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Reciprocal interactions among neuropeptides and adenosine in the cardiovascular system of rats: a role of K_{ATP} channels

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Abstract

Possible reciprocal interactions among vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and adenosine were investigated in anesthetized rats. Changes in arterial blood pressure were taken as a parameter to evaluate the interactions. The i.v. bolus injections of VIP (0.3 or 1 μg kg^{-1}), CGRP (0.1 or 0.3 μg kg^{-1}) and adenosine (1–100 μg kg^{-1}), like acetylcholine (0.1 μg kg^{-1}), produced reductions of blood pressure, accompanied by slight changes (less than 5% except for 100 μg kg^{-1} adenosine) in heart rate (HR). The vasodepressor responses to VIP and CGRP were significantly augmented by i.v. infusion of adenosine (3 μg kg^{-1} min^{-1}). The vasodepressor responses to adenosine and CGRP by VIP (0.03 μg kg^{-1} min^{-1}), and those to adenosine and VIP by CGRP (1 ng kg^{-1} min^{-1}) were also enhanced. The response to acetylcholine remained unchanged before and during i.v. infusion of either VIP, CGRP or adenosine. The i.v. infusion of cromakalim (0.1 μg kg^{-1} min^{-1}) also augmented the responses to VIP, CGRP and adenosine, but not to acetylcholine, whereas a single bolus i.v. injection of glibenclamide (20 mg kg^{-1}) significantly attenuated each one of them. The present results suggest that endogenous vasodilators, such as VIP, CGRP and adenosine, reciprocally interact in the body, at least partly through ATP-sensitive K^+ channels. © 1998 Elsevier Science B.V.

Keywords: VIP, Vasoactive intestinal polypeptide; CGRP, Calcitonin gene-related peptide; Adenosine; Reciprocal interaction; Cardiovascular system

1. Introduction

It is well known that for many years, studies on the neuronal control of cardiovascular function have focused on the antagonistic actions of the sympathetic and parasympathetic divisions of the autonomic nervous system. However, histochemical studies on the cardiovascular system have demonstrated that there are abundant nerve fibres containing vasoactive peptides, such as calcitonin gene-related peptide (CGRP), substance P and vasoactive intestinal polypeptide (VIP) (Mulderry et al., 1985; Shulkes, 1993; Rubino and Burnstock, 1996), which play an important role in the physiological control in the cardiovascular system (Rubino and Burnstock, 1996). On the other hand, adenosine, a metabolite of adenine nucleotides, is a ubiquitous biological substance found in every cell of the human body, and plays an important role in ischemic heart diseases (Hori and Kitakaze, 1991; Mubagwa et al., 1996). According to the description of Nelson (1993), it seems that a number of endogenous vasodilators, including VIP, CGRP and adenosine, act, at least in part, through membrane hyperpolarization caused by activating ATP-sensitive K^+ channels in vascular smooth muscle. Recently, we reported that CGRP enhances the vasodepressor response to adenosine in anesthetized rats (Sakai et al., 1998a), at least partly through the activation of ATP-sensitive K^+ channels. Thus, it is possible that endogenous vasodilators may reciprocally interact.

We have, therefore, designed the present study in rats to further extend the previous investigation (Sakai et al., 1998a) and to examine a possible reciprocal interaction among VIP, CGRP and adenosine, by studying their effects on arterial blood pressure.

2. Materials and methods

2.1. Chemicals

The chemicals used were: VIP (human, porcine); CGRP (human) (both from Peptide Institute, Osaka, Japan); cro-

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makalim; adenosine free base (both Sigma Chemical, St. Louis, MO, USA); acetylcholine chloride (RBI, Natick, MA, USA) and glibenclamide (Wako Junyaku, Osaka, Japan). CGRP was dissolved in distilled water, and diluted with 0.45% saline solution (Elhawary et al., 1995). Cromakalim was freshly dissolved in 99.5% ethanol at a concentration of 5 mg ml^{-1}. Glibenclamide was dissolved in 1 ml of 0.1 N 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). These solutions were diluted with 0.9% saline solution to the desired concentrations, just before the experiment. Other compounds were dissolved in 0.9% saline solution. The i.v. injections of these vehicles (0.2 ml/kg per 10 s) did not affect blood pressure and heart rate (HR).

