ELSEVIER

European Journal of Pharmacology 344 (1998) 153–159

ejp

Synergistic effect of calcitonin gene-related peptide on adenosine-induced vasodepression in rats

Kazushige Sakai *, Keiji Saito, Michitaka Akima

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

Received 26 August 1997; revised 28 November 1997; accepted 2 December 1997

Abstract

The action of calcitonin gene-related peptide (CGRP) on the vasodepressor response to adenosine was investigated in anesthetized rats. I.v. bolus injections of adenosine (1–100 μg/kg), acetylcholine (0.05–0.4 μg/kg), isoproterenol (1–30 ng/kg), nitroglycerin (0.3–10 μg/kg) and diltiazem (10–300 μg/kg) produced dose-dependent decreases in blood pressure, accompanied by changes in heart rate. Only the vasodepressor response elicited by adenosine, among the agents tested, was significantly enhanced by i.v. infusion of either CGRP (1 ng/kg per min) or cromakalim (0.1 μg/kg per min), which possesses glibenclamide-sensitive K^+ channel opening activity. After i.v. treatment with glibenclamide (20 mg/kg), the vasodepressor responses not only to adenosine but also to CGRP (0.5 μg/kg) and cromakalim (30 μg/kg) were significantly reduced, while those to acetylcholine and isoproterenol remained unchanged. The result indicates that the enhancement of the adenosine-induced vasodepression by CGRP, like that elicited by cromakalim, seems to be mediated at least partly through ATP-sensitive K^+ channel activation. © 1998 Elsevier Science B.V.

Keywords: CGRP (calcitonin gene-related peptide)-adenosine interaction; K^+ channel; ATP-sensitive; Vasodepressor response

1. Introduction

It is well known that calcitonin gene-related peptide (CGRP) is a potent vasodilator (Shulkes, 1993) and that it is widely distributed in the central and peripheral nervous systems and in the cardiovascular system, particularly in sensory nerve endings in the adventitia of coronary arteries (Saito et al., 1986; Shoji et al., 1987). Adenosine, a product of purine metabolism, is a ubiquitous biological compound found in every cell of the human body and also plays an important physiological role in the cardiovascular system as well as a biological role in cellular metabolism (Mubagwa et al., 1996). Nelson (1993) described that a number of endogenous vasodilators act at least in part through membrane hyperpolarization caused by K^+ channel activation, and that many of these vasodilators, including CGRP and adenosine, cause hyperpolarization by activating ATP-sensitive K^+ channels in arterial smooth muscle. It was of interest, therefore, to investigate whether there are reciprocal interactions between these endogenous vasodilators, especially in the cardiovascular system.

The aim of the present study was to examine the possible interaction between CGRP and adenosine, by studying changes in blood pressure in response to bolus i.v. doses of adenosine before and during i.v. infusion of CGRP in anesthetized rats. Cromakalim, an ATP-sensitive K^+ channel opener (Hamilton and Weston, 1989), was taken as a reference compound.

2. Materials and methods

2.1. Animal preparations

All experiments were performed according to the regulations of the Animal Research Committee of the Chugai Pharmaceutical Co., Ltd., Tokyo, Japan. Male Sprague–Dawley rats (Charles River Japan, Atsugi, Kanagawa) weighing about 400 g were allowed free access to food and water. The experiments were carried out in three sets. In the first set of experiments, the rats were divided into 15 groups (each $n = 5$) as follows: the effects of bolus i.v. injections of adenosine, acetylcholine, isoproterenol, nitroglycerin and diltiazem on blood pressure and heart rate

* Corresponding author. Tel.: +81-3-39870863; fax: +81-3-39857079.

were examined in the 5 groups infused i.v. with 0.9% saline solution (0.1 ml/kg per min), in the 5 groups infused i.v. with cromakalim (0.1 μg/kg per min) and in the 5 groups infused i.v. with calcitonin gene-related peptide (CGRP)(1 ng/kg per min), respectively. After stabilization of the preparations, the dose–response curve for bolus i.v. injections of each agent was recorded and immediately i.v. infusion of either 0.9% saline solution, cromakalim or CGRP was started. About 10–20 min later, bolus doses of the same agent were given i.v. and the dose–response curve for the agent was recorded again. From the preliminary experiment, the dose of CGRP or cromakalim used was chosen as being the dose that enhanced the action of adenosine, without affecting basal blood pressure and heart rate. In the second set of experiments, the effects of glibenclamide were examined on the

