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Involvement of calcitonin gene-related peptide in the development of tolerance to nitroglycerin in the rat

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Abstract

Previous studies have shown that the depressor effect of nitroglycerin is related to stimulation of endogenous calcitonin gene-related peptide (CGRP) release. In the present study, we explored whether endogenous CGRP is involved in the development of tolerance to nitroglycerin in the rat. Tolerance was induced by treatment with nitroglycerin (10 mg/kg, subcutaneous [s.c.]) three times a day for 8 days and confirmed by a reduction in hypotensive responses to intravenous (i.v.) nitroglycerin. Nitroglycerin (30 or 150 μg/kg, i.v.) significantly decreased blood pressure concomitantly with an increase in plasma concentration of nitric oxide (NO) and CGRP, and these effects of nitroglycerin disappeared after pretreatment with nitroglycerin for 8 days. However, the nitroglycerin-induced depressor effect and elevation of NO and CGRP content were restored, partially or completely, 4 or 8 days after nitroglycerin removal in the tolerant rat. The present study suggests that the development of tolerance to nitroglycerin is related to the decreased release of CGRP in the rat.

Keywords: Tolerance; Nitroglycerin; CGRP, calcitonin gene-related peptide; NO, nitric oxide; Rat

1. Introduction

Nitroglycerin is a classical drug used against angina pectoris. However, long-term administration of nitroglycerin can lead to tolerance development. The mechanisms responsible for tolerance to nitroglycerin are not clearly understood. There is substantial evidence to suggest that nitrate tolerance may be associated with a reduction of the content and activation of nitric oxide (NO) released from parent compounds (Persson et al., 1995; Slack et al., 1988; Munzel et al., 2000).

Calcitonin gene-related peptide (CGRP), the principal transmitter in capsaicin-sensitive sensory nerves, is widely distributed in vascular tissues and is a potent vasodilator (Franco-Cereceda, 1988). Results of previous investigations have suggested that nitroglycerin activates capsaicin-sensitive sensory nerves (Wei et al., 1992; Booth et al., 1997). Recently, our work has shown that the depressor effect of nitroglycerin is mediated by endogenous CGRP (Zhou et al., in press). In the present study, therefore, we examined whether the development of tolerance to nitroglycerin is related to endogenous CGRP.

2. Materials and methods

Animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 86-23, Revised 1986).

2.1. Nitroglycerin-induced tolerance

Male Wistar rats weighing 280–320 g were obtained from the Hunan Medical University Animal Center. Tolerance was induced by treatment with nitroglycerin (10 mg/kg, subcutaneous [s.c.]) three times a day for 8 days and its existence was confirmed by a reduction in hypotensive responses to intravenous (i.v.) nitroglycerin. In the control group, rats were given s.c. injections of 1% ethanol

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(0.5 ml/kg). In the case of the recovery of drug tolerance, the depressor effect of nitroglycerin was determined 4 or 8 days after drug removal in tolerant rats.

The rats were anesthetized with sodium pentobarbital (60 mg/kg). A polyethylene (PE 50) catheter was inserted into the left femoral artery to record blood pressure. An additional catheter was inserted into the right femoral artery for withdrawal of a reference arterial blood sample. Drugs were administered through a cannula inserted into the right femoral vein. After surgical procedures, at least 10 min was allowed for stabilization. Blood pressure was continuously monitored. The resulting electric signals were digitized by a MacLab analog to a digital converter and recorded by a Power Macintosh 7220 computer.

2.2. Measurement of plasma CGRP concentration

After a maximal depressor response to i.v. nitroglycerin was reached, blood samples (3 ml) were collected rapidly from the right femoral artery into tubes containing 10% Na_2EDTA 40 μl and aprotinin 500 mU/l. Plasma was obtained by centrifugation at 3000 rpm for 10 min at 4 °C. CGRP-like immunoreactivity in the plasma was measured