2.2. Animal preparations

All experiments were carried out under regulations of the Animal Research Committee of Chugai Pharmaceutical, Tokyo, Japan. Male Sprague–Dawley rats (Charles River Japan, Atsugi, Kanagawa, Japan) weighing about 400 g were allowed free access to food and water. The animals were anesthetized initially with pentobarbital sodium (55 mg kg^{-1} i.p.) and its additional dose (40 mg kg^{-1}) was injected s.c., as required. Polyethylene tubes (PE 10) were inserted into peripheral veins, usually to the right and left femoral veins for drug bolus i.v. injections or infusion, respectively. For i.v. bolus injection of the agents, 0.2 ml kg^{-1} of the solutions were given over a period of approximately 10 s and then the tubing was flushed with 0.9% saline solution. For i.v. infusion of the agents, the solutions were administered 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 with a Nihon Kohden pressure transducer (DX-360, Tokyo, Japan). HR was measured by means of a HR counter (Nihon Kohden, AT-601G) triggered by the arterial pressure pulse. 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 animals were divided into 12 groups (each $n = 5$). Groups I, II and III were given bolus i.v. doses of adenosine (1–100 μg kg^{-1}), VIP (0.3 or 1 μg kg^{-1}) and CGRP (0.1 or 0.3 μg kg^{-1}), respectively, before and during i.v. infusion of 0.9% saline solution (0.1 μl kg^{-1} min^{-1}). Groups IV and V were given bolus i.v. doses of adenosine and CGRP (0.1 μg kg^{-1}), respectively, before and during i.v. infusion of VIP (0.03 μg kg^{-1} min^{-1}). Groups VI and XII were given bolus i.v. doses of adenosine and VIP (0.3 μg kg^{-1}), respectively, before and during i.v. infusion of CGRP (1 ng kg^{-1} min^{-1}). Groups VIII and IX were given bolus i.v. doses of VIP (0.3 μg kg^{-1}) and CGRP (0.1 μg kg^{-1}), respectively, before and during i.v. infusion of adenosine (3 μg kg^{-1} min^{-1}). Group X was given bolus i.v. doses of adenosine (30 μg kg^{-1}), VIP (1 μg kg^{-1}), CGRP (0.3 μg kg^{-1}) and acetylcholine (0.1 μg kg^{-1}) before and during i.v. infusion of cromakalim (0.1 μg kg^{-1} min^{-1}). Groups XI and XII were given bolus i.v. doses of adenosine, respectively, before and after a bolus i.v. glibenclamide (20 mg/kg per 5 min), and thereafter followed by i.v. infusion of VIP and CGRP, respectively. In groups II and III, the effects of glibenclamide were also examined. After ending the control experiments with i.v. infusions of 0.9% saline solution, glibenclamide was given i.v. and 10 min later, bolus i.v. dose of VIP (group II) or CGRP (group III) was given. Then, an i.v. infusion of either CGRP (group II) or VIP (group III) was started, and the effects of bolus i.v. doses of VIP or CGRP were examined. Since tachyphylaxis might develop after the higher doses studied (Rubino and Burnstock, 1996), dose–response curves to the agents, except for adenosine (1–100 μg kg^{-1}), were not recorded, and the agents were given as follows: in each animal, single bolus injection of a relatively low dose of either VIP (0.3 or 1 μg kg^{-1}) or CGRP (0.1 or 0.3 μg kg^{-1}), following a single bolus i.v. injection of acetylcholine (0.1 μg kg^{-1}) was made. Then, the i.v. infusion of either 0.9% saline, VIP, CGRP or adenosine solution was started, and 15–20 min later, acetylcholine and successively the same dose of VIP or CGRP were injected i.v. again. The effects of bolus i.v. adenosine (1–100 μg kg^{-1}) were examined with similar procedures as described above. From preliminary experiments, the dose of either VIP or CGRP used for i.v. infusion was selected as the one that causes the enhancement of vasodepressor response to adenosine, without affecting basal blood pressure and HR. The i.v. infusion rate of adenosine was also determined, so that it would not have influence on basal blood pressure and HR. In principle, the i.v. administrations of each agent were time-matched.

