Mechanism of Action of Hypotensive Prostaglandins in Patients with Essential Hypertension

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Abstract

Despite extensive investigation, the biological mechanisms causing essential hypertension (EHT) remain unclear. To clarify the means by which hypotensive prostaglandins (Hypo-PGs, mainly PGE₁ and PGE₂) act in patients with EHT, the interaction between intravenously infused Hypo-PGs and pressor substances such as an adrenergic neurotransmitter, noradrenaline (NA) and angiotensin II (AII) was examined both in patients with EHT and in perfused isolated rabbit ear artery preparations. In patients with EHT, Hypo-PGs were shown to reduce the pressor responses to intravenously infused NA or AII, although no significant difference was found between the pressor responses to NA under basal conditions and the responses during intravenous infusion of Hypo-PGs. Animal studies were undertaken to investigate the inhibitory action of Hypo-PGs on the vasoconstrictive responses to electrical stimulation of the perivascular sympathetic nerves (VSNS) and to exogenous NA at pre- and postjunctional sites in blood vessel walls. The suppressive action of Hypo-PGs on the response to VSNS was shown to be more potent than that to their action on the response to exogenous NA. Thus, it was concluded that the hypotensive action of intravenously infused Hypo-PGs in patients with EHT may be more dependent on prejunctional sites than on the postjunctional sites in the walls of blood vessels.

Key words: essential hypertension, hypotensive prostaglandins, noradrenaline, angiotensin II, perivascular sympathetic nerve

Introduction

The etiology of essential hypertension (EHT) remains unclear despite extensive investigations in patients (Hickler et al., 1959; Nestel, 1969; Brunner et al., 1972; Kohon et al., 1990; Schichiri et al., 1990; John et al., 1995). What is known is that EHT involves an increase in peripheral vascular resistance (PVR) (Panza et al., 1990; Cockcroft et al., 1994), an increased pressor response to substances such as noradrenaline (NA) (Doyle et al., 1961; Eich et al., 1966; Sannerstedt, 1966) and angiotensin II (AII) (Mendelowitz, 1967; Redgrave et al., 1985), a
hyperdynamic $\beta$-adrenergic circulatory state in the initial stage (Frohlich et al., 1966), and a
decrease in renal blood flow (Lowenstein et al., 1967; Hollenberg et al., 1969). It has been
recently postulated that patients with EHT are deficient in hypotensive prostaglandins (Hypo-
PGs) (Papanicolaou et al., 1976; Tan et al., 1978; Michibayashi, 1980) and to understand the
pathogenesis of EHT, the interrelation between Hypo-PGs and adrenergic nerve
neurotransmitter NA must be understood. Hypo-PGs are ubiquitous, biologically active
unsaturated fatty acids that play a role in many physiological processes such as natriuresis and
water diuresis (Johnston et al., 1967; Stokes, 1983), inhibition of neurotransmitter release from
adrenergic nerve endings (Hedqvist, 1970; Stjärne, 1973), an hypotensive action based on
reduction in PVR (Bergström, 1967) and in antilipolysis (Bergström, 1967).

The present study focuses on the interaction between Hypo-PGs and peripheral adrenergic
nerve activity, and was carried out with the aim of clarifying the mechanism by which
intravenously administered Hypo-PGs act in patients with EHT.

Materials and Methods

Clinical study

All patients gave informed consent for this clinical investigation of the effects of the infusion
of pressor and depressor substances on their blood pressure. EHT was diagnosed on the basis
of supine systolic and diastolic blood pressures (Korotkoff phase V) and World Health
Organization (WHO) criteria. Thirteen patients (average age 47 ± 5 yrs; mean ± SD) with
uncomplicated mild to moderate EHT were included in this study. Systemic blood pressure was
measured in the supine position. All patients were confirmed to have systolic blood pressure
over 150 mmHg or diastolic pressure over 90 mmHg and to have been free of antihypertensive
medication for at least 2 weeks before the start of the study. Secondary causes of hypertension
were ruled out by the usual screening tests, including history, physical examination, urinalysis,
and blood chemistry. After an approximate 14-hr overnight fast, blood pressure was measured
three times by the same observer in each subject with a standard mercury sphygmomanometer.
Each subject had rested in a supine position for 30 min before the start of blood pressure
measurement. Hexamethonium ($\text{C}_6$, 0.27 mg/kg) was injected intramuscularly 30 min before
pressor testing (Aviado, 1971) and was followed by intravenous administration of either NA (3.0
$\mu$g/kg/min) or AII (10 ng/kg/min). After completion of either NA or AII infusion and when the
blood pressure had reverted to the basal level, Hypo-PG (PGE$_1$, 50 ng/kg/min) was infused into
the cubital vein. About 10 to 12 min after start of PGE$_1$ infusion, systemic blood pressure was
observed to have reduced and become stabilized, after which it remained constant. Following
cessation of the PGE$_1$ infusion and when the blood pressure had reverted to the basal level,
either NA (3 $\mu$g/kg/min) or AII (10 ng/kg/min) was again infused in combination with PGE$_1$ at
the dose previously used. Blood pressure stabilized and remained constant about 10 min after
infusion of NA and PGE$_1$ in combination. Blood pressure measurements were taken at intervals
of 30 s in the contralateral arm with the patient remaining in the supine position. This allowed
a comparison between the blood pressure during infusion of the pressor substance alone and
that in combination with PGE$_1$. The hypotensive effect of PGE$_1$ was determined as the
difference between the blood pressure levels (systolic [SBP], diastolic [DBP], and mean [MBP]) during the two infusions.

