-1}) was given i.v. over 5 min, and then the dose–response curve for adenosine was recorded again. In the 2nd, 3rd and 4th sets of the experiments, the animals were divided into 3–4 groups (each $n = 5$), and the dose–response curves for adenosine on blood pressure were recorded before and during i.v. infusion of either nicorandil (1, 3, 10 or 30 μg kg^{-1} min^{-1}) (2nd set), VIP (0.003, 0.01 or 0.03 μg kg^{-1} min^{-1}) (3rd set) or CGRP (0.1, 0.3 or 1 ng kg^{-1} min^{-1}) (4th set). After the effects of the lowest doses of nicorandil (1 μg kg^{-1} min^{-1}), VIP (0.003 μg kg^{-1} min^{-1}) and CGRP (0.1 ng kg^{-1} min^{-1}) were tested on adenosine-induced vasodepression, glibenclamide (20 mg kg^{-1}) was given i.v. over 5 min, and then i.v. infusion of the combination of either nicorandil (fixed on 1 μg kg^{-1} min^{-1}) and VIP (0.003 μg kg^{-1} min^{-1}) or CGRP (0.3 ng kg^{-1} min^{-1}) was started. Twenty minutes later, the vasodepressor effects of adenosine were examined again. The dose of glibenclamide was enough to completely prevent the vasodepressor response to a single bolus i.v. dose of cromakalim (30 μg kg^{-1}), which reduces mean arterial blood pressure by about 40 mmHg in rats (Saito and Sakai, 1998a). In the 5th and 6th sets of the experiments, the animals were divided into three groups (each $n = 5$) and

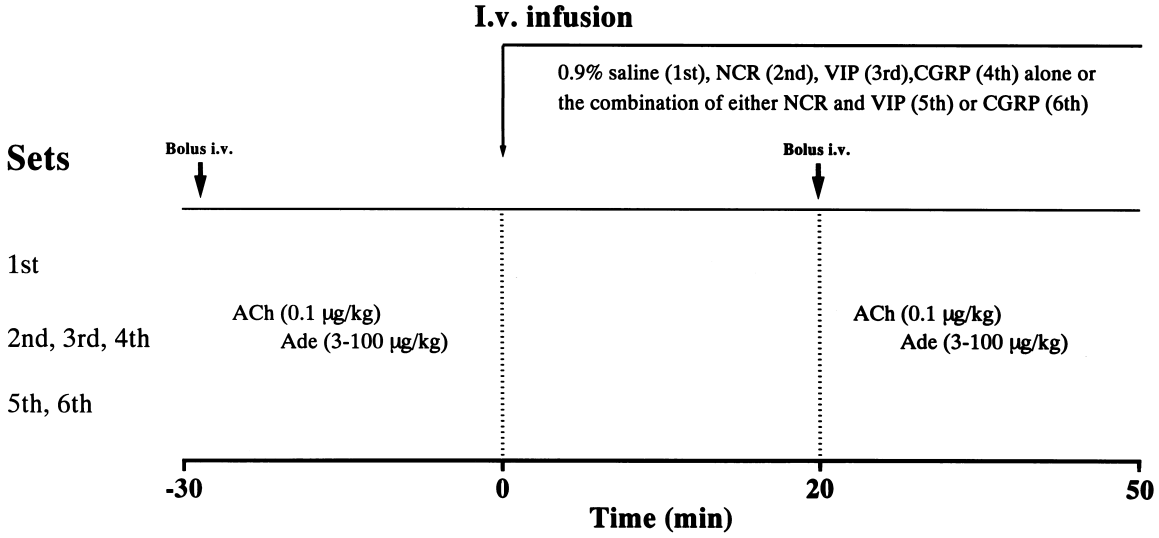

Fig. 1. Experimental protocols. NCR, nicorandil; ACh, acetylcholine; Ade, adenosine. See Section 2 for more details.