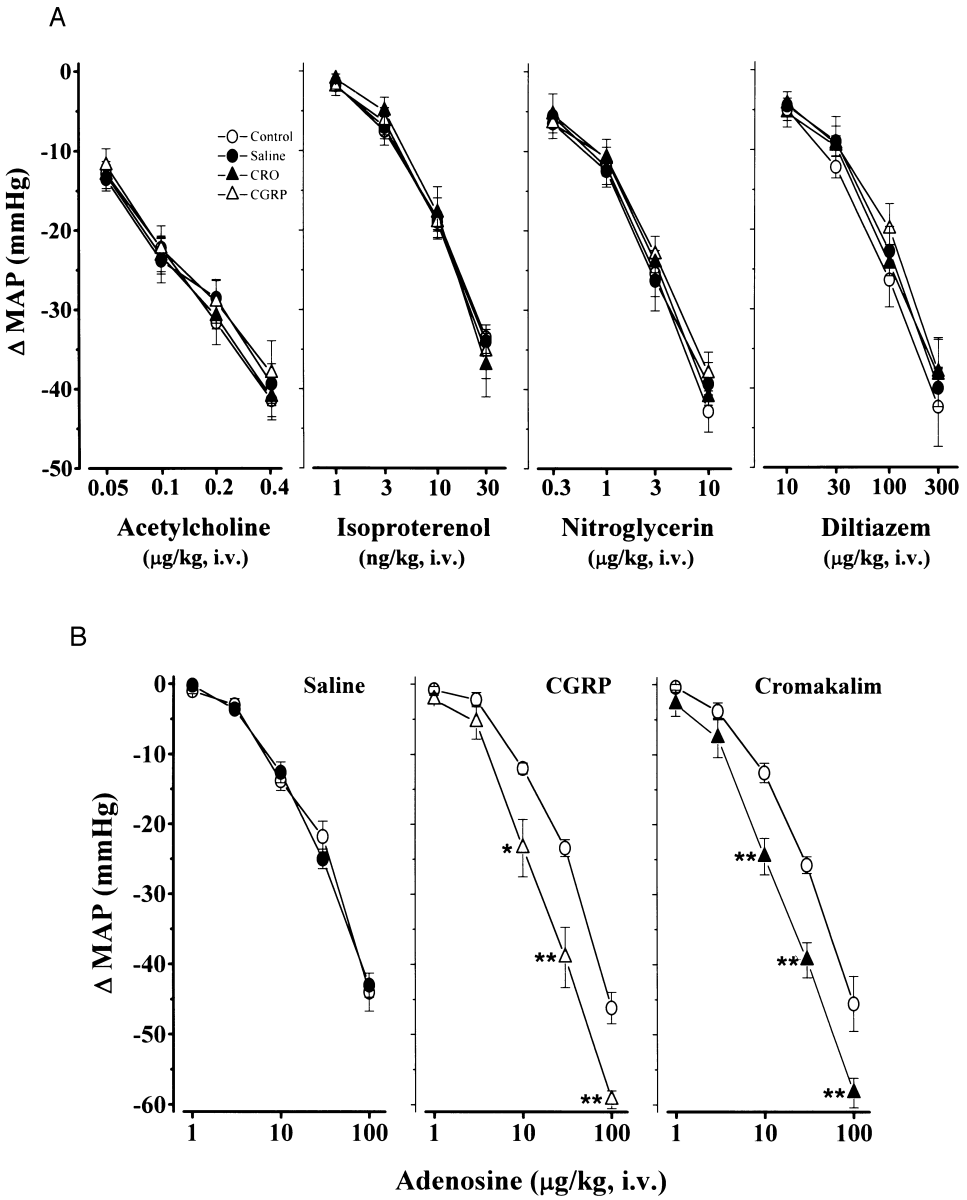

Fig. 1. Dose–response curves for peak decreases in mean systemic blood pressure (MAP) caused by bolus i.v. injections of acetylcholine, isoproterenol, nitroglycerin, diltiazem and adenosine before (control) and during i.v. infusion of either 0.9% saline solution, CGRP or cromakalim. (A) Immediately after the dose–response curves for bolus i.v. injections of acetylcholine, isoproterenol, nitroglycerin and diltiazem were recorded (control, ○), either 0.9% saline solution (0.1 ml/kg per min) (●), CGRP (1 ng/kg per min) (△) or cromakalim (0.1 μg/kg per min)(CRO, ▲) was infused i.v. and about 10–20 min later the dose–response curves for the tested agents were recorded again. (B) Just after the dose–response curve for adenosine was recorded (control, ○), each solution was infused i.v., as shown above, and the dose–response curve for adenosine was recorded again. Note that only the vasodepressor response to adenosine, of the agents tested, was significantly enhanced after the treatment with CGRP (△) and cromakalim (▲). The responses to the other agents remained unaltered before and during the infusion. Abscissa is shown in log scale. Vertical bars represent means ± S.E.M. from 5 animals. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the corresponding values from the control group (○).

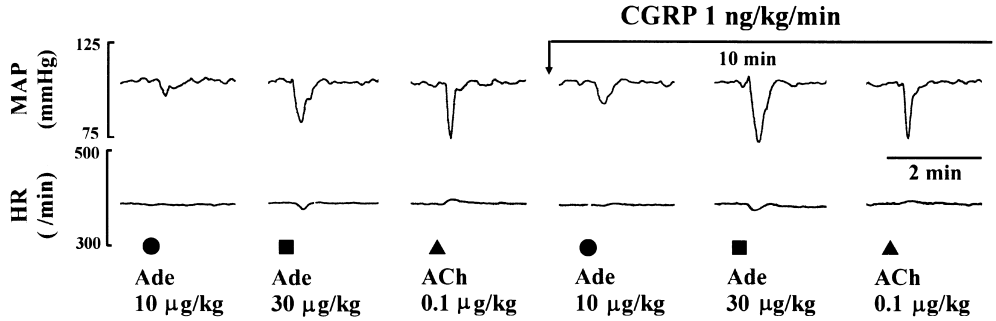

Fig. 2. Changes in mean systemic blood pressure (MAP) and heart rate (HR) caused by bolus i.v. injections of adenosine (Ade) and acetylcholine (ACh) in the absence and the presence of CGRP (1 ng/kg per min). I.v. bolus injections of adenosine (10 and 30 μg/kg) and acetylcholine (0.1 μg/kg) prominently reduced blood pressure