**Animal experiments**

Twenty male Japanese white rabbits, weighing from 2.0 to 3.0 kg, were stunned by a head blow and exanguinated via the carotid artery. Their ears were excised at the base and were freshly prepared according to the procedure described by de la Lande et al. (1968). The proximal portion of the central artery was then cannulated with fine polyethylene tubing. The ear preparations were perfused via a roller pump at a constant flow rate of 3 ml/min with a modified Krebs’ solution consisting of Na+ 137.0 mM, K+ 5.9 mM, Ca2+ 1.8 mM, Mg2+ 1.2 mM, Cl– 123.9 mM, HCO3– 25.0 mM, glucose 8.3 mM, and sucrose 20.0 mM. The modified Krebs’ solution (pH 7.4, 37°C) was equilibrated with a gas mixture of 95% O2 with 5% CO2, and then 0.5 µg/ml of atropine sulfate and 25 µg/ml of ascorbic acid were added (Michibayashi, 1992).

The proximal portion of the central artery was electrically stimulated for 15–30 s via two Ag-AgCl electrodes that contacted the arterial wall at an interelectrode distance of 10 mm. Stimulation was carried out by supramaximal rectangular pulses of 5–20 Hz in frequency and 1 ms in duration with an Electronic Stimulator (SEN-3101, Nihon Kohden, Japan). A suitable concentration of NA (0.1 ml), freshly prepared in the modified normal Krebs’ solution, was injected as a bolus into a rubber tube connected to the central arterial cannula. The vasocontractile response to NA (NA-R) was observed as a change in perfusion pressure (mmHg). This change was recorded on a kymograph with the use of a mercury manometer. Changes in the perfusion pressure were expressed as an alteration of PVR (mmHg/ml.min⁻¹.g⁻¹) based on a constant flow rate and the weight of the rabbit ear preparation, and was normalized to value per one gram of preparation weight for statistical comparison. The concentration of NA used was determined in each experiment so as to cause approximately the same change in perfusion pressure as that evoked by the preceding electrical stimulation of the perivascular sympathetic nerve (VSNS), because the inhibitory effects of Hypo-PGs on the vasocontractile response to VSNS or NA are influenced by the intensity or the dose, respectively (Malic et al., 1975; Michibayashi et al., 1981; Michibayashi, 1985). In another experiment, 1 ng/ml of AII, dissolved in distilled water, was infused into the arterial cannula to observe the effect of AII on either the response to VSNS or to a single bolus-injection of NA, followed by the inhibitory action of PGE₁ on these AII-induced effects.

In a third experiment, to investigate the interaction between the inhibitory action of Hypo-PGs and the enhancing action of AII (1.0 ng/ml) at pre- and postjunctional sites of adrenergic nerve endings in the artery, the inhibitory action of Hypo-PGs (PGE₁, 2–5 ng/ml) on the vasocontractile responses to VSNS was compared with those to exogenous NA in a perfused isolated rabbit ear artery preparation. Because the inhibitory effect of Hypo-PGs on the vasocontractile response to NA is influenced by the concentration of NA, concentrations of NA (from 5 to 25 ng) showing nearly the same response as the preceding vasocontractile response to VSNS were applied as a single bolus into the perfusing circuit.