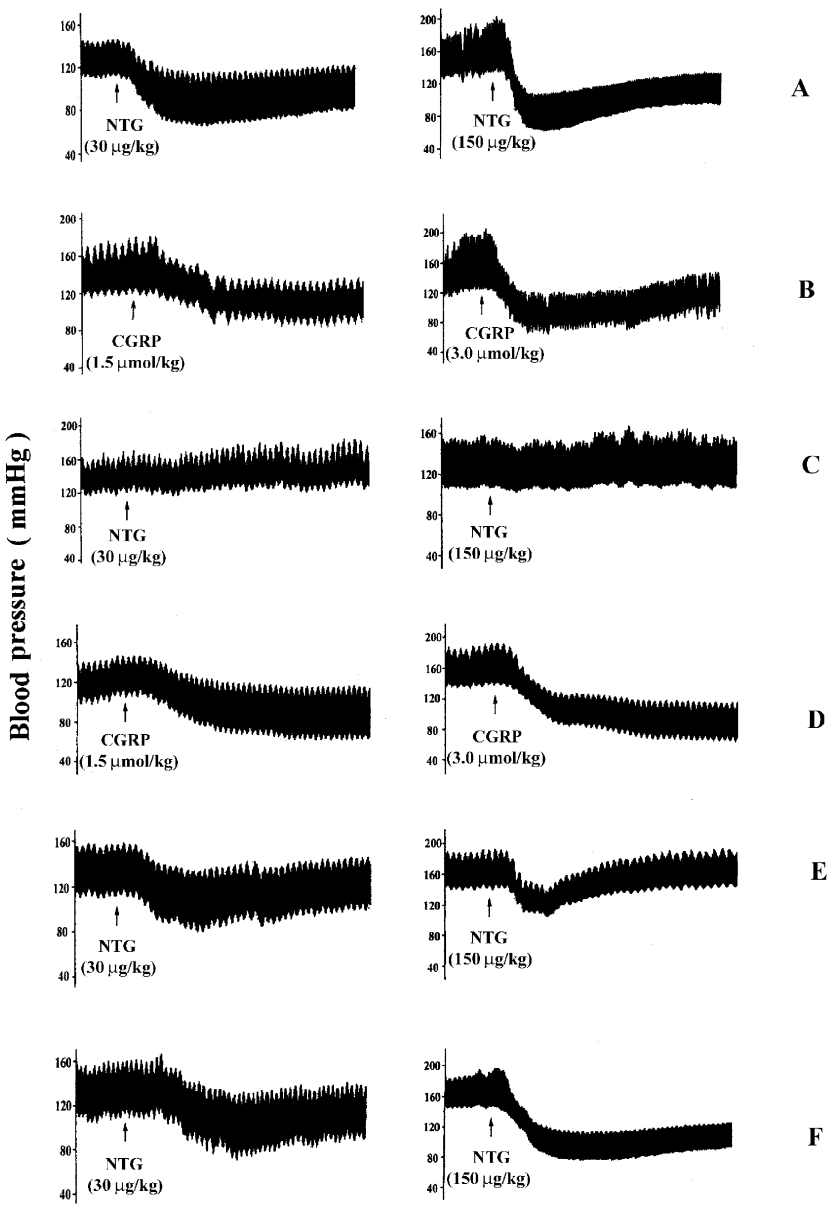

Fig. 1. Typical records of depressor effect induced by nitroglycerin in rats. (A and B) Nitroglycerin or CGRP was given intravenously. (C and D) Nitroglycerin or CGRP was given intravenously after pretreatment with nitroglycerin (10 mg/kg, s.c.) three times a day for 8 days. (E and F) Nitroglycerin was given intravenously 4 or 8 days after drug removal in the tolerant rat.

