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Adrenomedullin synergistically interacts with endogenous vasodilators in rats: a possible role of K_{ATP} channels

Kazushige Sakai *, Keiji Saito, Nobuhiko Ishizuka

Central Research Laboratories, Chugai Pharmaceutical, 3-41-8 Takada, Toshima-ku, Tokyo 171, Japan

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Abstract

To examine synergistic interactions among naturally occurring vasodilators, we investigated the effects of i.v. infusion of adrenomedullin (ADM) alone and in combination with low-dose vasoactive intestinal polypeptide (VIP) or calcitonin gene-related peptide (CGRP) on adenosine-induced vasodepression in rats. I.v. infusion of the combination of low-dose ADM (0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}), as well as that of ADM (1 ng kg^{-1} min^{-1}) alone, significantly enhanced the vasodepressor responses to bolus i.v. doses of adenosine (3–100 μg kg^{-1}), but not those to acetylcholine (0.1 μg kg^{-1}). The observed potentiation did not occur in the presence of glibenclamide (20 mg kg^{-1} i.v.), an antagonist of K_{ATP} channels. Simultaneous i.v. infusion of low-dose ADM and CGRP (0.1 ng kg^{-1} min^{-1}) failed to enhance the effects of adenosine as well as acetylcholine. In the whole-cell voltage clamp experiments using single cells of the rat mesenteric artery, ADM (10^{-11}–10^{-7} M) as well as CGRP (10^{-11}–10^{-7} M) produced increases of inward current in a concentration-dependent manner. The ADM-induced current was not affected by iberiotoxin, a specific blocker of large conductance Ca^{2+}-activated K^+ channels, but suppressed markedly by glibenclamide and $CGRP_{(8-37)}$, a selective antagonist of $CGRP_1$ receptors. From the results, we conclude that several naturally occurring vasodilators involving ADM synergistically interact, probably in link with K_{ATP} channels, and furthermore that ADM may act, in part through $CGRP_1$ receptor activation.

Keywords: Adrenomedullin; Vasodilator, endogenous; Synergistic interaction; K_{ATP} channel; $CGRP_1$ receptor

1. Introduction

Even though the neuronal regulation of cardiovascular function is intricate, it has been generally accepted that peptides, amines and other endogenous vasoactive substances play an important role in the cardiovascular regulation. We have recently reported that naturally occurring vasodilators such as adenosine, vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) synergistically interact in part through K_{ATP} channels in rats (Sakai and Saito, 1998; Sakai et al., 1998). Adrenomedullin (ADM) is one of endogenous peptides possessing a potent vasodilating activity, initially isolated from human pheochromocytoma tissue (Kitamura et al., 1993). Ichiki et al. (1994) detected immunoreactive ADM in multiple human tissues including adrenal medulla, heart, aorta, kidney, brain, lung, gastrointestinal organs, spleen and thyroid. Baskaya et al. (1995) reported that intracisternal administration of ADM to dogs induced dilation of the basilar and other major cerebral arteries, accompanied by an increase in the concentration of cAMP in the cerebrospinal fluid. Recently, Lang et al. (1997) and Sabates et al. (1997) suggested that the effect of ADM is in part linked with K_{ATP} channels.

We were interested in investigating the possibility of synergistic interaction between ADM and other naturally occurring vasodilators such as adenosine, VIP and CGRP, because they are widely distributed in various organs and may regulate pathophysiological control partly through opening of K_{ATP} channels in the cardiovascular system. The aim of the present study was to investigate in rats whether ADM alone and the combination with other endogenous vasodilators can enhance the adenosine-induced vasodepression, and if so, how mechanisms are involved in potentiating the vasodepression. Iberiotoxin, a specific blocker of large conductance Ca^{2+}-activated K^+ channels (Taguchi et al., 1995; Lang et al., 1997) and gliben-

* Corresponding author. Tel.: +81-3-3987-0863; Fax: +81-3-3985-7079.

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clamide, an antagonist of K_{ATP} channels (Standen et al., 1989) were utilized as useful pharmacological tools in the present experiment.

2. Materials and methods

2.1. Chemicals

The chemicals used were: ADM (human), CGRP (human), $CGRP_{(8-37)}$ (human) and VIP (human and porcine) (all from Peptide Institute, Osaka, Japan), acetylcholine chloride (RBI, Natick, MA, USA), glibenclamide (Wako Junyaku, Osaka, Japan), adenosine free base, iberiotoxin, and pinacidil (all from Sigma, St. Louis, MO, USA). Other reagents were also purchased from Sigma. CGRP was dissolved in distilled water, and diluted with 0.45% saline solution (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). The other drugs were freshly dissolved in and diluted with 0.9% saline solution, just before the experiment. For electrophysiological studies, all agents used were dissolved in deionized water and diluted to the final concentration in the superfusion solution.

2.2. Animal preparations

All experiments were conducted according to the guidelines outlined in the Regulations of the Animal Research Committee of the Chugai Pharmaceutical, Tokyo, Japan.