2.4. Statistical analysis

The values in the text are presented as means ± S.E.M. Peak responses to the agents are expressed as the changes from the preadministration levels. The dose–response curves for vasodepressor effects of adenosine (1, 3, 10, 30 and 100 μg kg^{-1} i.v.) were constructed on this basis. As the highest dose (100 μg kg^{-1}) of adenosine produced a near-maximal decrease (40–50 mmHg reduction from the preadministration value) in blood pressure, each dose–response curve for the decrease in blood pressure resulting from adenosine was computer-fitted by a nonlinear least squares routine to the equation (Furukawa et al., 1993) on the assumption that the maximum decrease in blood pressure induced by adenosine would be attained at 100 μg kg^{-1}. Thus, the doses of adenosine required to produce a

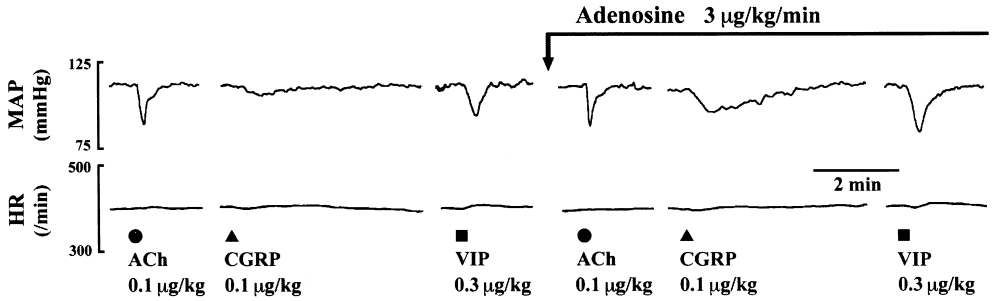

Fig. 1. Effects of acetylcholine (ACh), CGRP and VIP on MAP and HR before and during i.v. infusion of adenosine.

30-mmHg decrease in mean arterial blood pressure (MAP) before and after the i.v. treatment with VIP, CGRP and glibenclamide were determined from individual dose–response curves for adenosine, and given as means ± S.E.M. Differences between paired or unpaired mean values were analyzed by Student's *t*-test. Analysis of variance 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.

3. Results

Baseline values of MAP and HR for all of the rats tested ($n = 60$, all groups) were as follows: 115.9 ± 1.3 mmHg and 408.9 ± 3.9 beats per min, respectively, just before the first i.v. injection of test agents; 112.7 ± 1.6 mmHg and 404.2 ± 3.5 beats per min, respectively, just before the first i.v. injection of test agents following the start of i.v. infusion with either 0.9% saline (0.1 ml kg^{-1} min^{-1}), VIP (0.03 μg kg^{-1} min^{-1}), CGRP (1 ng kg^{-1} min^{-1}), adenosine (3 μg kg^{-1} min^{-1}), cromakalim (0.1 μg kg^{-1} min^{-1}) or single bolus i.v. injection of glibenclamide (20 mg kg^{-1} over 5 min). No significant differences were found between corresponding values for various groups. Thus, the parameter measured remained stable throughout the experimental period. The changes in basal blood pressure and HR even after the administration of the treatment were minor. The i.v. infusion of VIP (0.3 μg kg^{-1} min^{-1}), CGRP (3 ng kg^{-1} min^{-1}), adenosine (30 μg kg^{-1} min^{-1}) or cromakalim (3 μg kg^{-1} min^{-1}) produced significant reductions of blood pressure, accompanied by changes in HR.

Single bolus i.v. injections of CGRP (0.1 or 0.3 μg kg^{-1}), VIP (0.3 or 1 μg kg^{-1}), adenosine (10–100 μg kg^{-1}) and acetylcholine (0.1 μg kg^{-1}) elicited overt reductions of blood pressure, accompanied by a slight increase (for CGRP and VIP), a decrease (for large doses of adenosine) or virtually no change (for acetylcholine) in HR (Fig. 1). The magnitude of the vasodepressor responses to these agents was virtually the same before and during i.v. infusion of 0.9% saline solution (0.1 ml kg^{-1} min^{-1}) (groups I, II and III) (data not shown). Furthermore, the response to acetylcholine was not significantly modified when determined after VIP, CGRP, adenosine, cromakalim or glibenclamide.