and caused slight changes in heart rate. About 10 min after start of the i.v. infusion of CGRP at a rate of 1 ng/kg per min, the vasodepressor response to adenosine, but not to acetylcholine, was markedly enhanced. The effects of CGRP on changes in heart rate induced by adenosine were not clear.

vasodepressor responses to adenosine. After the dose–response curve for adenosine for the vasodepression was recorded, a single bolus dose of glibenclamide (20 mg/kg) was administered i.v. over 5 min to 5 rats and 5–10 min later the dose–response curve for adenosine was recorded again. In the third set of experiments, the effects of glibenclamide on the vasodepressor responses to bolus i.v. injections of acetylcholine, isoproterenol, cromakalim and CGRP were investigated in rats treated with 0.9% saline solution ($n = 5$) or glibenclamide ($n = 5$), as described above.

The animals were anesthetized initially with pentobarbital sodium (55 mg/kg i.p.) and an additional dose of pentobarbital (40 mg/kg) was injected s.c., as required. The polyethylene tubes (PE 10) were inserted into peripheral veins, usually the right and left femoral veins for drug bolus i.v. injections or infusion, respectively. For i.v. injection, 0.2 ml/kg of the drug solutions was given over a period of approximately 10 s and then the tubing was flushed with 0.9% saline solution. For i.v. infusion, drug solutions were given at a rate of 0.1 ml/kg per min by means of a Terumo syringe pump (STC-525, Tokyo, Japan). Systemic blood pressure was measured from the right femoral artery with a Nihon Kohden pressure transducer (DX-360, Tokyo, Japan). Heart rate was determined 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.