2.2.1. In vivo experiments

2.2.1.1. Measurements of arterial blood pressure and heart rate. Male Sprague–Dawley rats (Charles River Japan, Atsugi, Kanagawa) weighing 377 ± 4 ($n = 60$) were allowed free access to food and water. The rats were anesthetized initially with pentobarbital sodium (55 mg kg^{-1} i.p.) and an additional dose of pentobarbital (40 mg kg^{-1}) was injected s.c., as required. Polyethylene tubes (PE 10) were inserted into peripheral veins, the left jugular and femoral veins for i.v. drug injection and infusion, respectively. For i.v. bolus injection, 0.2 ml kg^{-1} of the drug solutions were given over a period of approximately 10 s and then flushed in with 0.9% saline solution. For i.v. infusion, drug solution was given at a rate of 0.1 ml kg^{-1} min^{-1} with a Terumo syringe pump (STC-525, Terumo, Tokyo, Japan). Arterial blood pressure was measured from the left femoral artery by means of a Nihon Kohden pressure transducer (DX-360, Nihon Kohden, Tokyo, Japan). Heart rate was measured by means of a heart rate counter (AT-601G, Nihon Kohden). All recordings were made on a chart by using a Graphtec Linearcorder (WR-3101, Nihon Kohden). Following surgery, a period of at least 30 min was allowed for stabilization of preparations.

2.2.1.2. Experimental protocols. The experiments were conducted in three sets. In a first set of experiments, the rats were divided into seven groups (each $n = 5$). The dose–response curve to bolus i.v. injections of adenosine (3–100 μg kg^{-1}), following single bolus i.v. injection of acetylcholine (0.1 μg kg^{-1}), for arterial blood pressure was recorded before and during i.v. infusion of either 0.9% saline solution (0.1 ml kg^{-1} min^{-1}), ADM (0.3 or 1 ng kg^{-1} min^{-1}), CGRP (0.3 or 1 ng kg^{-1} min^{-1}) or VIP (10 or 30 ng kg^{-1} min^{-1}). In a second set of experiments, the rats were divided into two groups (each $n = 5$). Just after the dose–response curve for bolus i.v. adenosine was recorded, the combination of either low-dose ADM (fixed on 0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}) or CGRP (0.1 ng kg^{-1} min^{-1}) was simultaneously infused i.v. The dose of each agent used was one-tenth of the minimum effective doses of the agents. In a third set of experiments, the rats were divided into three groups ($n = 5$). After the dose–response curve for bolus i.v. adenosine was recorded, a single dose of glibenclamide (20 mg kg^{-1}), which almost completely blocked 40 mmHg decrease in mean arterial blood pressure (MAP) caused by cromakalim (30 μg kg^{-1} i.v.), a potent K_{ATP} channel opener (Hamilton and Weston, 1989), in rats (Saito and Sakai, 1998), was given i.v. over 5 min, and 10 min later i.v. infusion of either 0.9% saline, ADM (1 ng kg^{-1} min^{-1}) alone or the combination of ADM (0.1 ng kg^{-1} min^{-1}) with VIP (3 ng kg^{-1} min^{-1}) was started. These agents in doses used induced virtually no effects on blood pressure and heart rate. In principle, i.v. administration of each agent was time-matched: after the dose–response curve for bolus i.v. adenosine (3–100 μg kg^{-1}), following a single bolus i.v. dose of acetylcholine (0.1 μg kg^{-1}), was recorded, 0.9% saline, ADM, VIP, CGRP or their combination was infused i.v., and 20 min later the vasodepressor effects of bolus i.v. injections of acetylcholine and adenosine were examined again in that order. Peak responses to the agents are expressed as the changes from the preadministration levels. The dose–response curves for vasodepressor responses to adenosine (3, 10, 30 and 100 μg kg^{-1} i.v.) were constructed on this basis. The effective doses ($ED_{30\ mmHg}$) of bolus i.v. adenosine required to produce a 30-mmHg decrease in MAP before and after i.v. treatment with ADM, VIP, CGRP and their combination were determined from individual dose–response curves for adenosine, as described elsewhere (Sakai and Saito, 1998), and given as means ± S.E.M.

2.2.2. In vitro experiments

2.2.2.1. Electrophysiological studies

Cell isolation. Single smooth muscle cells from rat mesenteric arteries were prepared by an enzymatic dissoci-

ation procedure (Quayle et al., 1994). Briefly, the rats were sacrificed by decapitation under ether anesthesia, and the mesenteric artery was quickly removed into an ice-cold normal Tyrode solution. A small piece of the mesenteric artery was dissected, and transferred into a low Ca^{2+} solution containing bovine serum albumin (1 mg ml^{-1}). After 10 min, the tissues were placed in the low Ca^{2+} solution, as described below, containing albumin (1 mg ml^{-1}), dithioerythritol (1 mg ml^{-1}) and papain (7 U ml^{-1}) for 20 min at 37°C, and further transferred into the solution containing albumin (1 mg ml^{-1}), type B collagenase (2 mg ml^{-1}) and type IV-S hyaluronidase (1 mg ml^{-1}) for 7 min at 37°C. The tissues were then rinsed with fresh low Ca^{2+} solution with albumin (1 mg ml^{-1}) for 10 min before single cells were obtained by gentle trituration with a polished wide-bore pipette, and stored in the same solution at 6°C for later use.