The drugs used were prostaglandin E₁ (Ono Pharmaceutical Co.), noradrenaline (Fluka, A.G.), noradrenaline injection (Sankyo), angiotensin II (CIBA-GEIGY), hexamethonium
bromide (Yamanouchi), ascorbic acid (Kishida), and atropine sulfate (Tanabe).

Results obtained in the present study were expressed as the mean ± SEM. All data were analyzed using the F-test and a difference with P<0.05 was considered to be significant.

The studies were conducted in compliance with the principles of the Declaration of Helsinki and prevailing amendments.

Results

Action of hypotensive prostaglandins on patients with essential hypertension

1) Hypotensive action of prostaglandin E₁ on the pressor response to intravenously administered noradrenaline.

Approximately 10 to 12 min after the start of NA infusion in patients with EHT, elevation of blood pressure ceased and then remained nearly constant. Changes in systemic blood pressure, i.e., SBP, DBP and MBP, were +42 ± 4% (P<0.001), +26 ± 4% (P<0.001) and +33 ± 4% (P<0.001, n=8), respectively (Fig. 1, white columns). After NA infusion was discontinued and the blood pressure had reverted to the basal level, Hypo-PGs (PGE₁, 50 ng/kg/min) were infused into the cubital vein. About 10 to 12 min after the start of PGE₁ infusion, systemic blood pressure stabilized and remained constant. Changes in systemic blood pressure, i.e., SBP, DBP and MBP, were –16 ± 3% (P<0.001), –19 ± 2% (P<0.001) and –17 ± 2% (P<0.001), respectively (Fig. 1, closed columns). After PGE₁ infusion was stopped and the blood pressure had reverted to the basal level, NA (3 µg/kg/min) was again infused in combination with PGE₁ at the concentration previously used. Blood pressure stabilized and remained constant about 10 min after infusion of NA and PGE₁ in combination. Changes in systemic blood pressure, i.e., SBP, DBP and MBP, were +10 ± 3% (P<0.05), +7 ± 2% (P<0.025) and +8 ± 2% (P<0.05), respectively (Fig. 1, stipple columns).

In all subsequent experiments, the comparison between pressor responses to NA under basal conditions and in the presence of Hypo-PGs in patients with EHT was made using the same data as presented in Fig. 1. ∆Pressor Response to NA under the basal conditions represents the difference between the elevated blood pressure observed during i.v. infusion of NA and the blood pressure under basal conditions. ∆Pressor Response to NA was expressed as a % change in systemic blood pressure under basal conditions without Hypo-PGs. Also, ∆Pressor Response to NA in the presence of Hypo-PGs represents the difference between the reduced blood pressure observed during i.v. infusion of Hypo-PGs alone and that observed during i.v. infusion of NA in combination with Hypo-PGs. ∆Pressor Response to NA was expressed as % change in systemic blood pressure during i.v. infusion of Hypo-PGs. While ∆Pressor Response (SBP) to NA under basal conditions was significantly higher than that in the presence of Hypo-PGs, ∆Pressor Responses (DBP and MBP) to NA were not significantly altered in the presence of Hypo-PGs (Fig. 2).

2) Hypotensive action of prostaglandin E₁ on the pressor response to intravenously administered angiotensin II in hospitalized patients.

All was infused into the cubital vein at 10 ng/kg/min. The treatment with AII elicited an increase in MBP of 19 ± 2 mmHg (n=5). After AII infusion was stopped and the blood pressure had reverted to the basal level, Hypo-PGs (PGE₁) were infused at a rate of 50 ng/kg/min for
approximately 10 to 12 min. After the blood pressure stabilized, the same concentration of AII was again infused in combination with PGE₁. This infusion of both AII and PGE₁ caused a significant (P<0.01) decrease in MBP of 14 ± 2 mmHg from the level obtained during the single infusion of AII, and MBP reverted to nearly the level observed under basal conditions.

**Experiments in isolated perfused rabbit ear artery preparations**

1) Enhancing effect of angiotensin II and inhibitory effect of hypotensive prostaglandins on
When the effect of AII (1.0 ng/ml) on the vasocontractile response to VSNS was examined, it was shown that the response is increased significantly (P<0.005) (Fig. 3, left panel). On the other hand, when the inhibitory action of Hypo-PG (PGE1) on the vasocontractile response to VSNS was examined, the response to VSNS was significantly (P<0.025) depressed in the presence of 2 to 5 ng/ml of PGE1 (Fig. 3, right panel).