2.2. Drugs

The drugs used were as follows: human calcitonin gene-related peptide (CGRP, Peptide Institute, Osaka, Japan), cromakalim, adenosine free base and diltiazem hydrochloride (all from the Sigma Chemical Co., St. Louis, MO), acetylcholine chloride (RBI, Natick, MA), *l*-isoproterenol hydrochloride (Nikken Kagaku, Tokyo), nitroglycerin (ampoule, Nihon Kayaku, Tokyo) 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 (Furukawa et al., 1993). Cromakalim was freshly dissolved in 99.5% ethanol at a concentration of 5 mg/ml. These solutions were diluted with 0.9% saline solution to the desired concentrations, just before the experiment. Other compounds were dissolved in and diluted with 0.9% saline solution to the desired concentrations.

2.3. Data analysis

Values in the text are presented as means ± S.E.M. Peak vasodepressor responses to drugs are expressed as the

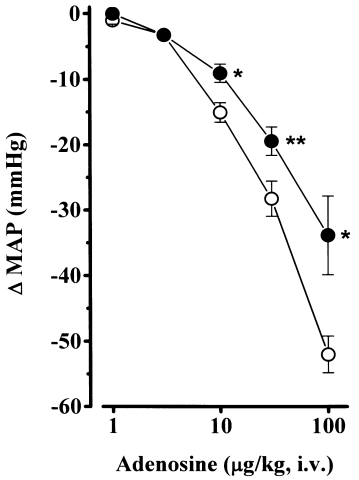

Fig. 3. Dose–response curve for peak decreases in mean systemic blood pressure (MAP) induced by bolus i.v. injections of adenosine before and after the i.v. treatment with a single bolus i.v. injection of glibenclamide. After the dose–response curve for adenosine was recorded (○), a single bolus dose of glibenclamide (20 mg/kg) was given i.v. over 5 min and about 10 min later the dose–response curve for adenosine was recorded again (●). The dose–response curve for adenosine was significantly shifted to the right after the treatment with glibenclamide. Vertical bars represent means ± S.E.M. from 5 animals. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the corresponding values from the control group (○). Abscissa is shown in log scale.

changes from preadministration levels. The doses of adenosine required to cause a 30 mmHg decrease in mean systemic blood pressure before and after the i.v. treatment with CGRP and glibenclamide were calculated from each dose–response curve to adenosine. Statistical evaluation was performed by use of Student's *t*-test for paired or