Solutions. The normal Tyrode solution contained (in mM): NaCl, 145; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 10; and HEPES, 10; sufficiently aerated with 95% O_2: 5% CO_2 and kept at 37°C. The pO_2 and pH of the solution were over 600 mmHg and 7.4, respectively (Radiometer, ABL System 625/620, Copenhagen, Denmark). The composition of the low Ca^{2+} solution (in mM) was: NaCl, 60; glutamate–Na, 80; KCl, 5; $CaCl_2$, 0.1; $MgCl_2$, 2; glucose, 10; and HEPES, 10 (pH = 7.4, adjusted with NaOH).

Electrophysiological recordings. Whole-cell currents were recorded by the conventional whole-cell configuration of the patch-clamp technique (Hamill et al., 1981), using an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA). Briefly, dispersed cells were dropped in a small bath chamber (0.2 ml) mounted on the stage of a Diaphot 200 inverted Nikon microscope (Nikon, Tokyo, Japan), and left to stick to the glass cover slip for 15 min before starting experiments. The composition of the external solution (in mM) in the bath chamber was: NaCl, 82; KCl, 60, $MgCl_2$, 1; $CaCl_2$, 0.1; glucose, 10; and HEPES, 10 (pH = 7.4, adjusted with NaOH). The 'intracellular' pipette solution contained (mM): KCl, 102; KOH, 38; $MgCl_2$, 1; $CaCl_2$, 1; EGTA, 10; ATP, 0.1; ADP, 0.1; NaGTP, 0.2; glucose, 10; and HEPES, 10; pH 7.4 (adjusted with NaOH) at 24°C; free concentrations estimated with 'MAXC' software: ATP, 12.9 μM; Ca^{2+}, 6.52 nM; Mg^{2+}, 0.42 mM. Micropipettes manufactured from borosilicate glass capillary tubes (1.5 mm, Narishige, Tokyo, Japan) by a programmable puller (Sutter Instrument, CA, USA) with electrode resistance of 0.8–1.3 MΩ. To minimize any activity of voltage-dependent and large conductance calcium-activated potassium channels, experiments were carried out at negative membrane potentials (−70 mV) with intracellular Ca^{2+} buffered to ~10 nM, while extracellular Ca^{2+} (0.1 mM) was also low. To enhance the amplitude of K^+ currents at hyperpolarized potentials, external K^+ was raised to 60 mM. K^+ currents were, therefore, inward and taken as downward deflections. Under theses conditions, the K^+ equilibrium potential (E_K) was −21.6 mV. All experiments were carried out at 24°C of room temperature. Whole-cell currents were low-pass filtered with a cut-off frequency of 2 Hz and recorded with a sampling frequency of 10 Hz. Voltage

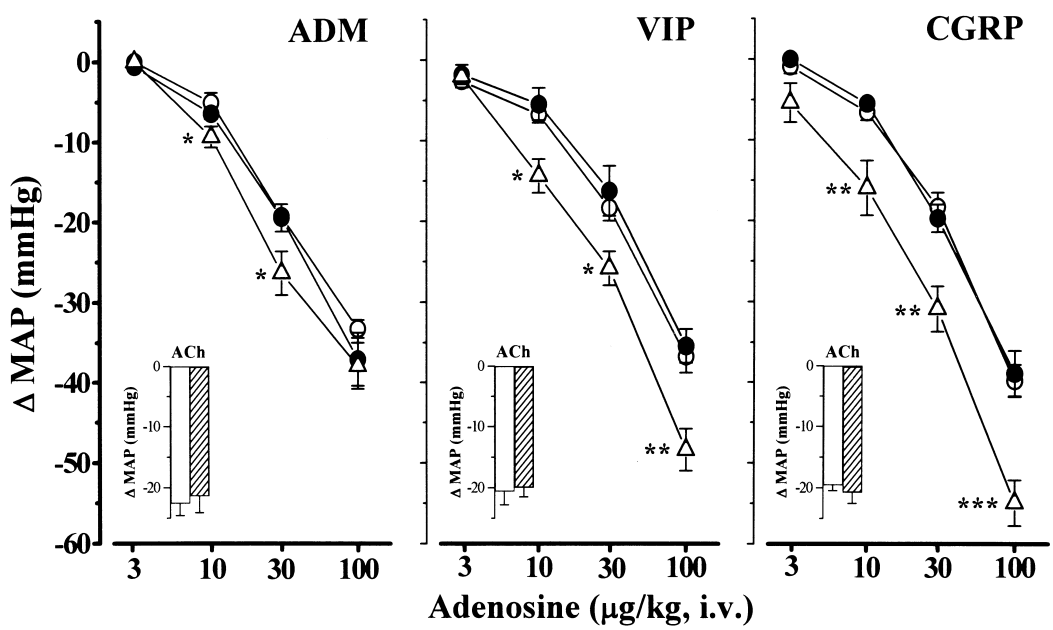

Fig. 1. Effects of i.v. infusion of ADM, VIP or CGRP on peak decreases in mean arterial blood pressure (MAP) caused by bolus i.v. injections of adenosine and acetylcholine (ACh). The effects of bolus i.v. injections of adenosine (3–100 μg kg^{-1}) and acetylcholine (0.1 μg kg^{-1}) were studied before (control) (○ or open columns) and during (hatched columns for ACh) the i.v. infusion of ADM (0.3, ●; 1 ng kg^{-1} min^{-1} △), VIP (10, ●; 30 ng kg^{-1} min^{-1}, △) or CGRP (0.3, ●; 1 ng kg^{-1} min^{-1}, △). Vertical bars represent 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.