3.1. Effects of an i.v. infusion of VIP on changes in blood pressure caused by bolus i.v. injections of CGRP and adenosine (groups IV and V)

The i.v. bolus injections of adenosine (3–100 μg kg^{-1}) elicited vasodepression in a dose-dependent fashion, with virtually no changes in HR, except for 30 (5–7% decrease) and 100 μg kg^{-1} (10–15% decrease). Single bolus i.v. injection of CGRP (0.1 μg kg^{-1}) caused also vasodepression, but this was accompanied by a slight HR increase (less than 5%). The vasodepressor responses to adenosine were significantly enhanced during an i.v. infusion of VIP (0.03 μg kg^{-1} min^{-1}). The doses of adenosine required to produce a 30-mmHg decrease in MAP before and during the VIP infusion were: 66.5 ± 5.2 μg kg^{-1} ($n = 5$); during, 34.5 ± 6.2 μg kg^{-1} ($n = 5$); respectively. The two

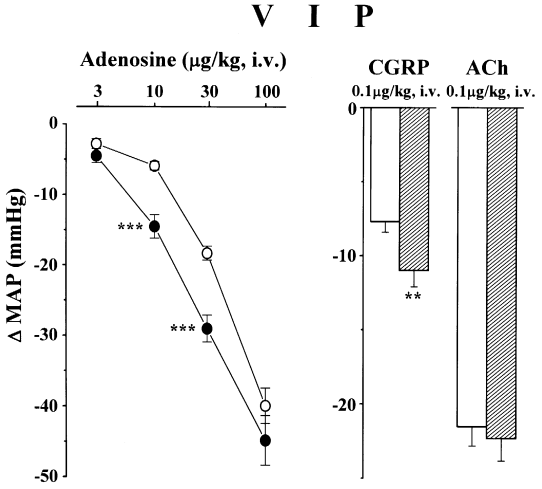

Fig. 2. Effects of i.v. infusion of VIP on peak decreases MAP caused by bolus i.v. injections of adenosine, CGRP and acetylcholine (ACh). ACh (0.1 μg kg^{-1}) and adenosine (3–100 μg kg^{-1}) or CGRP (0.1 μg kg^{-1}) were studied before (open columns, or ○) and during (hatched columns or ●) the infusion of VIP (0.03 μg kg^{-1} min^{-1}). Vertical bars represent means ± S.E.M. from five animals. $** P < 0.01$, $*** P < 0.001$, compared with the corresponding values before the treatment with VIP.

values are significantly ($P < 0.05$) different. Similarly, the vasodepressor response to CGRP was also significantly potentiated during an i.v. infusion of VIP, as summarized in Fig. 2. The changes in HR caused by adenosine and CGRP were not significantly modified by i.v. infusion of VIP (data not shown).

3.2. Effects of i.v. infusion of CGRP on changes in blood pressure caused by bolus i.v. injections of VIP and adenosine (groups VI and VII)

Just after the dose–response curve to adenosine (1–100 μg kg^{-1} i.v.) for vasodepression was recorded, i.v. infusion of CGRP at a rate of 1 ng kg^{-1} min^{-1} was started. CGRP shifted significantly the dose–vasodepressor response curve to the left of the control curve (Fig. 3). The doses of adenosine required to decrease the MAP by 30 mmHg before and during the CGRP infusion are 49.3 ± 5.5 μg kg^{-1} ($n = 5$), 20.5 ± 5.2 μg kg^{-1} ($n = 5$), respectively. The two values are significantly ($P < 0.01$) different. The vasodepression in response to a single bolus i.v. injection of VIP (0.3 μg kg^{-1}) was also significantly enhanced by the CGRP infusion (the right part in Fig. 3).