the dose–response curves for adenosine on blood pressure before and during simultaneous i.v. infusion of either nicorandil and VIP or CGRP were recorded. Either nicorandil (1 μg kg^{-1} min^{-1}) and VIP (0.001, 0.003 or 0.01 μg kg^{-1} min^{-1}) or CGRP (0.03, 0.1 or 0.3 ng kg^{-1} min^{-1}) were combined and infused i.v. simultaneously. In the 7th set of the experiment, five animals were used. Just after the dose–response curve for adenosine on blood pressure was obtained, a single bolus dose of DMPX (1 mg kg^{-1}) was given i.v. over 1 min. About 10 min later, the inhibitory effect of DMPX on the response to a single bolus i.v. injection of adenosine was examined, and then simultaneous i.v. infusion of nicorandil (1 μg kg^{-1} min^{-1}) and CGRP (0.3 ng kg^{-1} min^{-1}) was started. Twenty minutes later the dose–response curve for adenosine was recorded again. It has already been shown (Saito and Sakai, 1998c) that 1 mg kg^{-1} DMPX significantly prevents the vasodepression induced by 5′-(*N*-cyclopropyl)-carboxamidoadenosine (CPCA), a selective adenosine A_2 receptor agonist (Bruns et al., 1986). The i.v. infusions of these agents at the rates used, and the i.v. treatments with DMPX and glibenclamide had no influence on basal arterial blood pressure and heart rate. In principle, the time-matched i.v. administration of adenosine was done as follows: following a single bolus i.v. injection of acetylcholine (0.1 μg kg^{-1}), the dose–response curve for adenosine (3–100 μg kg^{-1}, i.v.) was recorded, and then i.v. infusion of the agents was started. Twenty minutes later the effects of acetylcholine and adenosine were examined again in that order.

2.4. Statistical analysis

Values in the text are presented as means ± S.E.M. Peak vasodepressor responses to the agents are expressed as the changes from the preadministration levels. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during/after i.v. treatment with 0.9% saline, nicorandil, VIP, CGRP alone and their combinations, and DMPX and glibenclamide were calculated from individual dose–response curves for adenosine (Tables 1–3), as described elsewhere (Sakai and Saito, 1998). Differences between paired or unpaired mean values were analyzed by Student's *t*-test. Analysis of variance (ANOVA) was used for the statistical analysis of multiple comparisons of data. When multiple comparisons were made with a single control, Dunnett's test was used to determine the level of statistical significance. A *P*-value less than 0.05 was considered to be statistically significant.

Table 1
The values given are the doses of adenosine required to decrease mean arterial blood pressure by 20 mmHg

Intravenous infusion (μg kg^{-1} min^{-1})	Intravenous bolus adenosine (μg kg^{-1})	
	Before	During
Saline	36.8 ± 3.4	35.3 ± 4.5
Nicorandil		
1	35.8 ± 3.1	37.3 ± 3.5
3	40.3 ± 4.6	39.0 ± 6.1
10	39.2 ± 4.1	20.7 ± 4.7[a]
30	42.3 ± 5.0	7.7 ± 2.0[c]

Values represent means ± S.E.M. (each $n = 5$).
[a] $P < 0.05$, [c] $P < 0.001$, compared with the corresponding values from the control (before) group.
The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during i.v. infusion of 0.9% saline or nicorandil solution were calculated from individual dose–response curves for adenosine. See Section 2.

Table 2
The values given are the doses of adenosine required to decrease mean arterial blood pressure by 20 mmHg

Intravenous infusion (μg kg^{-1} min^{-1})	Intravenous bolus adenosine (μg kg^{-1})	
	Before	During
VIP		
0.01	35.8 ± 3.1	38.7 ± 4.5
0.03	36.9 ± 3.3	20.4 ± 3.1[b]
Nicorandil (1 μg kg^{-1} min^{-1})		
0.001	40.2 ± 6.8	38.5 ± 4.0
0.003	37.8 ± 3.5	21.4 ± 1.5[b]
Glibenclamide (20 mg kg^{-1}) + Nicorandil (1 μg kg^{-1} min^{-1})		
0.003	35.5 ± 4.8	100 <

Values represent means ± S.E.M. (each $n = 5$).
[b] $P < 0.01$ (vs. before).
The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during i.v. infusion of VIP alone and in combination with nicorandil, in the absence and presence of glibenclamide, were calculated from individual dose–response curves for adenosine.

