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Differential Effects of Nicorandil on the Vasodepressor Responses to Vasoactive Polypeptides Administered Intravenously to Rats

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Abstract

The effects of nicorandil on vasodepressor responses to vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP) and substance P have been examined in anaesthetized rats.

Intravenous bolus injections of VIP (0·3 $\mu g\ kg^{-1}$), CGRP (0·1 $\mu g\ kg^{-1}$) and substance P (0·1 $\mu g\ kg^{-1}$) induced reductions of blood pressure accompanied by slight increases (less than 5%) in heart rate. Nicorandil infused intravenously at 10 or 30 $\mu g\ kg^{-1}\ min^{-1}$ significantly augmented the vasodepressor responses to VIP and CGRP but did not modify the responses to substance P and acetylcholine (0·1 $\mu g\ kg^{-1}$). Intravenous treatment with glibenclamide (20 $\mu g\ kg^{-1}$) significantly attenuated not only the vasodepression caused by VIP and CGRP, but also the enhancement of the effects of the agents by nicorandil.

These results indicate that nicorandil can enhance the action of VIP and CGRP, in rats, at least partly through ATP-sensitive K^+-channel activation.

It has recently been reported that abundant sensory-motor nerve fibres containing peptides, such as calcitonin gene-related peptide (CGRP), substance P and vasoactive intestinal polypeptide (VIP), are widely distributed in vascular smooth muscle and in the heart, and might be involved in the physiological control of vascular tone and blood flow (Rubino & Burnstock 1996). Vascular smooth muscle cells can be stimulated or inhibited by a variety of vasoactive agents that activate a network of signal-transduction pathways. In the cardiovascular system, CGRP and substance P have been shown to co-exist in many nerve terminals contained in the same granular vesicles, probably providing the morphological basis for their co-release (Gibbins et al 1985; Gulbenkian et al 1986). Thus, through these endogenous vasodilators, reciprocal interactions (cross-talk) between different types of cell might be responsible for physiological control of the cardiovascular system. It was of interest, therefore, to investigate a possible interaction between endogenous vasodilators and cardiovascular drugs. In this study we have examined the effect of nicorandil, an orally efficacious anti-anginal drug (Sakai 1989; Kinoshita & Sakai 1990; Krumenacker & Roland 1992) with the properties of an ATP-sensitive K^+-channel opener and of a nitrate (Taira 1989), on vasodepressor responses to VIP, CGRP and substance P in anaesthetized rats.

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Materials and Methods

Nicorandil (*N*-(2-hydroxyethyl)nicotinamide nitrate ester) was synthesized in the Chugai Organic Chemistry Laboratory. Human and porcine vasoactive intestinal polypeptide (VIP) and human calcitonin gene-related peptide (CGRP) were from The Peptide Institute (Osaka, Japan), substance P was from Bachem (Bubendorf, Switzerland), acetyl choline chloride was from RBI (Natick, MA, USA) and glibenclamide was from Wako Junyaku (Osaka, Japan). Glibenclamide was dissolved in NaOH (0·1 M; 1 mL) then glucose solution (5%; 4 mL) was slowly added under sonication to give a final concentration of 5 mg mL^{-1} (Furukawa et al 1993). CGRP was dissolved in distilled water and diluted with 0·45% saline solution, according to the report of Elhawary et al (1995). Other compounds were dissolved in 0·9% NaCl solution (saline) and diluted with the same solution to the desired concentrations; fresh solutions were prepared immediately before the experiments.

Experiments were performed in accordance with the Guidelines for Animal Experiments of Chugai Pharmaceutical, Tokyo, Japan, and the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society. Male Sprague-Dawley rats (Charles River Japan, Atsugi, Kanagawa), approximately 350 g, were allowed free access to food and water. The animals were initially anaesthetized with pentobarbitone-Na (55 mg kg^{-1}, i.p.); a supplementary dose of pentobarbitone (40 mg kg^{-1}, s.c.) was then given as necessary to maintain stable anaesthesia. Two polyethylene tubes (PE 10) were inserted into the left jugular vein for intravenous drug bolus injection and infusion. The right femoral artery was cannulated for measurement of systemic blood pressure and heart rate. After surgery a period of approximately 30 min was allowed for stabilization of preparations. Systemic blood pressure was measured with a Nihon Kohden (Tokyo, Japan) DX-360 pressure-transducer, heart rate by means of a heart-rate counter (Nihon Kohden; AT-601G). All data were recorded on a chart by use of a Graphtec (Tokyo, Japan) WR-3101 linearcorder.