Table 1
Effects of i.v. infusion of ADM, VIP, CGRP or the combination of either ADM and VIP or CGRP on adenosine-induced vasodepression

I.v. infusion (ng kg^{-1} min^{-1})	$ED_{30\ mmHg}$	
	Before	During
Saline	81.4±4.8	78.8±5.8
ADM		
0.3	77.9±7.4	64.7±5.0
1	86.2±3.9	49.0±10.9**
VIP		
10	79.0±4.1	78.4±9.6
30	81.4±4.8	42.2±4.1***
CGRP		
0.3	79.1±7.2	68.7±7.3
1	81.6±7.0	31.0±6.4***
ADM (0.1)+VIP (3)	81.7±5.3	59.2±2.2**
ADM (0.1)+CGRP (0.1)	77.2±8.9	66.0±9.1

Values represent means±S.E.M. (each $n=5$). ** $P<0.01$, *** $P<0.001$, compared with corresponding values from the control (before) group. The effective doses ($ED_{30\ mmHg}$) of bolus i.v. adenosine (μg kg^{-1}) required to cause a 30-mm Hg decrease in MAP before and during i.v. infusion of 0.9% saline solution, ADM, VIP, CGRP or the combination of either ADM and VIP or CGRP were calculated from individual dose–response curves for adenosine.

dependency of glibenclamide-sensitive currents in the presence of ADM was examined using 200-ms voltage-clamp ramps from −100 to +50 mV. Whole-cell currents evoked during ramp pulses were low-pass filtered with a cut-off frequency of 2 kHz recorded with a sampling frequency of 10 kHz. Digitizing and analyzing were made by the 'pCLAMP' software (Axon Instruments, Burlingame, CA, USA) on an IBM personal computer.

2.3. Statistical analysis

Values in the text are presented as means ± S.E.M. 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 statistical significant.

3. Results

3.1. In vivo experiments

Baseline values of MAP and heart rate for all of the rats tested ($n=60$, three sets, 12 groups) were as follows: 110 ± 1 mmHg and 418 ± 4 bpm, respectively, just before the first bolus i.v. injection of acetylcholine (0.1 μg kg^{-1}); 106 ± 2 mmHg and 406 ± 5 bpm, respectively, just before the first i.v. injection of acetylcholine following i.v. infu-

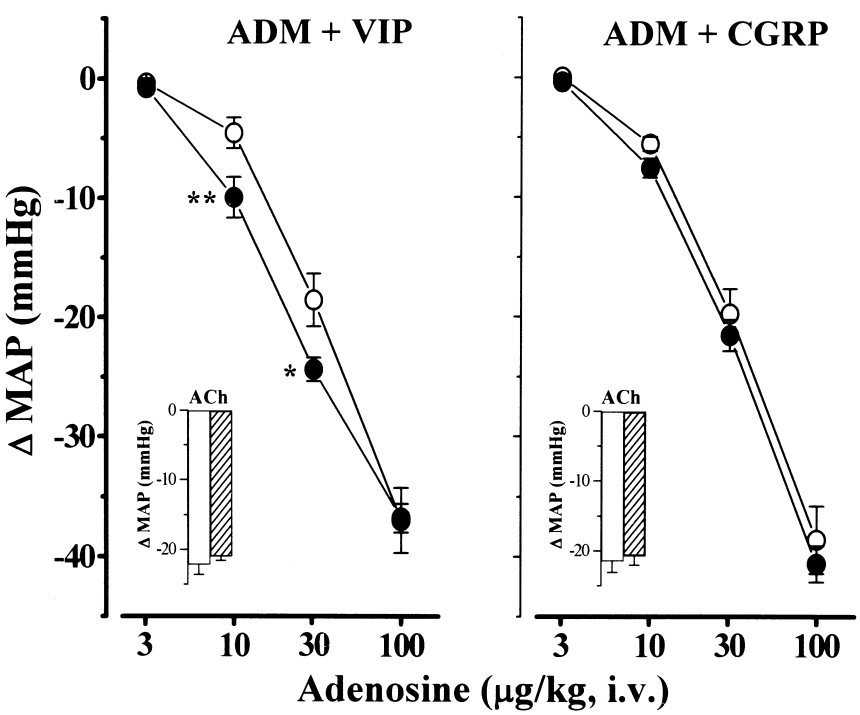

Fig. 2. Effects of simultaneous i.v. infusion of either low-dose ADM and VIP or CGRP on peak decreases in MAP caused by bolus i.v. injections of adenosine and acetylcholine (ACh). The effects of bolus i.v. injections of adenosine (3–100 μg kg^{-1}) and acetylcholine (0.1 μg kg^{-1}) were examined before (control) (○ or open columns) and during (● or hatched columns) the combined i.v. infusion of either ADM (0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}) or CGRP (0.1 ng kg^{-1} min^{-1}). Vertical bars represent means ± S.E.M. from five animals. * $P<0.05$, ** $P<0.01$, compared with the corresponding values from the control group.

sion of 0.9% saline (0.1 ml kg^{-1} min^{-1}), ADM (0.3 or 1 ng kg^{-1} min^{-1}), VIP (10 or 30 ng kg^{-1} min^{-1}), CGRP (0.3 or 1 ng kg^{-1} min^{-1}) or the combination of either ADM and VIP or CGRP. Significant differences were not observed between corresponding values. Also, there were no significant differences in baseline values of MAP and heart rate among the 12 groups (each $n = 5$). Thus, the preparations remained stable throughout the experimental period, virtually with little changes in the parameters even after the agents above were treated.