3.3. Effects of i.v. infusion of adenosine on changes in blood pressure induced by bolus i.v. injections of VIP and CGRP (groups VIII and IX)

Single bolus i.v. injections of VIP (0.3 μg kg^{-1}) and CGRP (0.1 μg kg^{-1}) reduced blood pressure, and slightly increased HR. Just after the effects of either VIP or CGRP, following i.v. injection of acetylcholine (0.1 μg kg^{-1}), had been examined, adenosine was infused i.v. at a rate of 3 μg kg^{-1} min^{-1}, and 15–20 min later, the same doses of acetylcholine and VIP or CGRP were given i.v. The vasodepressor response to VIP and CGRP, but not to acetylcholine, were significantly enhanced during adenosine infusion (Fig. 4).

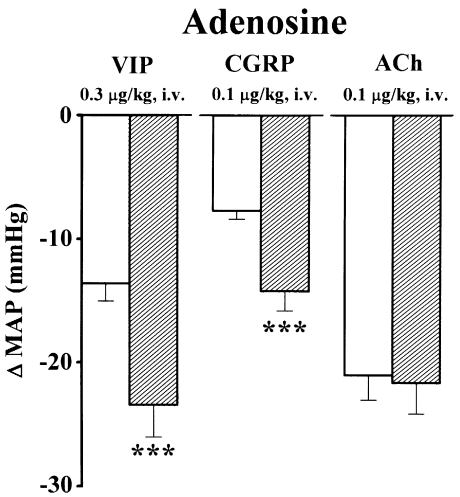

Fig. 4. Effects of i.v. infusion of adenosine on peak decrease in MAP elicited by bolus i.v. injections of VIP, CGRP and acetylcholine (ACh). Adenosine (3 μg kg^{-1} min^{-1}) was infused i.v. Vertical bars represent means ± S.E.M. from five animals. $***P < 0.001$, compared with the corresponding values before (open columns) the treatment with adenosine.

3.4. Effects of i.v. infusion of cromakalim on changes in blood pressure caused by bolus i.v. injections of VIP, CGRP, adenosine and acetylcholine (group X)

An i.v. infusion of cromakalim at a rate of 0.1 μg kg^{-1} min^{-1} significantly augmented the vasodepressor responses to VIP (1 μg kg^{-1}), CGRP (0.3 μg kg^{-1}) and

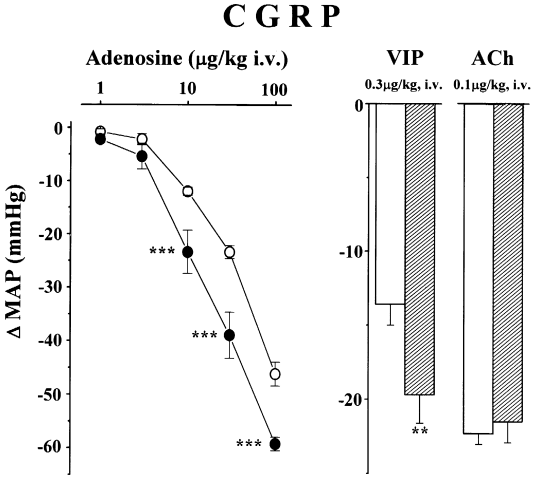

Fig. 3. Effects of i.v. infusion of CGRP on peak decreases in MAP caused by bolus i.v. injections of adenosine and VIP, acetylcholine (ACh). CGRP (1 ng kg^{-1} min^{-1}) was infused i.v. Vertical bars represent means ± S.E.M. from five animals. $**P < 0.01$, $***P < 0.001$, compared with the corresponding values before (open columns or ○) the treatment with CGRP.