Table 3
The values given are the doses of adenosine required to decrease mean arterial blood pressure by 20 mmHg

Intravenous infusion (ng kg^{-1} min^{-1})	Intravenous bolus adenosine (μg kg^{-1})	
	Before	During
CGRP		
0.1	40.7 ± 6.1	34.5 ± 4.7
0.3	40.3 ± 4.6	33.4 ± 4.7
1.0	36.9 ± 3.5	18.7 ± 2.3[b]
Nicorandil (1 μg kg^{-1} min^{-1})		
0.03	42.2 ± 6.8	32.6 ± 5.8
0.1	38.2 ± 4.1	23.4 ± 3.1[a]
0.3	38.3 ± 4.5	23.7 ± 1.5[a]
DMPX (1 mg kg^{-1}) + Nicorandil (1 μg kg^{-1} min^{-1})		
0.3	35.5 ± 4.8	81.2 ± 5.4[c]
Glibenclamide (20 mg kg^{-1}) + Nicorandil (1 μg kg^{-1} min^{-1})		
0.3	41.0 ± 4.5	100 <

Values represent means ± S.E.M. (each $n = 5$).
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ (vs. before).
The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during i.v. infusion of CGRP alone and in combination with nicorandil, in the absence and presence of DMPX or glibenclamide, were calculated from individual dose–response curves for adenosine.
DMPX: 3,7-dimethyl-1-propargylxanthine.

3. Results

Baseline mean arterial blood pressure and heart rate in all of the rats tested ($n = 90$, all sets) were as follows: 118 ± 2 mmHg and 416 ± 4 beats min^{-1}, respectively, just before the first i.v. injection of agents; 110 ± 2 mmHg and 402 ± 5 beats min^{-1}, respectively, just before the first i.v. injection of agents following the start of the i.v. treatment with either 0.9% saline, nicorandil, VIP, CGRP, and their combinations, and DMPX and glibenclamide. No significant differences were observed between the corresponding values. Thus, the preparations remained stable throughout the entire experimental period, with little change in basal arterial blood pressure and heart rate being recorded even after these agents were administered.

Intravenous bolus injections of adenosine (3–100 $\mu g\ kg^{-1}$) produced dose-dependent decreases in blood pressure, accompanied by a slight decrease (less than 10% except for 100 $\mu g\ kg^{-1}$) in heart rate, as represented in Fig. 2. The vasodepression caused by adenosine remained unchanged before (control) and during i.v. infusion of 0.9% saline solution (Table 1) (1st set).

3.1. Effects of nicorandil on adenosine-induced vasodepression (2nd set)

Just after the dose–response curve for bolus i.v. adenosine (3–100 $\mu g\ kg^{-1}$) was recorded, i.v. infusion of nicorandil at a rate of either 1, 3, 10 or 30 $\mu g\ kg^{-1}\ min^{-1}$ was started. During the infusion of nicorandil at rates of 10 and 30 $\mu g\ kg^{-1}\ min^{-1}$, the vasodepression elicited by adenosine, unlike that elicited by acetylcholine (0.1 $\mu g\ kg^{-1}$) (data not shown), was significantly augmented but was hardly affected by i.v. infusion at either 1 or 3 $\mu g\ kg^{-1}\ min^{-1}$. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during the nicorandil infusion are presented in Table 1. The changes in heart rate caused by adenosine were not significantly modified by i.v. infusion of nicorandil (data not shown). On the basis of the finding that the vasodepressor responses to adenosine were not modified by i.v. infusion of 1 or 3 $\mu g\ kg^{-1}\ min^{-1}$, the i.v. infusion rate of nicorandil used in the combination experiment was 1 $\mu g\ kg^{-1}\ min^{-1}$.

3.2. Effects of VIP on adenosine-induced vasodepression (3rd set)

Before and during i.v. infusion of VIP (0.003, 0.01 or 0.03 $\mu g\ kg^{-1}\ min^{-1}$), the vasodepressor effects of bolus i.v. adenosine (3–100 $\mu g\ kg^{-1}$) were compared. After the dose–response curve for adenosine was recorded, the infusion of VIP was started. The infusion rates of VIP did not affect the basal arterial blood pressure (e.g., 0.03 $\mu g\ kg^{-1}\ min^{-1}$: before, 120 ± 4 mmHg; 20 min after the start of infusion, 113 ± 5 mmHg; $n = 5$) and heart rate (before, 428 ± 14 beats min^{-1}; 20 min after the start of infusion, 402 ± 9 beats min^{-1}; $n = 5$). As shown in the left part of Fig. 3, i.v. infusion of VIP at a rate of 0.03 $\mu g\ kg^{-1}\ min^{-1}$ significantly shifted the dose–response curve for adenosine to the left, but the lower doses hardly affected the curve. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during the VIP infusion were calculated and are presented in Table 2.