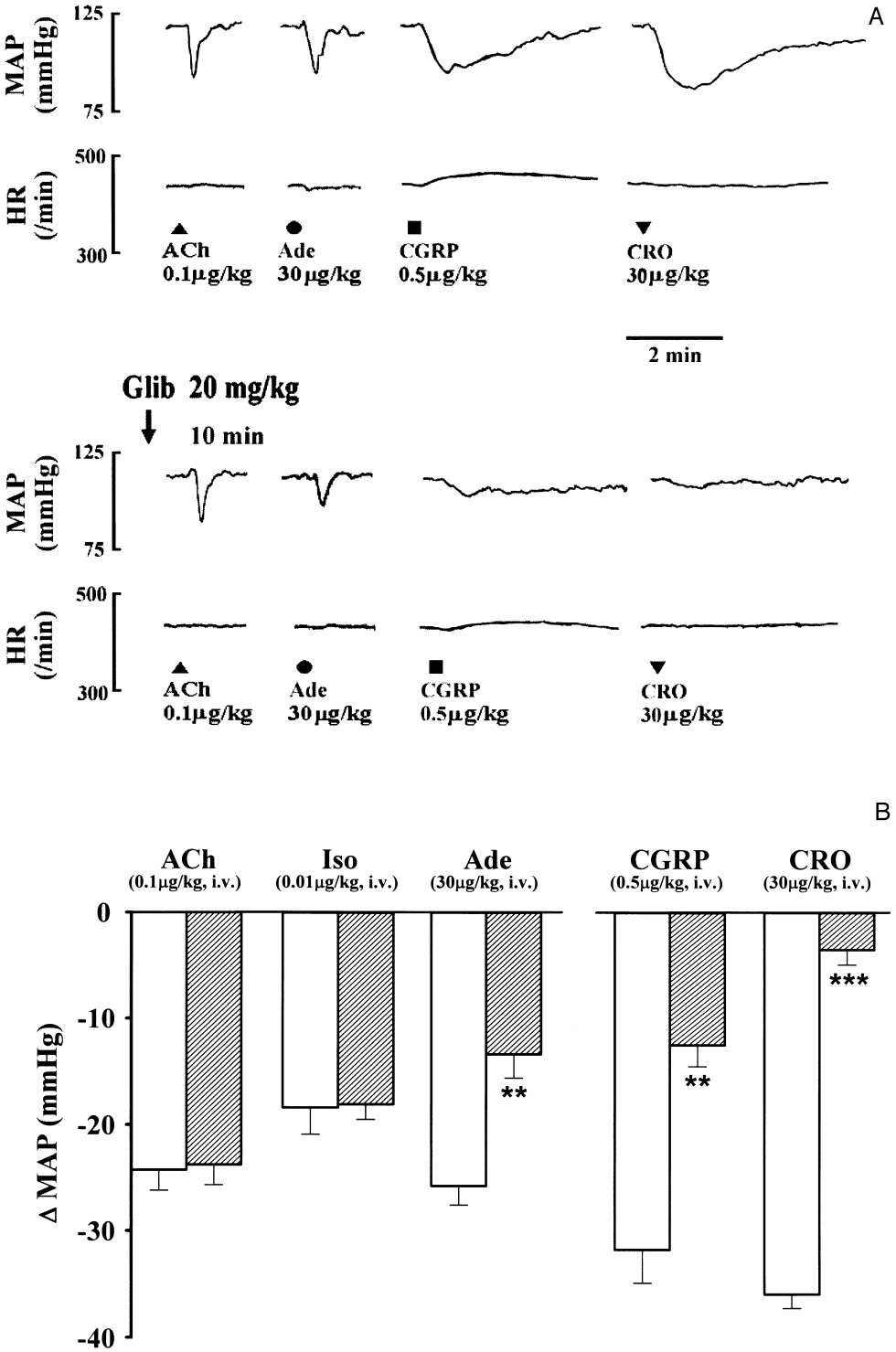

Fig. 4. Effects of a bolus i.v. injection of glibenclamide on mean systemic blood pressure (MAP) and heart rate (HR) induced by bolus i.v. injections of acetylcholine (ACh), isoproterenol (Iso), adenosine (Ade), CGRP and cromakalim (CRO). (A) A representative example. (B) Summarized data. Peak decreases in mean systemic blood pressure (MAP) induced by bolus i.v. injections of acetylcholine (0.1 μg/kg), isoproterenol (10 ng/kg), adenosine (30 μg/kg), CGRP (0.5 μg/kg) and cromakalim (30 μg/kg) before (open columns, control) and after (hatched columns) the i.v. treatment with glibenclamide are illustrated. See Fig. 3 for more details. Vertical bars means ± S.E.M. from 5 animals. $^{**}P < 0.01$, $^{***}P < 0.001$, compared with the corresponding values from the control group.

unpaired values; *P*-values lower than 0.05 were considered to be statistically significant.

3. Results

Baseline values of mean systemic blood pressure and heart rate in all of the rats tested ($n = 90$) were as follows: 115.8 ± 1.5 mmHg and 405.0 ± 4.0 beats/min, respectively, just before the first injection of drugs and 113.9 ± 1.0 mmHg and 401.0 ± 4.5 beats/min, respectively, just before the first injection of drugs following the start of the treatment with either 0.9% saline, cromakalim, CGRP or glibenclamide. No significant differences were observed between the corresponding values. Thus, the preparations remained stable throughout the entire experimental period.