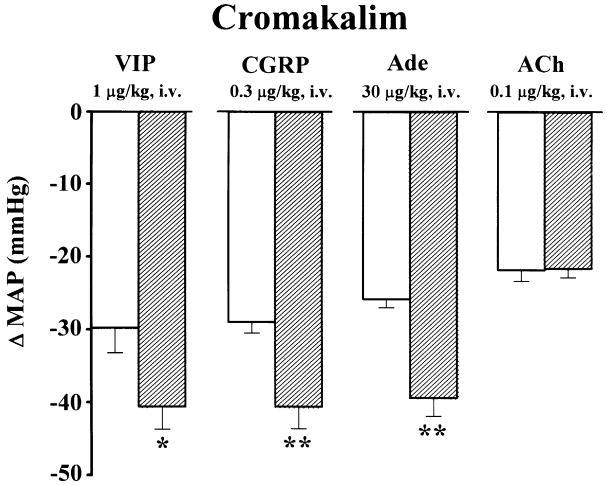

Fig. 5. Effects of i.v. infusion of cromakalim on peak changes in MAP caused by bolus i.v. injections of VIP, CGRP, adenosine (Ade) and acetylcholine (ACh). Cromakalim (0.1 μg kg^{-1} min^{-1}) was infused i.v. Vertical bars means ± S.E.M. from five animals. $*P < 0.05$, $**P < 0.01$, compared with the corresponding values before (open columns) the treatment with cromakalim.

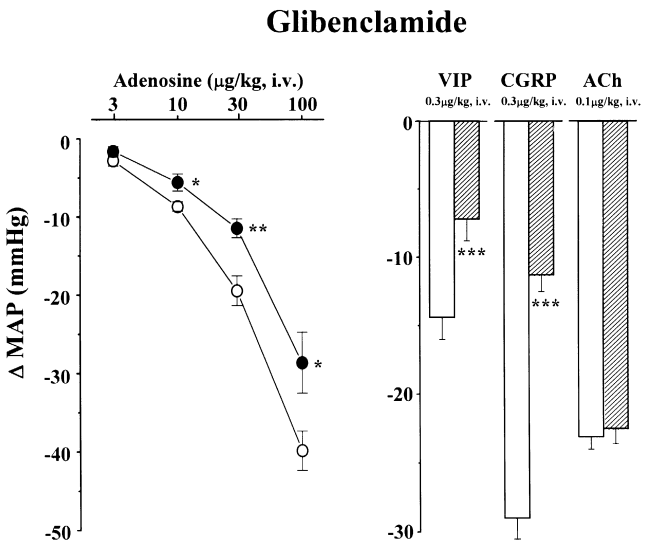

Fig. 6. Effects of a single bolus i.v. injection of glibenclamide on peak decreases in MAP caused by bolus i.v. injections of adenosine, VIP, CGRP and acetylcholine (ACh). Glibenclamide (20 mg kg^{-1}) was given i.v. for 5 min. Vertical bars means ± S.E.M. from five animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared with the corresponding values before (open columns or ○) the treatment with glibenclamide.

adenosine (30 μg kg^{-1}), but not to acetylcholine (0.1 μg kg^{-1}) (Fig. 5).

3.5. Effects of bolus i.v. injection of glibenclamide on changes in blood pressure caused by bolus i.v. injections of VIP, CGRP, adenosine and acetylcholine (groups XI and XII)

A single bolus i.v. injection of glibenclamide (20 mg/kg per 5 min) significantly attenuated the vasodepressor responses to VIP (0.3 μg kg^{-1}), CGRP (0.3 μg kg^{-1}) and adenosine (3–100 μg kg^{-1}). The response to acetylcholine (0.1 μg kg^{-1}) remained unchanged even after the treatment with glibenclamide. The doses of adenosine (44.2 ± 4.9 μg kg^{-1}; $n = 5$, 111.3 ± 20.6 μg kg^{-1}; $n = 5$, respectively) required to decrease the MAP by 30 mmHg before and after the treatment with glibenclamide were significantly different ($P < 0.05$) (Fig. 6). Furthermore, the reciprocal interactions among adenosine, VIP and CGRP in vasodepression did not occur after glibenclamide (20 mg kg^{-1}) (data not shown).

4. Discussion

Single bolus i.v. injections of VIP, CGRP, adenosine, and acetylcholine definitely reduced blood pressure in anesthetized rats. VIP and CGRP slightly increased HR, whereas adenosine, especially in larger doses studied, decreased it, whereas the dose of acetylcholine used did not modify this parameter. The present study indicates that VIP, CGRP and adenosine can reciprocally interact to lower blood pressure. Indeed, VIP enhanced the vasodepressor response to CGRP and adenosine, CGRP enhanced the response to VIP and adenosine, and adenosine increased the vasodepressor effects of VIP and CGRP.