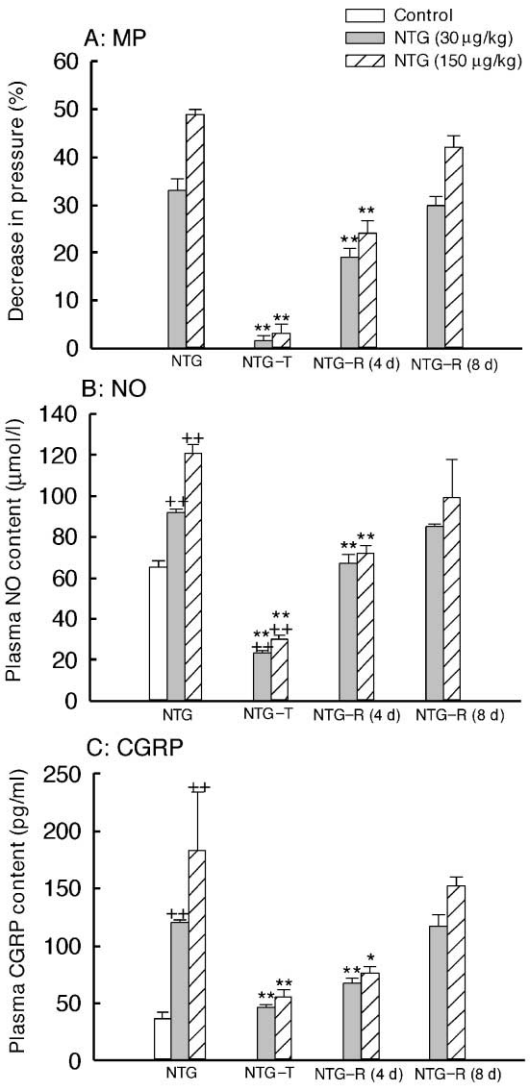

Fig. 2. Effect of nitroglycerin on blood pressure and plasma concentrations of nitric oxide and CGRP. Nitroglycerin was injected intravenously in all groups. MP: mean arterial pressure; NTG: nitroglycerin; NTG-T: tolerance was induced by pretreatment with nitroglycerin (10 mg/kg, s.c.) three times a day for 8 days; NTG-R (4 d) or NTG-R (8 d): 4 or 8 days after nitroglycerin removal in the tolerant rat. Values are means ± S.E.M. ($n = 7$). $^{++}P < 0.01$ vs. control; $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. NTG.

using antisera raised against rat CGRP, ^{125}I-labelled CGRP, and rat CGRP standard.

2.3. Measurement of plasma NO concentration

Blood samples (3 ml) were collected rapidly in the same way as above except that aprotinin was added. The plasma level of NO was measured indirectly, spectrophotometrically, by the content of nitrite and nitrate.

2.4. Reagents

CGRP was purchased from Sigma (St. Louis, MO, USA). Nitroglycerin was purchased from Beijing Yiming Pharmaceutical Factory (Beijing, PR China). Nitroglycerin was dissolved in 99% ethanol and further diluted in 0.9% saline to the proper final concentration. CGRP was dissolved in 0.9% saline. Radioimmunoassay kits for the measurement of CGRP were obtained from Dongya Immunity Technology Institute (Beijing, PR China). Supplies for the NO assay were obtained from Nanjing Ju-Li Biological Medical Engineering Institute (PR China).

2.5. Statistics

All values were expressed as means ± S.E.M. Statistical analysis was carried out by analysis of variance and the Newman–Keuls test. The level of significance was chosen as $P < 0.05$.

3. Results

There were no differences in baseline values for blood pressure among groups (Table 1). Nitroglycerin, in a single bolus dose of 30 or 150 μg/kg (i.v.), significantly decreased blood pressure in a dose-dependent manner, an effect that had almost completely disappeared in the rats pretreated with nitroglycerin (10 mg/kg, s.c.) three times a day for 8 days. However, the depressor effect of nitroglycerin was restored, partially or completely, 4 or 8 days after drug removal in the tolerant rats (Figs. 1 and 2A). The depressor effect of exogenous CGRP was unaltered by prolonged nitroglycerin treatment (Fig. 3).

Nitroglycerin (30 or 150 μg/kg, i.v.) caused a significant increase in plasma concentrations of NO. However, after prolonged drug treatment, nitroglycerin no longer produced the elevated concentration of NO. The elevated level of NO was also gradually restored after drug removal in the tolerant rats (Fig. 2B).

As has been reported previously (Zhou et al., in press), various doses of i.v. nitroglycerin significantly increased plasma concentrations of CGRP. The increase in the level of CGRP with nitroglycerin also disappeared after long-

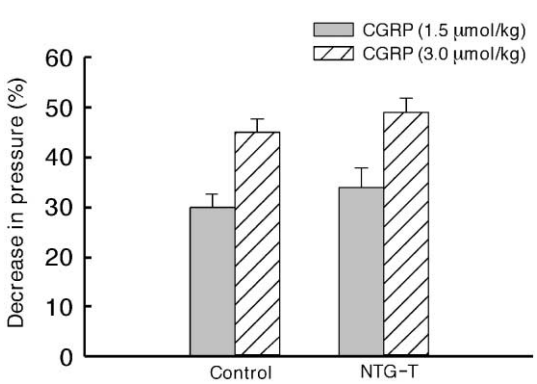

Fig. 3. Effect of CGRP on blood pressure. CGRP was injected intravenously. NTG-T: tolerance was induced by pretreatment with nitroglycerin for 8 days. Values are means ± S.E.M. ($n = 6$–8).