3.1. Effects of cromakalim and CGRP on vasodepressor responses to adenosine, acetylcholine, isoproterenol, nitroglycerin or diltiazem

I.v. bolus injections of adenosine (1–100 μg/kg), acetylcholine (0.05–0.4 μg/kg), isoproterenol (1–30 ng/kg), nitroglycerin (0.3–10 μg/kg) and diltiazem (10–300 μg/kg) caused dose-dependent decreases in blood pressure, accompanied by changes (less than 10%, except for a 10–15% decrease produced by 100 μg/kg adenosine) in heart rate (Figs. 1 and 2). The dose–response curves for these agents causing the vasodepression remained unaltered before and during i.v. infusion of 0.9% saline solution at a rate of 0.1 ml/kg per min (Fig. 1A and B). As shown in Fig. 1B, the vasodepressor response to adenosine was significantly enhanced during the i.v. infusion of cromakalim (0.1 μg/kg per min) or CGRP (1 ng/kg per min), while the responses to acetylcholine, isoproterenol, nitroglycerin and diltiazem remained unmodified (Fig. 1A), despite infusion of cromakalim and CGRP at a rate which significantly augmented the vasodepressor response to adenosine. A representative result is shown in Fig. 2. The doses of adenosine required to cause a 30 mm Hg decrease in mean systemic blood pressure before and during the i.v. infusion of cromakalim or CGRP were calculated as follows: before, 45.0 ± 5.9 μg/kg; during, 18.3 ± 3.5 μg/kg; for cromakalim ($P < 0.01$ versus before): before, 50.4 ± 3.7 μg/kg; during, 20.2 ± 4.0 μg/kg and for CGRP ($P < 0.001$ versus before) (each $n = 5$).

3.2. Effects of glibenclamide on vasodepressor response to adenosine

After the dose–response curves for the vasodepressor response to bolus i.v. injection of adenosine (1–100 μg/kg) were recorded, a single bolus i.v. injection of glibenclamide (20 mg/kg) was given over 5 min. The dose of glibenclamide hardly affected basal blood pressure and heart rate. About 5–10 min later, the vasodepressor response to adenosine was significantly attenuated by the treatment with glibenclamide (Fig. 3). The doses of adenosine required to decrease the mean systemic blood pressure by 30 mm Hg before and after the treatment with glibenclamide were calculated as follows: before, 50.0 ± 8.2 μg/kg; after, 113.2 ± 22.2 μg/kg; $P < 0.05$ (each $n = 5$).

3.3. Effects of glibenclamide on vasodepressor responses to acetylcholine, isoproterenol, CGRP and cromakalim

Bolus doses of acetylcholine (0.1 μg/kg), isoproterenol (10 ng/kg), CGRP (0.5 μg/kg) and cromakalim (30 μg/kg) were administered i.v. As shown in Fig. 4A and B, these agents decreased the blood pressure by 20–40 mm Hg. About 10 min after the treatment with a single bolus i.v. dose of glibenclamide (20 mg/kg over 5 min), the vasodepressor responses to cromakalim and CGRP, like those to adenosine, were significantly attenuated, but the response to acetylcholine and isoproterenol remained virtually unaltered. It was confirmed that the vasodepressor responses to acetylcholine, isoproterenol, cromakalim and CGRP in the doses tested were not significantly changed in magnitude or duration before and after i.v. injection of 0.9% saline solution.

4. Discussion

The results of the present investigation revealed that bolus i.v. injections of adenosine, isoproterenol, acetylcholine, nitroglycerin and diltiazem elicited dose-dependent decreases in blood pressure, accompanied by changes in heart rate, in anesthetized rats. Only the vasodepressor response to adenosine was significantly enhanced by i.v. infusion of CGRP or cromakalim.

It has been reported that, like isoproterenol, an adrenergic β-receptor stimulant (Lefkowitz et al., 1995), CGRP elicits formation of cAMP in the vasculature (Edwards et al., 1991; Jansen et al., 1992) as well as in the myocardium (Ishikawa et al., 1987, 1988) and also that the interaction of adenosine with the A_2 receptor leads to a stimulation of adenylate cyclase activity and a subsequent increase in intracellular cAMP levels (Linden et al., 1993), resulting in cAMP-dependent vasodilatation (Silver et al., 1984). According to the investigation by Nelson et al. (1990) in rabbit mesenteric arteries, the vasorelaxation elicited by CGRP seems to be related to activation of ATP-sensitive K^+ channels. Kitazono et al. (1993), using a cranial window in anesthetized rats, examined the responses of the basilar artery to CGRP in vivo and obtained similar results and came to a similar conclusion as Nelson et al. (1990). The vasodilator mechanism of adenosine is thought in part to involve the opening of ATP-sensitive K^+ channels in vascular smooth muscle (Daut et al., 1990; Aversano et al., 1991; Belloni and Hintze, 1991). Thus, the vasodilatation