The experiments were performed in three sets. Because tachyphylaxis might be developed after high doses of, or consecutive administration of, the polypeptides (Rubino & Burnstock 1996), dose-response curves for the agents tested were not constructed and instead the agents were given as follows: in a single preparation, a single bolus injection (0·2 mL kg^{-1}over 10 s) of a relatively low dose of either intestinal vasoactive polypeptide (VIP; 0·3 $\mu g\ kg^{-1}$), calcitonin gene-related peptide (CGRP; 0·1 $\mu g\ kg^{-1}$) or substance P (0·1 $\mu g\ kg^{-1}$) after a single intravenous bolus injection of acetylcholine (0·1 $\mu g\ kg^{-1}$). Continuous intravenous infusion of 0·9% saline or nicorandil solution was then started at 0·1 mL $kg^{-1}\ min^{-1}$ by means of a Terumo (Tokyo, Japan) STC-525 syringe pump, and 20–30 min later acetylcholine (0·1 $\mu g\ kg^{-1}$), then the same dose of VIP, CGRP or substance P, were again injected intravenously. In principle, the administration of each agent was time-matched.

In a first set of experiments, the animals were divided into three groups (n = 7 for each). Immediately after intravenous administration of bolus doses of acetylcholine and VIP, 0·9% saline solution was infused intravenously at 0·1 mL $kg^{-1}\ min^{-1}$, and 20–30 min later the same doses of acetylcholine and VIP, in that order, were again given intravenously. Similarly, the vasodepressor effects of CGRP and substance P were examined before and during intravenous infusion of 0·9% saline solution. In a second set of experiments the rats were divided into three groups (again n = 7) and nicorandil solution, instead of 0·9% saline, was infused intravenously at 10 or 30 $\mu g\ kg^{-1}\ min^{-1}$. From our recent study (Saito & Sakai 1997) the dose of nicorandil used for intravenous infusion was selected as that causing enhancement of adenosine action but hardly affecting basal systemic blood pressure and heart rate. In a third set of experiments the rats were also divided into three groups (n = 7), and a single bolus dose of glibenclamide (20 mg kg^{-1}) was given intravenously over 5 min. Before and after administration single bolus doses of either VIP, CGRP or substance P, and acetylcholine were administered intravenously. Thereafter, intravenous infusion of nicorandil was started and approximately 20 min later the same dose of VIP or CGRP was again given intravenously.

Values in the text are expressed as means ± s.e.m. Peak vasodepressor responses to the agents are expressed as changes from pre-administration levels. Statistical evaluation was performed by use of Student's *t*-test for paired values. A P value $< 0·05$ was considered to be indicative of statistical significance.

Results

The basal values of mean systemic blood pressure and heart rate of the rats (n = 63) were 118·4 ± 1·7 mm Hg and 409·7 ± 6·0 beats min^{-1}, respectively, just before the first injection of the agents and 116·4 ± 1·9 mm Hg and 407·6 ± 7·9 beats min^{-1}, respectively, just before the first injection of the agents after the start of intravenous treatment with 0·9% saline (0·1 mL $kg^{-1}\ min^{-1}$), nicorandil (10 or 30 $\mu g\ kg^{-1}\ min^{-1}$) or glibenclamide (20 mg kg^{-1} over 5 min). No significant differences were observed between the corresponding values and so the preparations remained stable through the entire experimental periods.