3.1.1. Effects of i.v. infusion of ADM, VIP or CGRP on blood pressure and heart rate caused by bolus i.v. injections of adenosine (first set)

I.v. bolus injections of adenosine (3–100 μg kg^{-1}) caused vasodepression in a dose-related fashion, with virtually no changes in heart rate, except for 30 (5–7% decrease) and 100 μg kg^{-1} (10–15% decrease). Just after the vasodepressor effects of acetylcholine (0.1 μg kg^{-1} i.v.) and adenosine were examined, i.v. infusion of 0.9% saline solution (0.1 ml kg^{-1} min^{-1}), ADM (0.3 or 1 ng kg^{-1} min^{-1}), VIP (10 or 30 ng kg^{-1} min^{-1}) or CGRP (0.3 or 1 ng kg^{-1} min^{-1}) was started. As depicted in Fig. 1, the vasodepressor response to adenosine, but not that to acetylcholine, was significantly enhanced during i.v. infusion of ADM (1 ng kg^{-1} min^{-1}), VIP (30 ng kg^{-1} min^{-1}) or CGRP (1 ng kg^{-1} min^{-1}). In smaller doses of each agent, the enhancement of the adenosine-induced action did not occur. The changes in heart rate caused by adenosine were not significantly modified during i.v. infusion of ADM as well as VIP and CGRP (data not shown). The responses to adenosine remained virtually unchanged before and during i.v. infusion of 0.9% saline solution (0.1 ml kg^{-1} min^{-1}) (Table 1). The $ED_{30\ mmHg}$ values of adenosine before and during i.v. infusion of either 0.9% saline, ADM, VIP or CGRP are presented in Table 1.

3.1.2. Effects of simultaneous i.v. infusion of either low-dose ADM and VIP or CGRP on adenosine-induced vasodepression (second set)

Just after the vasodepressor effects of bolus i.v. doses of adenosine (3–100 μg kg^{-1}), following bolus i.v. injection of acetylcholine (0.1 μg kg^{-1}), were examined, either ADM (0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}) or CGRP (0.1 ng kg^{-1} min^{-1}) were simultaneously infused i.v. During the combined infusion of ADM and VIP, which had no influence on basal arterial blood pressure and heart rate, the vasodepressor responses to adenosine, but not those to acetylcholine, were significantly potentiated (Fig. 2). However, the simultaneous infusion of ADM and CGRP failed to potentiate the responses to adenosine as well as acetylcholine. The $ED_{30\ mmHg}$ values of adenosine before

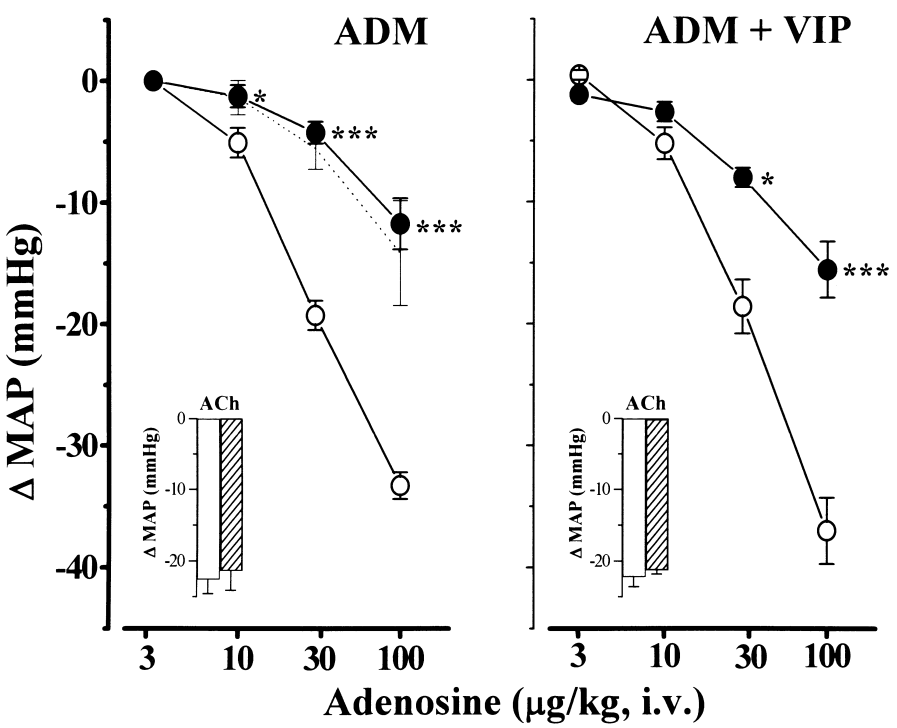

Fig. 3. Effects of i.v. infusion of either 0.9% saline, ADM alone or the combination with VIP on peak decreases in MAP elicited by bolus i.v. injections of adenosine and acetylcholine (ACh) in the presence of glibenclamide. The effects of adenosine (3–100 μg kg^{-1}) and acetylcholine (0.1 μg kg^{-1}) were examined during i.v. infusion of 0.9% saline (dotted line), ADM (1 ng kg^{-1} min^{-1}) alone or the combination of low-dose ADM (0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}) in the presence (● or hatched columns) of glibenclamide (20 mg kg^{-1} i.v.). Vertical bars represent means ± S.E.M. from five animals. $* P < 0.05$, $*** P < 0.001$, compared with the corresponding values before (○, open columns) the treatment with glibenclamide.

and during the combined i.v. infusion of either low-dose ADM and VIP or CGRP are presented in Table 1.