elicited by CGRP and adenosine seems to be mediated, in part, by a cAMP-dependent mechanism and activation of ATP-sensitive K^+ channels. The question arises as to whether the potentiation of adenosine action by CGRP is linked to ATP-sensitive K^+ channels or to an increase in cAMP levels.

In the present study, it was noted that the vasodepressor response to adenosine was significantly augmented by the treatment with cromakalim, an ATP-sensitive K^+ channel opener (Hamilton and Weston, 1989), as well as with CGRP and that the effects of adenosine, like those of CGRP and cromakalim, were markedly prevented by glibenclamide, an antagonist of ATP-sensitive K^+ channels (Cavero et al., 1989; Standen et al., 1989; Clapham et al., 1991), indicating that the opening of ATP-sensitive K^+ channels plays an important role in the interaction between adenosine and CGRP. The vasodepressor response to isoproterenol, an adrenergic β-receptor stimulant, like that of acetylcholine, nitroglycerin and diltiazem, was not influenced by treatment with either cromakalim and CGRP or glibenclamide. Thus, it appears that the enhancement of the adenosine-induced vasodepression by cromakalim and CGRP was elicited through a common mechanism, i.e. the synergistic activation of ATP-sensitive K^+ channels, but not by a cAMP-dependent mechanism.

It has been described that the vasorelaxation and hyperpolarization elicited by CGRP seem to involve activation of peptide receptors on vascular smooth muscle cells and subsequent second messenger activation of ATP-sensitive K^+ channels (Nelson, 1993). Even though cromakalim directly activates these channels, it is presumed that adenosine opens ATP-sensitive K^+ channels indirectly through activation of adenosine A_2 receptors (Saito and Sakai, 1998a,b). Thus, several endogenous vasodilators could synergistically amplify membrane hyperpolarization by directly or indirectly activating ATP-sensitive K^+ channels through different pathways in vascular smooth muscle cells, leading to more potent vasodilatation or vasodepression.

In summary, the present experiment demonstrated that CGRP augmented the vasodepressor response to adenosine, at least partly through activation of ATP-sensitive K^+ channels. This finding indicates that endogenous vasoactive substances may have reciprocal interactions in various organs, through ATP-sensitive K^+ channel activation and contribute to the regulation of the cardiovascular system.

References

Aversano, T., Ouyang, P., Silverman, H., 1991. Blockade of the ATP-sensitive potassium channel modulates reactive hyperemia in the canine coronary circulation. Circ. Res. 69, 618–622.

Belloni, F.L., Hintze, T.H., 1991. Glibenclamide attenuates adenosine-induced bradycardia and coronary vasodilatation. Am. J. Physiol. 261, H720–H727.

Cavero, I., Mondot, S., Mestre, M., 1989. Vasorelaxant effects of cromakalim in rats are mediated by glibenclamide-sensitive potassium channels. J. Pharmacol. Exp. Ther. 248, 1261–1268.

Clapham, J.C., Hamilton, T.C., Longman, S.D., Buckingham, R.E., Campbell, C.A., 1991. Antihypertensive and hemodynamic properties of the potassium channel activating (−)enantiomer of cromakalim in animal models. Arzneim. Forsch. 41, 385–391.

Daut, J., Maier-Rudolph, W., von Beckerath, N., Mehrke, G., Gunther, K., Goedel-Meinen, L., 1990. Hypoxic dilation of coronary arteries is mediated by ATP-sensitive potassium channels. Science 247, 1341–1344.

Edwards, R.M., Stack, E.J., Trizna, W., 1991. Calcitonin gene-related peptide stimulates adenylate cyclase and relaxes intracerebral arterioles. J. Pharmacol. Exp. Ther. 257, 1020–1024.

