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European Journal of Pharmacology 286 (1995) 201–204

ejp

Short communication

Rapid increase in plasma nitrite concentration following intravenous administration of nipradilol

Takeshi Adachi [a], Shingo Hori [b,*], Koji Miyazaki [a], Eiichi Takahashi [a], Masahiro Nakagawa [a], Akira Udagawa [c], Nariyuki Hayashi [c], Naoki Aikawa [b], Satoshi Ogawa [a]

[a] *Cardiopulmonary Division, Department of Internal Medicine, Keio University, School of Medicine, Tokyo, Japan*
[b] *Department of Emergency Medicine, Keio University, School of Medicine, Tokyo, Japan*
[c] *Nihon University, Memorial Critical Emergency Center, Tokyo, Japan*

Received 16 March 1995; revised 29 August 1995; accepted 1 September 1995

Abstract

Nipradilol, a β-adrenoceptor antagonist with vasodilator activity, has a structure which contains a NO_2 group. In anesthetized dogs, the time course of the systemic vasodilatation and plasma nitrite (NO_2^-) concentration was studied following intravenous administration of nipradilol (1 mg/kg). A fall in systemic vascular resistance was observed at 1 min, which was rapidly followed by a significant increase in the plasma NO_2^- concentration. It is indicated that nipradilol exerts systemic vasodilatation via nitric oxide (NO) release in vivo.

Keywords: Nipradilol; Nitric oxide (NO); Nitrite; cGMP

1. Introduction

Nipradilol (3,4-dihydro-8-[2-hydroxy-3-isopropyl amino] propoxy-3-nitroxy-2*H*-1-benzopyran) is a non-selective β-adrenoceptor antagonist with a vasodilation effect. It exerts systemic vasodilation via a reduction of left ventricular end-diastolic pressure in vivo (Uchida, 1982; Fujii et al., 1986), and is widely used in the treatment of hypertension and angina pectoris. However, the precise mechanism of its vasodilator effect is yet to be elucidated. It is reported that its α-adrenoceptor blocking effect is minimal (Fujii et al., 1986). In the spontaneously hypertensive rat, the vasodilation is not mediated by the α-blocking effect, as indicated by the vasopressor response to phenylephrine which was not inhibited with nipradilol (Imai et al., 1988).

Nipradilol contains a nitroxy group within its molecular structure. Moreover, it increases the intracellular cyclic guanosine monophosphate (cGMP) concentration in rat cultured mesangial cells in vitro (Koya et al., 1993). Accordingly, it is assumed that its vasodilator effect may be via nitric oxide (NO) release. However, NO has a very short half-life and is nearly impossible to measure directly in vivo (Archer, 1993). It is reported that NO reacts rapidly with oxygen and is metabolized to NO_x (nitrite/nitrate [NO_2^-/NO_3^-]) via NO_2 production (Forni et al., 1986). The NO_x concentration has been proposed as a marker to assess NO production (Suzuki et al., 1992).

$$NO + 1/2O_2 \rightarrow NO_2$$

$$NO + NO_2 \leftrightarrow N_2O_3 \qquad N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+$$
$$2NO_2 \leftrightarrow N_2O_4 \qquad N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+$$

The aim of the present study was to investigate the mechanism of the vasodilating effect of intravenous administration of nipradilol in vivo, by comparing the time course of vasodilation and the plasma NO_x level.

2. Materials and methods

2.1. Animal preparation

Closed-chest dogs ($n = 11$, $14 \sim 22$ kg) were anesthetized with pentobarbital sodium (25 mg/kg) and ventilated artificially to maintain PaO_2 and $PaCO_2$

* Corresponding author. Department of Emergency Medicine, Keio University Hospital, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan. Tel.: 81-3-3225-1323; fax: 81-3-3225-1320.

SSDI 0014-2999(95)00559-5

within the physiological range. Catheters (7F) were inserted from the right femoral vein for drug administration and in the bilateral femoral arteries for the measurement of aortic pressure and for blood sampling. In 6 dogs, an 8F Opti-Swan-Gantz catheter was inserted from the right jugular vein for measurement of cardiac output and mixed venous blood oxygen saturation (Vigilance VGSSYS oxymeter continuous cardiac output monitoring, Baxter Healthcare Corporation, Deerfield, IL, USA). Limb lead ECG and aortic pressure were recorded on a polygraph (RM 6000, Nihon Kohden, Tokyo, Japan).

2.2. Protocol

Following a sufficient stabilization period of 30 min, 10 ml of blood was sampled and the same amount of saline was administered at a control, 3, 5, 10 and 30 min after intravenous administration of 1 mg/kg nipradilol (Kowa, Nagoya, Japan). Arterial blood pH, hematocrit and oxygen saturation were measured before and after the experiments (pH/Blood Gas Analyzer 1306, Instrumentation Laboratory, Tokyo, Japan).