3.1.3. Effects of i.v. infusion of ADM alone or the combination of low-dose ADM and VIP on adenosine-induced vasodepression in the presence of glibenclamide (third set)

In the presence of glibenclamide (20 mg kg^{-1} i.v. over 5 min), the dose–response curve to bolus i.v. adenosine (3–100 μg kg^{-1}) for arterial blood pressure during i.v. infusion of 0.9% saline solution was significantly shifted rightwards. As shown in Fig. 3, the enhancement of the vasodepressor response to adenosine did not occur during i.v. infusion of either ADM (1 ng kg^{-1} min^{-1}) alone or the combination of ADM (0.1 ng kg^{-1} min^{-1}) and VIP (3 ng kg^{-1} min^{-1}). The vasodepressor response to single bolus i.v. dose of acetylcholine (0.1 μg kg^{-1}) remained unchanged before and after i.v. treatment with glibenclamide.

3.2. In vitro experiments

3.2.1. Effects of ADM and CGRP on smooth muscle cell membrane of the mesenteric artery

By use of the conventional whole-cell patch-clamp technique, single cells of the mesenteric artery were dialysed with a low-ATP containing solution (0.1 mM) that elicited a slowly developing inward currents. ADM (10^{-8} M) increased the inward currents from -33.1 ± 6.6 pA (current density, -2.84 ± 0.49 pA/pF) to -97.1 ± 12.1 pA (current density, -8.67 ± 1.38 pA/pF) (each $n = 5$). ADM and CGRP (both 10^{-11}–10^{-7} M) produced concentration-dependent increases in the amplitudes of an inward current, when the membrane was held at -70 mV (Fig. 4).

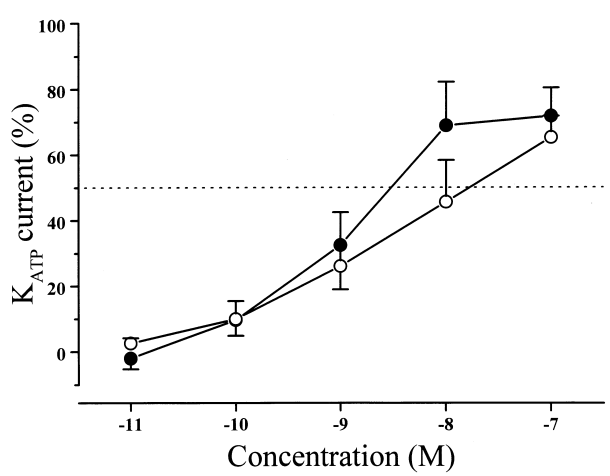

Fig. 4. Concentration-dependent increases in the membrane current induced by cumulative exposure of ADM (○) or CGRP (●) in single cells of the mesenteric artery from several rats. The response are expressed as percent changes from the maximum one evoked by 10^{-4} M pinacidil. Vertical bars represent means ± S.E.M. from five observations.