3.3. Effects of CGRP on adenosine-induced vasodepression (4th set)

Immediately after the vasodepressor responses to adenosine (3–100 $\mu g\ kg^{-1}$) were examined, i.v. infusion of CGRP (0.1, 0.3 or 1 ng $kg^{-1}\ min^{-1}$) was started. These infusion rates of CGRP had no influence on basal arterial blood pressure (e.g., 1 ng $kg^{-1}\ min^{-1}$: before, 116 ± 6 mmHg; 20 min after the start of infusion, 110 ± 5 mmHg; $n = 5$) and heart rate (before, 420 ± 15 beats min^{-1}; 20 min after the start of infusion, 414 ± 13 beats min^{-1}; $n = 5$). During the CGRP infusion at a rate of 1 ng $kg^{-1}\ min^{-1}$, the vasodepressor response to adenosine was significantly enhanced, while it virtually remained unchanged

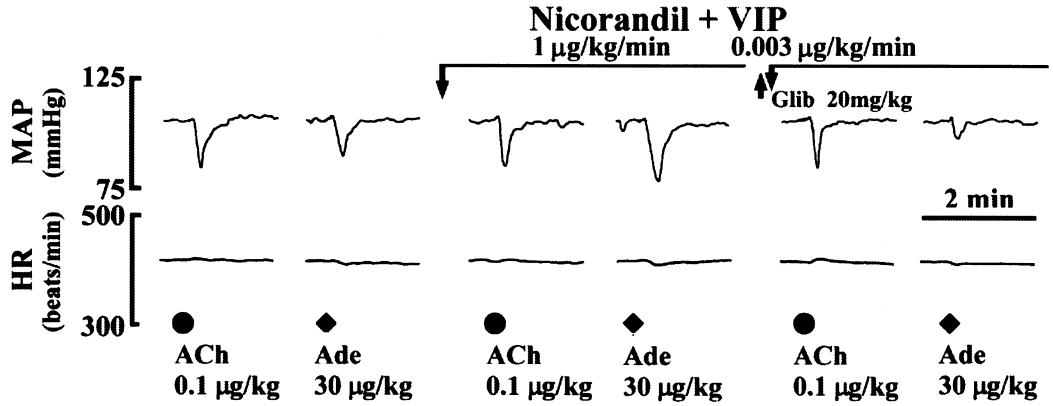

Fig. 2. Changes in mean arterial blood pressure (MAP) and heart rate (HR) elicited by bolus i.v. injections of acetylcholine (ACh) and adenosine (Ade) before and during simultaneous i.v. infusion of nicorandil and VIP in the absence or the presence of glibenclamide (Glib).