2.3. Determination of cGMP, human atrial natriuretic polypeptide (human ANP), norepinephrine and epinephrine

The sampled blood was prepared by centrifugation at 3000 rpm for 10 min. cGMP and human ANP were measured by radioimmunoassay.

Plasma norepinephrine and epinephrine concentration were measured by the HPLC-THI (high performance liquid chromatography-trihydroxy indole) method.

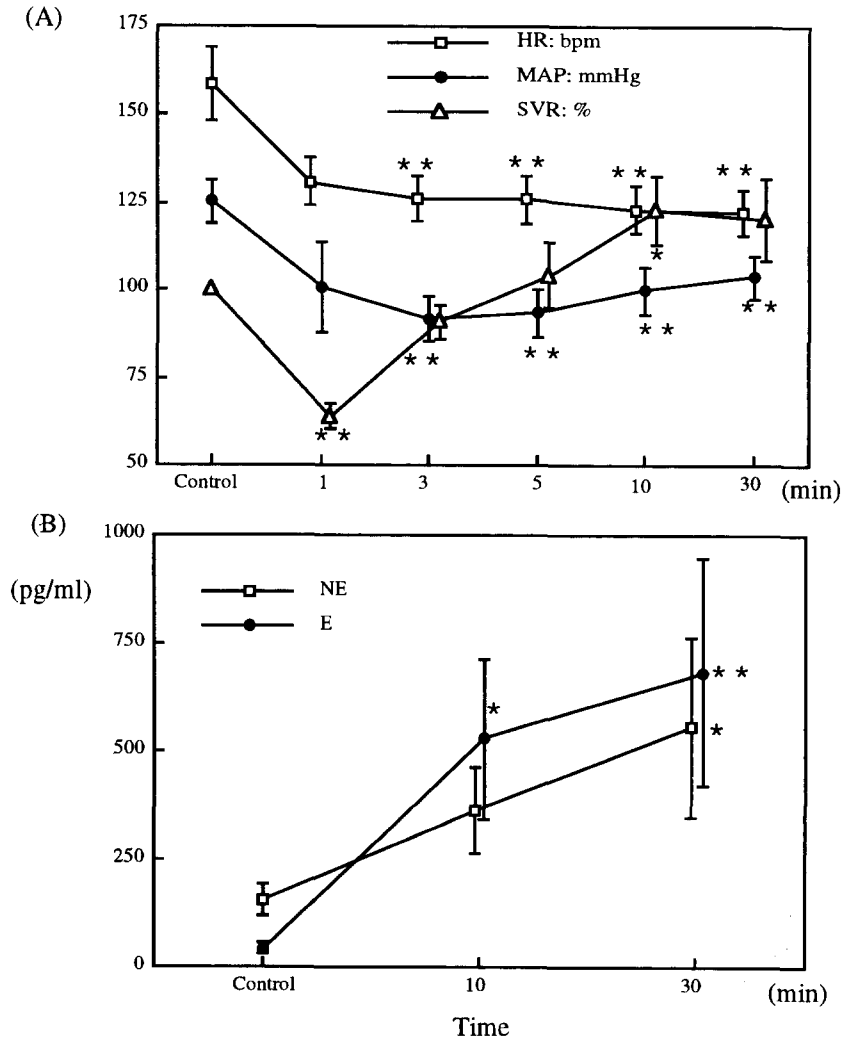

Fig. 1. Hemodynamic changes and plasma catecholamine concentrations following intravenous administration of nipradilol. (A) HR: heart rate, MAP: mean aortic pressure, SVR: systemic vascular resistance. (B) NE: norepinephrine, E: epinephrine. HR: $P < 0.001$, MAP: $P < 0.001$, SVR: $P < 0.001$, NE: $P < 0.05$, E: $P < 0.05$ (two-way ANOVA), * $P < 0.05$, ** $P < 0.01$ vs. control (Dunnet's method).

2.4. Determination of NO_x

An automated system based on the Griess method was applied for the analysis of NO_x (TRAACS-800: Technicon, Bran-Luebbe, Norderstedt, Germany). This system is equipped with a microdialysis membrane for the removal of plasma protein and has the capability to measure the plasma NO_x concentration with high accuracy (Green et al., 1982).

Because the plasma NO_2^- level is low in canine preparations, the accuracy of the measurement by the automated system in the concentration range less than 1 μM was verified in a preliminary experiment. NO_2^- levels were measured repeatedly in pooled human serum which was diluted with saline. A good linearity between NO_2^- levels and dilution scale was obtained as low as 0.5 μM and standard deviation values were adequately small (0.01–0.06 μM).

2.5. Statistical analysis

All data are presented as means ± S.E.M. Any two groups were compared using Student's paired *t*-test. The time-course data were compared by two-way analysis of variance (ANOVA) followed by Dunnett's method to evaluate the statistical significance of the difference between any two groups. Differences were considered statistically significant when *P* values were less than 0.05.