For all the rats tested single intravenous bolus doses of VIP (0·3 $\mu g\ kg^{-1}$), CGRP (0·1 $\mu g\ kg^{-1}$), and substance P (0·1 mg kg^{-1}) elicited substantial vasodepression, with slight increases (less than 5%) in heart rate; acetylcholine (0·10 $\mu g\ kg^{-1}$) has a similar effect. The vasodepressor response to each agent remained virtually unchanged before and during intravenous infusion of 0·9% saline solution, as shown in Table 1. The vasodepressor responses to VIP and CGRP were significantly enhanced by intravenous infusion of nicorandil (10 or 30 $\mu g\ kg^{-1}\ min^{-1}$) whereas responses to substance P and acetylcholine remained unchanged, as shown in Figure 1 and Table 1. Infusion of nicor-

Table 1. Effects of nicorandil and glibenclamide on the peak reductions of mean systemic blood pressure caused by vasoactive intestinal polypeptide, calcitonin gene-related peptide, substance P and acetylcholine given intravenously to rats.

Treatment	Vasoactive intestinal polypeptide (0·3 μg kg^{-1})	Calcitonin gene-related peptide (0·1 μg kg^{-1})	Substance P (0·1 μg kg^{-1})	Acetylcholine (0·1 μg kg^{-1})
None	−29·4 ± 2·0	−17·0 ± 1·0	−28·1 ± 1·3	−21·9 ± 1·5
Saline	−30·7 ± 2·0	−17·8 ± 1·2	−27·6 ± 1·3	−21·4 ± 0·9
Nicorandil	−41·3 ± 2·3‡	−27·9 ± 2·5	−27·3 ± 1·2	−20·7 ± 0·9
Glibenclamide	−10·0 ± 1·3‡	−6·6 ± 1·2‡	−26·3 ± 1·7	−19·9 ± 1·0
Glibenclamide + nicorandil	−11·1 ± 1·1‡	−7·1 ± 1·2‡	−27·9 ± 1·4	−21·1 ± 0·9

Intravenous infusions of 0·9% saline (control) and nicorandil or glibenclamide solution, or both, were performed at 0·1 mL kg^{-1} min^{-1} and 10 or 30 μg kg^{-1} min^{-1}, respectively (see text for experimental protocol and basal values of mean blood pressure). Values are means ± s.e.m. (n = 7). *P < 0·05, ‡P < 0·01 significantly different from the corresponding result from saline-treated group.

andil had no influence on basal systemic blood pressure or heart rate. On the other hand, treatment with glibenclamide (20 mg kg^{-1}, i.v.), which did not affect basal systemic blood pressure and heart rate, significantly inhibited not only the responses to VIP and CGRP, but also the augmentation of the effects of the agents by nicorandil (Table 1). It was confirmed that the effect of glibenclamide lasted for more than 1 h.

Discussion

Vasodepressor responses to VIP and CGRP were significantly enhanced during intravenous infusion of nicorandil whereas responses to substance P and acetylcholine remained virtually unchanged. It is widely accepted that nicorandil has ATP-sensitive K^+-channel opening activity and nitrate-like properties (Taira 1989). As has been clearly revealed by our recent investigation (Saito & Sakai 1998), the vasodepressor response of rats to a single bolus injection of nicorandil (300 μg kg^{-1}, i.v.) was significantly attenuated by treatment with glibenclamide (20 mg kg^{-1}, i.v.), an antagonist of ATP-sensitive K^+ channels (Cavero et al 1989; Standen et al 1989), indicating that the opening of ATP-sensitive K^+ channels is involved in the