Interestingly, the vasodepressor responses to VIP, CGRP and adenosine, but not to acetylcholine, were also significantly enhanced by cromakalim, an ATP-sensitive K^+ channel opener (Hamilton and Weston, 1989), while in agreement with current findings on VIP (Standen et al., 1989), CGRP (Nelson et al., 1990a) and adenosine (Daut et al., 1990; Belloni and Hintze, 1991), the vasodepressor effects of these agents were significantly attenuated by glibenclamide, an antagonist of ATP-sensitive K^+ channels. Furthermore, the interactions between adenosine, VIP and CGRP in decreasing blood pressure were blocked by glibenclamide (data not shown). This supports our recent reports (Sakai et al., 1998b; Saito and Sakai, 1998a,b) that nicorandil and cromakalim do not enhance the vasodepressor responses to adenosine, VIP and CGRP in animals pretreated with glibenclamide. Taken together, these findings may indicate that ATP-sensitive K^+ channels may play a role in the reciprocal interaction among these substances. Thus, as described by Nelson (1993), it seems that endogenous vasodilators such as VIP, CGRP and adenosine, cooperate in the control of vascular tone through a common pathway, which is the opening of ATP-sensitive K^+ channels.

The vasodilatation or vasodepression caused by adenosine (Kusachi et al., 1983; Cushing et al., 1991), CGRP (Edvinsson, 1985; Edwards et al., 1991; Jansen et al., 1992) and VIP (Edvinsson, 1985; Ignarro et al., 1987) has been proposed to be partly mediated by a cAMP-dependent mechanism. According to our recent study (Sakai et al., 1998a) using anesthetized rats, the vasodepressor response to isoproterenol, an adrenergic β-receptor stimulant, known to increase cAMP (Lefkowitz et al., 1995), was not affected by either cromakalim or glibenclamide. This supports the view that an increase in cAMP level does not participate to the vasodepression caused by VIP, CGRP and adenosine.

Cromakalim and pinacidil activate ATP-sensitive K^+ channels in vascular smooth muscle, whereas glibenclamide blocks them (Standen et al., 1989; Nelson et al., 1990b). On the other hand, vasorelaxation and hyperpolarization to VIP (Standen et al., 1989) and CGRP (Nelson et al., 1990a) appear to involve activation of peptide receptors on the vascular smooth muscle cells which appear to be coupled with the activation of ATP-sensitive K^+ channels, even though the nature of the coupling messenger system remains to be elucidated. Recently, we reported the potentiation of the adenosine-induced vasodepression by nicorandil and cromakalim, which may depend on adenosine A_2 receptor stimulation and vascular ATP-sensitive K^+ channels, since the potentiating effect of either cromakalim or nicorandil on the vasodepressor response to adenosine was not observed after the treatment with

glibenclamide or a selective antagonist of adenosine A_2 receptors (Sebastiao and Ribeiro, 1989), 3,7-dimethyl-1-propargylxanthine (DMPX) (Saito and Sakai, 1998c). Thus, it seems that VIP, CGRP, and adenosine activate ATP-sensitive K^+ channels through peptide and adenosine A_2 receptor stimulation, respectively and that these vasodilators activate distinct receptors which are coupled with ATP-sensitive K^+ channels.

Taken together, these findings indicate that through ATP-sensitive K^+ channels, these endogenous vasodilators intervene in the chemical communication between the different cell types of resistance arterioles. The present results lead also to propose that endogenous substances such as VIP, CGRP and adenosine—locally released at the level of the effector tissue, from stimulated nerve terminals or from organs—contribute to the physiological regulation of blood flow and vascular tone through reciprocal interaction.

In conclusion, the present results demonstrated that VIP, CGRP and adenosine interact reciprocally in vasodepression in rats. The reciprocal enhancement of blood pressure response is partly mediated through ATP-sensitive K^+ channels. Since these substances are endogenous physiological agents, it is reasonable to propose that this interaction may intervene in the regulation of regional blood flow during health and diseases.

Acknowledgements

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