3. Results

3.1. Hemodynamic changes and plasma catecholamine concentrations (Fig. 1)

Soon after intravenous administration of nipradilol, the mean arterial pressure decreased significantly and tended to recover after 30 min. The heart rate decreased simultaneously and remained at the same level thereafter. In contrast to the aortic pressure and heart rate, systemic vascular resistance decreased rapidly at 1 min, then recovered and increased at 10 min. At 1 min, a transient rise in oxygen saturation of the mixed venous blood was observed. The plasma catecholamine level was increased in parallel with the changes in the systemic vascular resistance. The plasma epinephrine level was significantly increased at 10 min, whereas the plasma norepinephrine level was significantly increased at 30 min.

Blood pH (7.54 ± 0.03 vs. 7.56 ± 0.03), arterial blood oxygen saturation (99.8 ± 0.1% vs. 99.3 ± 0.4%) and hematocrit (36 ± 1% vs. 34 ± 2%) did not change significantly before and after the experiment.

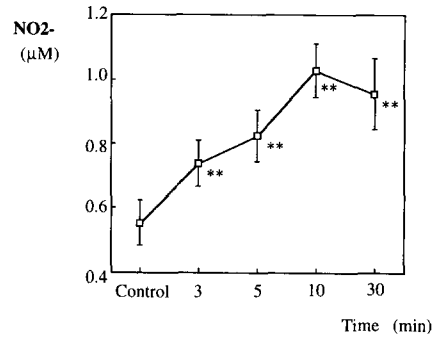

Fig. 2. Plasma NO_2^- concentration following intravenous administration of nipradilol. $P < 0.001$ by two-way ANOVA, ** $P < 0.01$ vs. control by Dunnet's method.

3.2. Plasma NO_x, cGMP, human ANP (Fig. 2)

The plasma NO_2^- level was significantly increased at 3 min and reached its peak at 10 min following the administration of nipradilol, whereas the NO_3^- level did not change significantly (16.2 ± 3.4 μM at control vs. 16.9 ± 3.4 μM at 30 min).

The plasma cGMP level was increased from 20.7 ± 2.8 pmol/ml at the control sampling to 23.1 ± 3.1 pmol/ml at 30 min; however, the increase was not statistically significant (N.S. by two-way ANOVA, $P <$ 0.05 by Dunnet's method). Human ANP was not changed (63.7 ± 22.6 pmol/ml control vs. 62.9 ± 15.4 pmol/ml at 30 min).

4. Discussion

The major finding of the present study was that NO_2^- increased rapidly and significantly following nipradilol-induced systemic vasodilatation. It is suggested that nipradilol exerts its vasodilator effect by acting as a NO donor.

Nipradilol has a nitroxy group in its molecular structure, therefore it is speculated that NO is released through the same metabolic pathway as that operating with nitroglycerin. Ignarro reported that nitroglycerin produces NO_2^- via an enzymatic reaction with glutathione *S*-transferase and then NO_2^- releases NO with SH groups in the smooth muscle cell (Ignarro, 1989). It was recently reported that NO_2^- synthesis may not be related to NO release itself, but is a product of the degradation process of nitroglycerin (Kurz et al., 1993). However, intracellular NO_2^- is unlikely to be released into the bloodstream due to its negative ionic charge (Ignarro, 1989). Accordingly, it is considered that the plasma NO_2^- measured in this study was the product of the chemical reaction, in the extracellular space, of NO which had been synthesized

in the intracellular space and then released into the bloodstream.

Two reports have described the changes in the urinary NO_x level following the administration of NO donor. In spontaneously hypertensive rats, the long-term administration of molisidomine increases urinary NO_3^- and cGMP excretion (Boger et al., 1994). With oral administration of FK409 in Sprague-Dawley rats, the plasma cGMP concentration increases simultaneously with vasodilation and an increase in urinary excretion of NO_x (Kita et al., 1994). In their reports, urinary NO_x excretion was considered to be a useful marker of NO release. Based on our literature search, the present report appears to be the first of the change in plasma NO_x concentration and its relation to the acute hemodynamic responses following administration of a NO donor.

In the present study, plasma cGMP was immediately increased by 12% following nipradilol administration, but not statistically significant. The possible cause for the minimal change was that plasma cGMP is released from the intracellular compartment and is an indirect marker of NO metabolism (Kita et al., 1994).

A transient systemic vasodilation succeeded by vasoconstriction was observed in anesthetized closed chest dogs in the present experiment, a finding compatible with the two-phase hemodynamic responses previously described in open chest dogs (Fujii et al., 1986). It is considered that the vasoconstriction in the late phase is attributable to the β-blocking effect of nipradilol, associated with the reflex-mediated α-stimulating effect as shown by the release of catecholamines (Fig. 1).

Acknowledgements

The authors wish to express their thanks to Ms. Eiko Takeshita of the Clinical Laboratory of Keio University Hospital for measurement of catecholamines and to Mr. Katsuyuki Takahashi of the Clinical Laboratory of Nihon University for his excellent support with the measurement of NO_x.

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