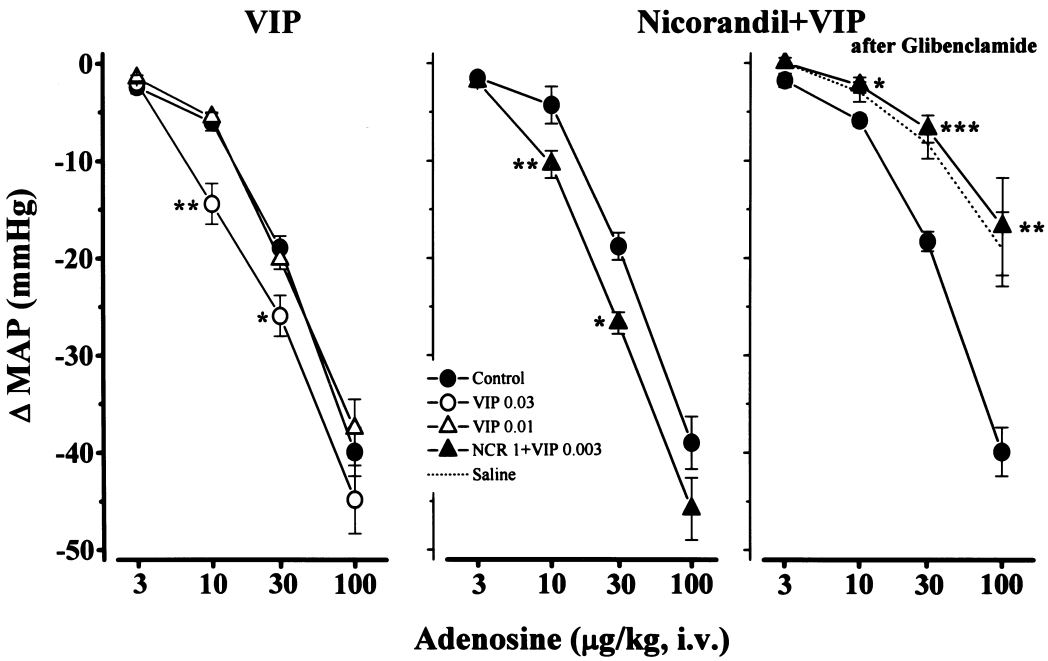

Fig. 3. Dose–response curves for peak decreases in mean arterial blood pressure (MAP) caused by bolus i.v. injections of adenosine before (control) and during i.v. infusion (μg kg^{-1} min^{-1}) of VIP alone (left part) and in combination with nicorandil (NCR) in the absence (middle part) or the presence (right part) of glibenclamide (20 mg kg^{-1} i.v. over 5 min). Vertical bars present means ± S.E.M. from five animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the corresponding values from the control group (●).

during infusion of CGRP at lower doses. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during the infusion of CGRP are summarized in Table 3.

3.4. Effects of simultaneous infusion of low-dose nicorandil and VIP on adenosine-induced vasodepression (5th set)

Just after the dose–response curve for adenosine (3–100 μg kg^{-1}, i.v.) was recorded, nicorandil (1 μg kg^{-1} min^{-1}) and VIP (0.003 μg kg^{-1} min^{-1}), which did not affect the basal arterial blood pressure and heart rate, were concomitantly infused i.v. As depicted in Fig. 2 and in the middle part of Fig. 3, the vasodepressor response to adenosine, unlike that to acetylcholine (0.1 μg kg^{-1}), was significantly potentiated by the combined administration of nicorandil and VIP. Simultaneous i.v. infusion of nicorandil (1 μg kg^{-1} min^{-1}) and VIP (0.001 μg kg^{-1} min^{-1}) had no effect on vasodepressor responses to adenosine. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during the combined infusion of nicorandil and VIP are shown in Table 2.

3.5. Effects of simultaneous infusion of low-dose nicorandil and CGRP on adenosine-induced vasodepression (6th set)

The vasodepressor effects of adenosine (3–100 μg kg^{-1}, i.v.) were examined before and during simultaneous i.v. infusion of nicorandil (1 μg kg^{-1} min^{-1}) and CGRP (0.1 or 0.3 ng kg^{-1} min^{-1}), which had no influence on basal arterial blood pressure and heart rate. During the combined infusion of nicorandil and CGRP, the vasodepressor responses to adenosine were significantly enhanced. However, with the combination of nicorandil (1 μg kg^{-1} min^{-1}) and CGRP in a lower dose (0.03 ng kg^{-1} min^{-1}), the response to adenosine remained unmodified. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during the combined infusion of nicorandil and CGRP are presented in Table 3.

3.6. Effects of simultaneous infusion of nicorandil and VIP on adenosine-induced vasodepression in the absence and presence of glibenclamide (1st and 3rd sets)

Immediately after the dose–response curve for vasodepression elicited by adenosine was recorded, a single bolus dose of glibenclamide (20 mg kg^{-1}) was injected i.v. over 5 min. The administration of glibenclamide, which had no influence on basal arterial blood pressure and heart rate, significantly attenuated the vasodepressor response to adenosine (3–100 μg kg^{-1}, i.v.) (dotted line, the right part in Fig. 3), but did not modify the response to acetylcholine (0.1 μg kg^{-1}, i.v.) (Fig. 2). As demonstrated in Fig. 2 and in the right part of Fig. 3, there was no enhancement of the vasodepressor responses to adenosine in the presence of glibenclamide, even during the combined i.v. infusion of nicorandil (1 μg kg^{-1} min^{-1}) and VIP (0.003 μg kg^{-1}

min^{-1}). The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during simultaneous infusion of nicorandil and VIP in the absence and presence of glibenclamide are summarized in Table 2.