Table 1
Basal values for blood pressure (mm Hg). NTG: nitroglycerin; NTG-T: tolerance was induced by pretreatment with nitroglycerin (10 mg/kg, s.c.) three times a day for 8 days; NTG-R: 4 or 8 days after nitroglycerin removal in the tolerant rat. Values are means ± S.E.M.

	n	Mean arterial pressure
NTG (30 μg/kg)	7	134 ± 6.3
NTG (150 μg/kg)	7	132 ± 4.0
NTG-T (30 μg/kg)	7	131 ± 4.3
NTG-T (150 μg/kg)	7	135 ± 11.0
NTG-R (4 days, 30 μg/kg)	7	132 ± 4.4
NTG-R (4 days, 150 μg/kg)	7	129 ± 11.7
NTG-R (8 days, 30 μg/kg)	7	140 ± 4.7
NTG-R (8 days, 150 μg/kg)	7	134 ± 11.3
CGRP (1.5 μmol/kg)	8	140 ± 11.3
CGRP (3.0 μmol/kg)	6	128 ± 11.0

term drug treatment. Similarly, the release of CGRP stimulated by nitroglycerin was also restored, partially or completely, 4 or 8 days after drug removal in the tolerant rat (Fig. 2C).

4. Discussion

It has been demonstrated that nitrovasodilators relax vascular smooth muscle by stimulating soluble guanylate cyclase and thus by increasing the formation of cyclic guanosine monophosphate (cGMP), and that repeated administration of organic nitrates causes tolerance development characterized by a diminished relaxing effect and decreased formation of NO (Kukovetz and Holzmann, 1990; Persson et al., 1995; Slack et al., 1988). Recently, it has been reported that vasodilator responses to nitroglycerin are attenuated or abolished by CGRP-(8–37), a selective CGRP receptor antagonist, in feline cerebral arterioles or rat aorta (Wei et al., 1992; Booth et al., 2000). The results of the present study confirmed previous observations that i.v. nitroglycerin causes a depressor effect concomitantly with an increase in concentrations of CGRP, and these effects of nitroglycerin are attenuated by pretreatment with capsaicin or methylene blue, an inhibitor of guanylate cyclase (Zhou et al., in press). These results suggest that cardiovascular effects of nitroglycerin are mediated by CGRP. Based on the regulatory effect of NO on CGRP release, we postulate that the decrease in hypotensive efficacy of nitroglycerin in the tolerant animals may alter the activation of capsaicin-sensitive sensory nerves. The results revealed that nitroglycerin caused a decrease in blood pressure concomitantly with an increase in plasma concentrations of NO and CGRP, and these effects of nitroglycerin disappeared after prolonged drug treatment. However, the nitroglycerin-induced depressor effect and the elevation of NO and CGRP content were restored, partially or completely, 4 or 8 days after drug removal in the tolerant rat. These findings suggest that the tolerance to nitroglycerin is related to the decreased release of CGRP in rats.

It has been reported that some neuropeptides, such as substance P, which coexist with CGRP in capsaicin-sensitive sensory nerves, induce rapid tolerance development (Strobel et al., 1996). Others have shown that long-term nitroglycerin treatment is associated with cross-tolerance to endothelium-dependent vasodilators such as acetylcholine (De la Lande et al., 1999). CGRP produces endothelium-dependent or endothelium-independent vasorelaxation in various vessels (Hirata et al., 1988). Therefore, another possibility we considered was that long-term nitroglycerin treatment induces tolerance via a postjunctional site of action. However, in the present study, prolonged nitroglycerin treatment had no effect on the depressor effect of exogenous CGRP. This suggests that the development of tolerance to nitroglycerin may result from an action at prejunctional sites.

The mechanism responsible for alterations of CGRP release by long-term nitroglycerin treatment is unclear. There is evidence to suggest that the development of tolerance to nitroglycerin is associated with the desensitization of guanylate cyclase, leading to an attenuated rise in cGMP (Lawson et al., 1996). Previous investigations have suggested that NO regulates substance P release through the activation of guanylate cyclase and the subsequent increase in cGMP levels (Kamisaki et al., 1995). Our recent work has also shown that the depressor effect and elevated concentrations of CGRP with nitroglycerin were significantly reduced by methylene blue (Zhou et al., in press). It is likely that long-term nitroglycerin treatment decreases the release of CGRP through the decreased production of NO and the reduced activity of guanylate cyclase, resulting in attenuation of the hypotensive effect. However, the exact mechanism for alterations of CGRP release in nitroglycerin-induced tolerant rats needs to be investigated.

In summary, the present study suggests that, in the rat, the development of tolerance to nitroglycerin is related to the decreased release of CGRP.

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