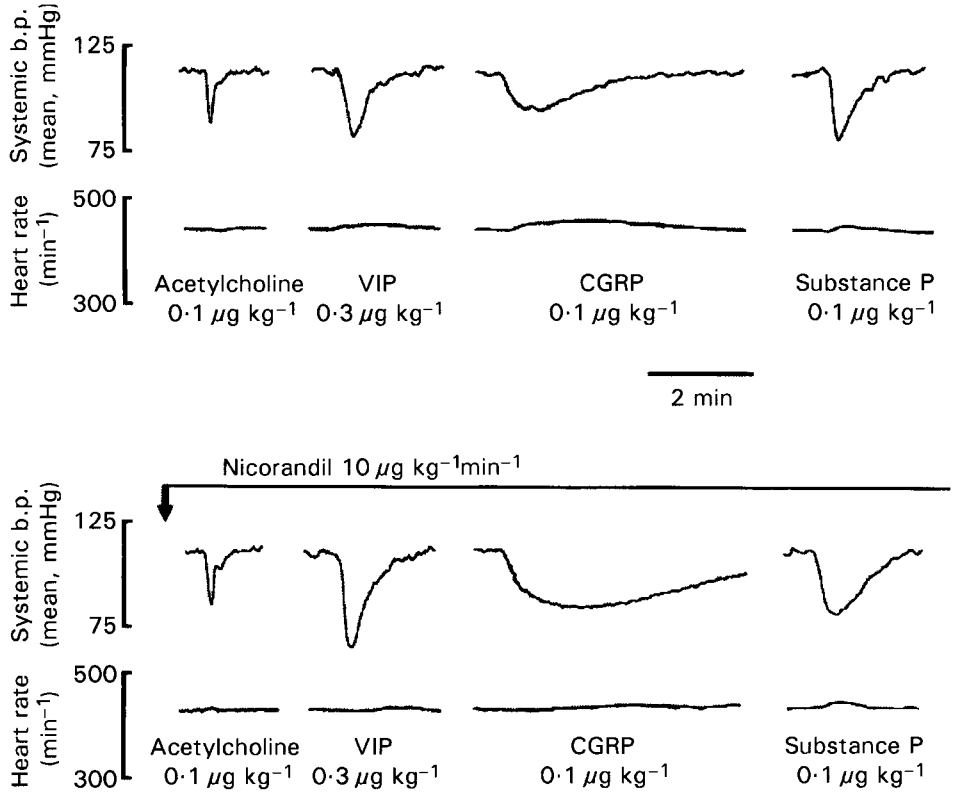

Figure 1. Effects of vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), substance P and acetylcholine on mean systemic blood pressure (b.p.) and heart rate before and during intravenous infusion of nicorandil solution.

mechanism of nicorandil-mediated vasodepression. This finding was in agreement with results obtained by Borg et al (1991).

It has been reported that hyperpolarization and part of the vasorelaxation induced by the neuropeptides CGRP (Nelson 1993) and VIP (Standen et al 1989) were blocked by glibenclamide but were not affected by blockers of Ca^{2+}-activated K^+ channels. In the current experiment we also found that vasodepressor responses to VIP and CGRP were significantly attenuated by administration of glibenclamide. Nelson (1993) has recently reported that several endogenous vasodilators act at least in part through membrane hyperpolarization caused by K^+-channel activation, and many of these vasodilators hyperpolarize by activating ATP-sensitive K^+ channels in vascular smooth muscle. According to Nelson vasorelaxation and hyperpolarization in response to CGRP and VIP seem to involve activation of peptide receptors on vascular smooth-muscle cells and subsequent second-messenger activation of ATP-sensitive K^+ channels. Thus, it is assumed that potentiation of vasodepressor responses to CGRP and VIP by nicorandil occurs through a common mechanism, i.e. opening of ATP-sensitive K^+ channels. Indeed, the enhancement of the vasodepressor response to CGRP and VIP by nicorandil was not observed after treatment with glibenclamide.

In conclusion, this study clearly revealed that nicorandil significantly potentiated vasodepressor responses to VIP and CGRP in anaesthetized rats, but not those to substance P and acetylcholine. It seems that nicorandil, in common with the ATP-sensitive K^+-channel openers cromakalim and pinacidil (Hamilton & Weston 1989), directly activates ATP-sensitive K^+ channels, whereas several endogenous vasodilators, for example VIP and CGRP, stimulate the channels indirectly, probably by release of second messengers as a result of activation of peptide receptors on vascular smooth-muscle cells (Nelson 1993). It is possible, therefore, that enhancement of the vasodepressor response occurs as a result of synergistic activation with nicorandil and CGRP or VIP on ATP-sensitive K^+ channels in vascular smooth muscle.

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