3.7. Effects of simultaneous infusion of nicorandil and CGRP on adenosine-induced vasodepression in the absence and presence of DMPX or glibenclamide (4th and 7th sets)

After the vasodepressor effects of adenosine (3–100 $\mu g\ kg^{-1}$, i.v.) were examined, a single dose of DMPX (1 mg kg^{-1} over 1 min) or glibenclamide (20 mg kg^{-1} over 5 min) was injected i.v. DMPX and glibenclamide caused virtually little change in arterial blood pressure and heart rate. About 10 min later, simultaneous i.v. infusion of nicorandil (1 $\mu g\ kg^{-1}\ min^{-1}$) and CGRP (0.3 ng $kg^{-1}\ min^{-1}$) was started, and 20 min later the effects of adenosine were examined again. Potentiation of the vasodepressor responses to adenosine was not observed during simultaneous infusion of nicorandil and CGRP. The doses of adenosine required to cause a 20-mmHg decrease in mean arterial blood pressure before and during simultaneous infusion of nicorandil and CGRP in the absence and presence of DMPX or glibenclamide are presented in Table 3.

4. Discussion

The present results revealed that single bolus i.v. injections of adenosine elicited dose-dependent decreases in arterial blood pressure but hardly affected heart rate (except for the largest dose of adenosine). Intravenous infusion of nicorandil or i.v. infusion of the combination of nicorandil with low doses of either VIP or CGRP, which alone had no effects on the adenosine-induced vasodepression, significantly enhanced the vasodepressor response caused by adenosine, but not that caused by acetylcholine.

According to the present experiment, the minimum effective doses of nicorandil, VIP and CGRP that potentiated the adenosine-induced vasodepression were 10, 0.03 $\mu g\ kg^{-1}$ and 1 ng $kg^{-1}\ min^{-1}$, respectively. It should be noted that the combined effects of the agents were examined using one-tenth of these doses: nicorandil, 1 $\mu g\ kg^{-1}\ min^{-1}$; VIP, 0.003 $\mu g\ kg^{-1}\ min^{-1}$; and CGRP, 0.1 ng $kg^{-1}\ min^{-1}$. Interestingly, nicorandil even in combination with either VIP or CGRP in extremely low doses caused a pronounced potentiation of the adenosine-induced vasodepression.

As has been reported already by us, the vasodepressor effects of VIP, CGRP and adenosine are significantly enhanced by treatment with cromakalim (Saito and Sakai, 1998a,b) and nicorandil (Sakai et al., 1998a), both K_{ATP} channel openers (Hamilton and Weston, 1989), whereas the vasodepressor effects of these agents, including nicorandil, were significantly, but not completely, prevented by the treatment with glibenclamide (Saito and Sakai, 1998a,b), an antagonist of K_{ATP} channels (Standen et al., 1989; Daut et al., 1990; Nelson et al., 1990; Belloni and Hintze, 1991), although the dose of glibenclamide (20 mg kg^{-1} over 5 min i.v.) used completely inhibited a 40-mmHg decrease in mean arterial blood pressure induced by a bolus i.v. injection of cromakalim (30 $\mu g\ kg^{-1}$) in rats (Sakai et al., 1998b). This probably indicates that the remaining, glibenclamide-insensitive vasodepression induced by these agents is mediated by additional mechanism, e.g., nitric oxide pathway or/and some other ion channels. In the presence of glibenclamide, potentiation of the adenosine-induced vasodepression by the combined administration of either nicorandil and VIP or CGRP was not observed.

Recently, we reported that the vasodepression caused by adenosine in rats is mediated via A_2-receptors (Saito and Sakai, 1998c). This view is consistent with the findings of Furukawa et al. (1993) for pithed rats. In the present experiment, the combined administration of either nicorandil and VIP (data not shown) or CGRP failed to enhance the adenosine-induced vasodepression in the presence of DMPX, a potent antagonist of adenosine A_2 receptors (Seale et al., 1988; Sebastiao and Ribeiro, 1989), in a dose sufficient to block the effect of CPCA, a potent adenosine A_2 receptor agonist (Bruns et al., 1986). Thus, it is more likely that the potentiation of the adenosine-induced vasodepression caused by simultaneous administration of either nicorandil and VIP or CGRP was closely related to activation of adenosine A_2 receptors which are coupled with K_{ATP} channels.