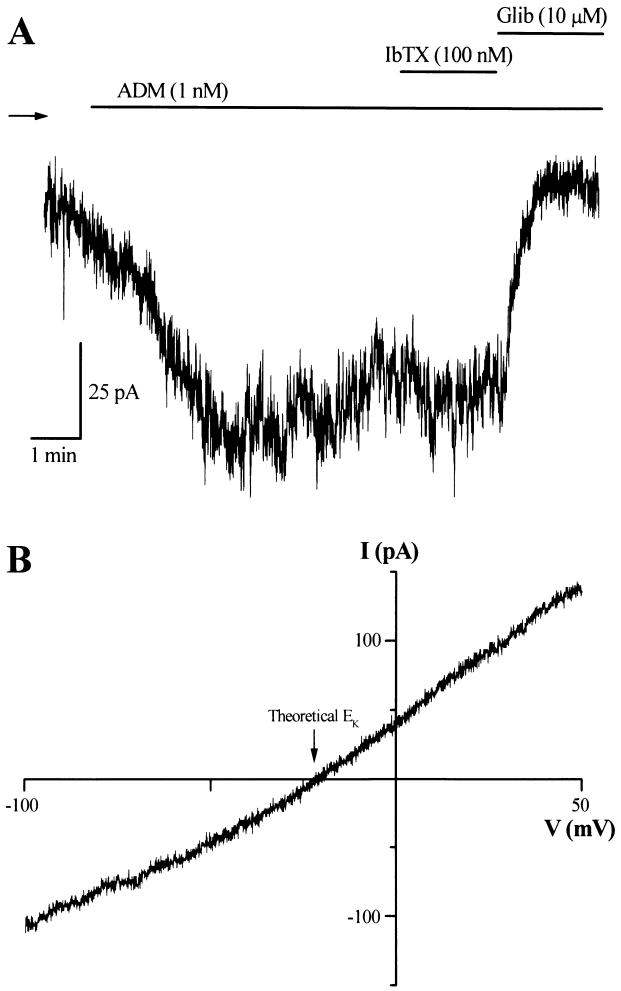

Fig. 5. Effects of ADM on membrane current in the absence and presence of iberiotoxin (IbTX) and glibenclamide (Glib) in single cells of the mesenteric artery. (A) Original record. Iberiotoxin and glibenclamide were applied externally. The holding potential was -70 mV. Zero current level is indicated by arrow. (B) Voltage-dependence of ADM-induced currents. Glibenclamide-sensitive currents evoked by ADM (difference between currents in the presence of ADM 1 nM and of ADM 1 nM plus glibenclamide 10 μM) are shown. Arrow indicates theoretical potassium equilibrium potentials (E_K) with 60 mM external potassium. Reversal potentials of ADM-induced currents were -19.6 ± 0.9 mV ($n = 5$).

3.2.2. Effects of iberiotoxin, glibenclamide and $CGRP_{(8-37)}$ on currents induced by ADM on smooth muscle cell membrane of the mesenteric artery

The effects of iberiotoxin (10^{-7} M) and glibenclamide (10^{-5} M) were observed on the properties of the inward current induced by ADM (10^{-9} M) (Fig. 5). Glibenclamide (10^{-5} M) significantly inhibited the current evoked by ADM, although iberiotoxin (10^{-7} M) did not affect the currents (part A). The reversal potentials of the glibenclamide-sensitive current in the presence of ADM (10^{-9} M) was -19.6 ± 0.9 mV ($n = 5$), near the theoretical potassium equilibrium potential, indicating that ADM-induced currents were through K^+ selective channels (part

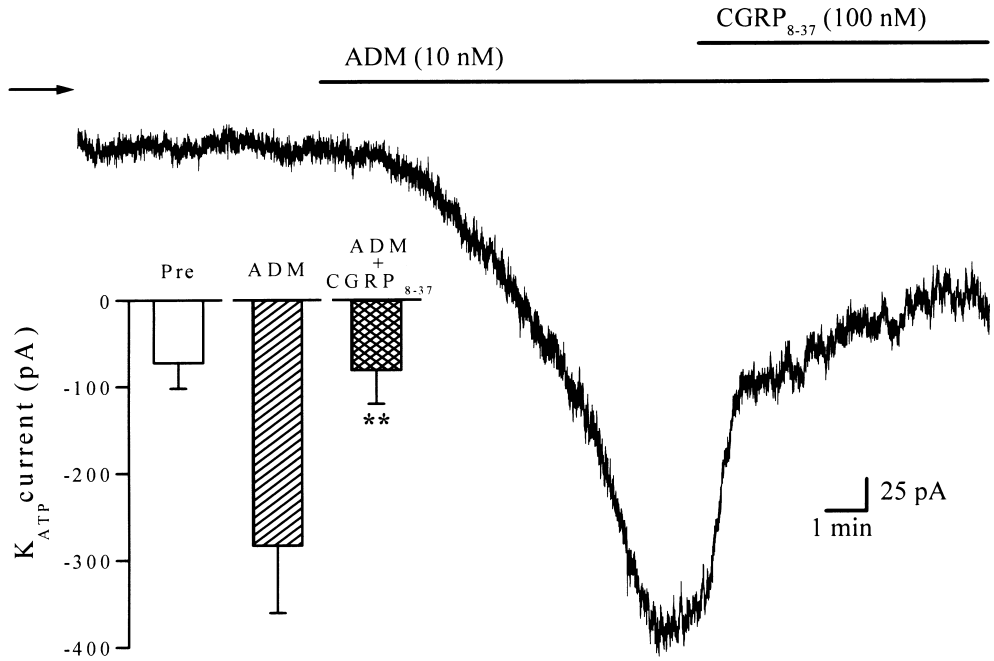

Fig. 6. Effects of ADM on membrane current in the absence and presence of $CGRP_{(8-37)}$ in single cells of the mesenteric artery from several rats. $CGRP_{(8-37)}$ was applied externally. The condition was the same as that in Fig. 5. Zero current level is indicated by arrow. Inset: summarized data. Vertical bars present means ± S.E.M. from five observations. $**P < 0.01$, compared with the corresponding values from the ADM-treated group. Pre, preadministration.

B). As shown in Fig. 6, $CGRP_{(8-37)}$ (10^{-7} M) almost completely blocked the inward currents evoked by ADM (10^{-8} M) as well as CGRP (10^{-8} M) (data not shown).

4. Discussion

The present experiments demonstrated that single bolus i.v. injections of adenosine elicited dose-dependent decreases in arterial blood pressure but scarcely affected heart rate (except for large doses of adenosine). I.v. infusion of ADM alone or the combination of low-dose ADM with low-dose of VIP, but not with that of CGRP, which alone had no effects on the adenosine-induced vasodepression, significantly potentiated the vasodepressor response to adenosine, but not that to acetylcholine.