Elhawary, A.M., Poon, J., Pang, C.C.Y., 1995. Effects of calcitonin gene-related peptide receptor antagonists on renal actions of adrenomedullin. Br. J. Pharmacol. 115, 1133–1140.

Furukawa, S., Satoh, K., Taira, N., 1993. Opening of ATP-sensitive K^+ channels responsible for adenosine A_2 receptor-mediated vasodepression does not involve a pertussis toxin-sensitive G protein. Eur. J. Pharmacol. 236, 255–262.

Hamilton, T.C., Weston, A.H., 1989. Cromakalim, nicorandil and pinacidil: Novel drugs which open potassium channels in smooth muscle. Gen. Pharmacol. 20, 1–9.

Ishikawa, T., Okamura, N., Saito, A., Goto, K., 1987. Effects of calcitonin gene-related peptide (CGRP) and isoproterenol on the contractility and adenylate cyclase activity in the rat heart. J. Mol. Cell. Cardiol. 19, 723–727.

Ishikawa, T., Okamura, N., Saito, A., Masaki, T., Goto, K., 1988. Positive inotropic effect of calcitonin gene-related peptide mediated by cyclic AMP in guinea-pig heart. Circ. Res. 63, 726–734.

Jansen, I., Mortensen, A., Edvinsson, L., 1992. Characterization of calcitonin gene-related peptide receptors in human cerebral vessels: Vasomotor responses and cAMP accumulation. Ann. N. Y. Acad. Sci. 657, 435–440.

Kitazono, T., Heistad, D.D., Faraci, F.M., 1993. Role of ATP-sensitive K^+ channels in CGRP-induced dilatation of basilar artery in vivo. Am. J. Physiol. 265, H581–H585.

Lefkowitz, R.J., Hoffman, B.B., Taylor, P., 1995. The autonomic and somatic motor nervous systems. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, P.W., Gilman, A.G. (Eds.), The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp. 127–128.

Linden, J., Tucker, A.L., Robeva, A.S., Graber, S.G., Munshi, R., 1993. Properties of recombinant adenosine receptors. Drug Dev. Res. 28, 232–236.

Mubagwa, K., Mullane, K., Flameng, W., 1996. Role of adenosine in the heart and circulation. Cardiovasc. Res. 32, 797–813.

Nelson, M.T., 1993. The role of potassium channels in the regulation of peripheral resistance. In: Escande, D., Standen, N. (Eds.), K^+ Channels in Cardiovascular Medicine. Springer-Verlag, France, pp. 95–106.

Nelson, M.T., Huang, Y., Brayden, J.E., Hescheler, J., Standen, N.B., 1990. Arterial dilations in response to calcitonin gene-related peptide involve activation of K^+ channels. Nature 344, 770–773.

Saito, K., Sakai, K., 1998a. Enhancement of the vasodepressor response to adenosine by nicorandil in rats: Comparison with cromakalim. Fundam. Clin. Pharmacol. 12, 37–43.

Saito, K., Sakai, K., 1998b. Potentiating effects of nicorandil on the adenosine A_2 receptor-mediated vasodepression in rats: Potential role for K_{ATP} channels. Fundam. Clin. Pharmacol. In press.

Saito, A., Kimura, S., Goto, K., 1986. Calcitonin gene-related peptide as potential neurotransmitter in guinea pig right atrium. Am. J. Physiol. 250, H693–H698.

Shoji, T., Ishihara, H., Ishihara, T., Saito, A., Goto, K., 1987. Vasodilating effects of human and rat calcitonin gene-related peptides in isolated porcine coronary arteries. Naunyn Schmiedebergs Arch. Pharmacol. 336, 438–444.

Shulkes, A., 1993. Calcitonin gene-related peptide: Potential role in vascular disorders. Drugs Aging 3, 189–194.

Silver, P.J., Walus, K., DiSalvo, J., 1984. Adenosine-mediated relaxation and activation of cyclic AMP-dependent protein kinase in coronary arterial smooth muscle. J. Pharmacol. Exp. Ther. 228, 342–347.

Standen, N.B., Quayle, J.M., Davies, N.W., Brayden, J.E., Huang, Y., Nelson, M.T., 1989. Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. Science 245, 177–180.