It has been reported that, like isoproterenol, an adrenergic β-receptor stimulant (Lefkowitz et al., 1995), VIP (Ignarro et al., 1987) and CGRP (Rubino and Burnstock, 1996) stimulate the formation of cAMP in the vasculature and the myocardium, and also that the coupling of adenosine to the A_2 receptor leads to stimulation of adenylate cyclase activity and to a subsequent increase in intracellular cAMP levels (Linden et al., 1993), resulting in cAMP-dependent vasodilatation (Silver et al., 1984). Kleppisch and Nelson (1995) described that adenosine activates K_{ATP} channels in arterial smooth muscle through A_2 receptors, leading to activation of adenylate cyclase, and that the resulting increase in intracellular cAMP opens K_{ATP} channels by stimulating protein kinase A. Similar findings have been reported by Mubagwa et al. (1996). However, our recent study (Sakai et al., 1998b) revealed in anesthetized rats that the vasodepressor response to isoproterenol was not affected by either cromakalim or glibenclamide, suggesting that the observed potentiation in the adenosine-induced vasodepression caused by nicorandil, VIP and CGRP was induced via a pathway that does not involve adenylate cyclase/protein kinase A.

It has been suggested by Nelson (1993) that nicorandil activates K_{ATP} channels, and that adenosine and VIP as well as CGRP stimulate them throughout activation of purinergic and peptide receptors, respectively, on vascular smooth muscle cells with subsequent second messenger activation. Indeed, glibenclamide blocks K_{ATP} channels, whereas DMPX seems to block the channels via the adenosine A_2 receptors which are coupled to K_{ATP} channels. Thus, it is presumed that several naturally occurring vasodilators, such as VIP, CGRP and adenosine, reciprocally interact partly through a common mechanism, i.e., K_{ATP} channels (Sakai and Saito, 1998), and that nicorandil modulates them, probably through different signal transduction pathways in vascular smooth muscles and amplifies membrane hyperpolarization, leading to more potent vasodilatation.

It is accepted that nicorandil is an orally effective anti-anginal drug (Sakai, 1989; Kinoshita and Sakai, 1990; Krumenacker and Roland, 1992). Endogenous vasodilators such as VIP, CGRP and adenosine seem to regulate the physiological control of the cardiovascular system (Ignarro et al., 1987; Rubino and Burnstock, 1996; Mubagwa et al., 1996). Adenosine is released into the coronary sinus blood during myocardial ischemia and plays an important role in the physiological control of the cardiovascular system (Hori and Kitakaze, 1991; Mubagwa et al., 1996). Furthermore, release of CGRP has been demonstrated in experimental myocardial ischemia (Franco-Cereceda et al., 1989) and increased blood concentrations of CGRP have been detected in patients with acute myocardial infarction (Mair et al., 1990). If endogenous vasodilators released during myocardial ischemia are not enough to produce maximal coronary vasodilatation and prevention of reperfusion injury, then synergism between nicorandil and endogenous vasodilators such as VIP, CGRP and adenosine, even in low concentrations, will be of great value in the therapy of ischemic heart diseases. Even though the present study was limited to the interaction of the agents on the blood pressure and heart rate of rats, this view is strongly supported by a brief report by Komamura and Inoue (1995) that nicorandil, like cromakalim, infused into the left anterior descending coronary artery in anesthetized dogs significantly enhances coronary blood flow responses to adenosine and adenosine release from the myocardium, both of which attenuate myocardial ischemia.

In conclusion, the present results demonstrated that in extremely low doses nicorandil in combination with VIP or CGRP enhanced adenosine-induced vasodepression in rats, partly through K_{ATP} channels in vascular smooth muscle. VIP (Ignarro et al., 1987), CGRP (Rubino and Burnstock, 1996) and adenosine (Mubagwa et al., 1996) are potent endogenous vasodilators, locally released in the effector tissue from stimulated nerve terminals or from organs, and contribute to the physiological regulation of blood flow and vascular tone in the cardiovascular system. It is possible that nicorandil has a synergistic interaction with these agents to increase vasodilator activity, which has a strong cardioprotective action against ischemic insult.

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