According to the present study, the minimum effective doses of ADM, VIP and CGRP that potentiated the adenosine-induced vasodepression were approximately 1, 30 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: ADM, 0.1 ng kg^{-1} min^{-1}; VIP, 3 ng kg^{-1} min^{-1}; and CGRP, 0.1 ng kg^{-1} min^{-1}. Interestingly, ADM even in combination with VIP in extremely low-dose caused a pronounced potentiation of the adenosine-induced vasodepression, whereas the combination of ADM and CGRP did not elicit the observed potentiation. Understanding the mechanisms by which these endogenous substances potentiate the adenosine effect should provide important insights into the control of blood pressure and blood flow.

It has been reported that ADM as well as a number of other endogenous vasodilators (e.g., adenosine by Daut et al. (1990) and Belloni and Hintze (1991); VIP by Ignarro et al. (1987); CGRP by Rubino and Burnstock (1996)) increase intracellular levels of cAMP through activation of adenyl cyclase (Ishizaka et al., 1994; Richards et al., 1996). Recently, Sabates et al. (1997) reported that in anesthetized, open-chest dogs bolus injections of ADM into the coronary artery, as well as those of adenosine, pinacidil and CGRP, caused significant increases in coronary blood flow in a dose-related fashion, without altering systemic hemodynamic measurements, which were blocked by intracoronary injection of U37883A, an antagonist of K_{ATP} channels. Thus, it appears that ADM acts in part through activation of K_{ATP} channels, similar to adenosine (Daut et al., 1990; Belloni and Hintze, 1991), VIP (Standen et al., 1989) and CGRP (Nelson et al., 1990; Kitazono et al., 1993).

The present electrophysiological experiments demonstrated that ADM evoked inward currents in a concentration-dependent manner, in single cells of the rat mesenteric artery. The currents were not affected by treatment with iberiotoxin, a specific blocker of large conductance calcium-activated K^+ channels (Taguchi et al., 1995; Lang et al., 1997), but was significantly blocked by glibenclamide, an antagonist of K_{ATP} channels (Standen et al., 1989). Additionally, it should be noted that the reversal potentials

of the glibenclamide-sensitive currents in the presence of ADM was approximately −19.6 mV, near the theoretical potassium equilibrium potential. Taken together, the findings may indicate that ADM-induced currents were through K^+ selective channels. Thus, our experiments support the suggestion by Lang et al. (1997) and Sabates et al. (1997) that the effect of ADM is in part linked with K_{ATP} channels.

In the present experiments, i.v. infusion of ADM, VIP or CGRP alone and the combination with low-dose of ADM and VIP significantly potentiated the adenosine-induced vasodepression. On the basis of our recent finding (Sakai et al., 1998) that the vasodepressor response to isoproterenol, β-adrenoceptor stimulant, which increases the formation of cAMP in tissues (Lefkowitz et al., 1995), was not modified by either cromakalim or glibenclamide in rats, it is possible that the observed potentiation in the adenosine-induced vasodepression caused by ADM, VIP and CGRP was induced via a pathway that does not involve adenylate cyclase/protein kinase A. Additionally, the potentiation was not observed after treatment with glibenclamide, suggesting that it is at least in part coupled to K_{ATP} channels. Interestingly, in the present experiment, the combination of low-dose ADM and CGRP did not enhance the adenosine-induced vasodepression. As has been reviewed by Richards et al. (1996), ADM has 27% similarity to CGRP, in its C-terminal portion (amino acid residues 16–52), and may belong to the CGRP super-family of peptides. Taken together, even though further investigation is needed, it is possible that synergistic effects of ADM and CGRP did not occur, because both agents might have acted through the same ($CGRP_1$) receptors or the same signal transduction pathway. Actually, it was found in the present electrophysiological experiment that K_{ATP} currents evoked by ADM were significantly reversed by $CGRP_{(8-37)}$, a selective antagonist of $CGRP_1$ receptors (McMurdo et al., 1997). Similar findings have been reported in various animal models in vitro and in vivo (Baskaya et al., 1995; Elhawary et al., 1995; Lang et al., 1997; Sabates et al., 1997).

It is known that the effects of several naturally occurring vasodilators, which synergistically interact (Sakai and Saito, 1998; Sakai et al., 1998) probably through individual signal transduction pathways in the cells, are at least in part coupled with K_{ATP} channels (Nelson, 1993). Therefore, it is more likely that endogenous vasodilators such as adenosine, VIP and CGRP, which are widely distributed in various organs, synergistically act in part through K_{ATP} channel activation in the cardiovascular system, and regulate the control of blood flow and vascular tone. ADM also is one of biologically active peptides. Although the clinical significance still remain to be elucidated, it is known that plasma ADM levels are increased in proportion to each condition's severity in many cardiovascular diseases, such as hypertension, renal failure, heart failure, and acute myocardial infarction (Nishikimi et al., 1994). Thus, it is presumed that increased ADM may be involved in the defense mechanism against further deterioration of diseases, through ADM alone or synergistic interactions with other vasodilators.

In summary, the present experiments in vivo and in vitro revealed that ADM activates K_{ATP} channels probably through $CGRP_1$ receptors, and that ADM alone or the combination of low-dose ADM and VIP, but not CGRP, significantly potentiates the adenosine-induced vasodepression, in part through K_{ATP} channel activation. As ADM possesses a potent vasodilating property (Baskaya et al., 1995; Gardiner et al., 1995; Lang et al., 1997; Sabates et al., 1997), it is possible that this agent alone or in combination with other endogenous vasodilators may contribute to the physiological regulation of blood flow and vascular tone in the cardiovascular system.

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