2) Comparison between inhibitory actions of hypotensive prostaglandins on vasocontractile responses to electrical stimulation of perivascular sympathetic nerves and to exogenous NA in the presence of angiotensin II.

Hypo-PGs (PGE1, 2–5 ng/ml) were shown to completely and significantly (P<0.005) suppress the AII-induced enhancing response to VSNS (Fig. 4, left panel). Subsequently, the action of Hypo-PGs on the vasocontractile response to exogenous NA was examined. In contrast to VSNS, the inhibitory action of Hypo-PGs (PGE1, 2–5 ng/ml) on the AII-induced enhanced response to NA was depressed, but not significantly (Fig. 4, right panel).

Fig. 2. Comparison between pressor responses to noradrenaline under basal conditions and in the presence of hypotensive PGs in patients with essential hypertension. The Δ Pressor Response to NA under basal conditions is the difference between the blood pressures (SBP, DBP and MBP) measured during i.v. infusion of NA and those under basal conditions ( ). The Δ Pressor Response to NA in the presence of Hypo-PGs is the difference between the reduced blood pressures (SBP, DBP and MBP) measured during i.v. infusion of Hypo-PGs alone and those measured during i.v. infusion of NA in combination with Hypo-PGs ( ). The values of the Δ Pressor Response to NA were expressed as % change in systemic blood pressure (SBP, DBP and MBP) in the supine position under basal conditions and during i.v. infusion of Hypo-PGs alone, respectively. Each value and vertical bar indicates the mean and standard error of the mean, respectively. Number of determination was eight patients. ※ P<0.01, ♦ P<0.1, NS not significant.
When Hypo-PGs are given intravenously, they elicit natriuresis, water diuresis, an increase in renal blood flow (Jonston et al., 1969; Stokes, 1983) and a decrease in systemic blood pressure (Bergström, 1967; Michibayashi, 1981). It is generally accepted that this decrease in blood pressure during *i.v.* infusion of Hypo-PGs is due mainly to a decrease in the total peripheral vascular resistance (TPVR) (Bergström, 1967), and to natriuresis and water diuresis, rather than to suppression of the cardiac work load. Among factors contributing to the hypotensive change, the decrease in TPVR is the major factor responsible for the immediate hypotension observed just after *i.v.* infusion of Hypo-PGs, whereas renal factors, *i.e.*, natriuresis and water diuresis, cause a slow lowering of blood pressure, *i.e.*, delayed hypotension, during the infusion. In the present study, there was a little time lag between the decrease in blood pressure and the beginning of Hypo-PGs infusion in patients with EHT. So, the action of Hypo-PGs in the present study is considered to be entirely dependent on the reduction in TPVR and only slightly dependent on renal factors.

TPVR is generally accepted to involve humoral, vascular myogenic, neurogenic, and structural factors (Uchida, 1969). Thus, animal experiments were undertaken to investigate whether any of the circulatory factors described above participate in the hypotensive action of *i.v.* infusion of Hypo-PGs. Because central administration of these PGs is known to elicit a slight

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**Discussion**

Fig. 3. Enhancing action of angiotensin II and inhibitory action of hypotensive PGs on vasocontractile responses to electrical stimulation of perivascular sympathetic nerves. Each value and vertical bar indicates the mean and standard error of the mean (n=5 to 9), respectively. ※, P<0.025–0.005. Vasocontractile response to VSNS is expressed as peripheral vascular resistance (mmHg/ml.min⁻¹.g⁻¹).
elevation in blood pressure rather than a fall (Leksell, 1976), in vitro experiments in isolated perfused rabbit ear artery preparations were carried out with the aim of clarifying the mechanism by which Hypo-PGs elicit the decrease in TPVR and which of the four factors is affected most by the Hypo-PGs. The present animal experiments focused mainly on the inhibitory actions of exogenous Hypo-PGs on the vasocontractile response to VSNS and to pressor substances such as NA and AII. That the Hypo-PGs acted more strongly at the prejunctional sites leads to a notion that the hypotensive action of the Hypo-PGs is intimately dependent on suppression of adrenergic neurotransmitter release from prejunctional sites. Hypo-PGs cause an inhibitory action on vasocontractile response to VSNS at concentrations of 2–5 ng/ml, which are nearly equal to physiological levels in blood vessel walls (Nekrasova et al., 1980; Maggi et al., 1981). The pharmacological concentrations of 10–50 ng/ml, however, appear to be necessary for restricting the response to exogenous NA (Michibayashi, 1978). Also, since AII facilitates NA release from the adrenergic nerve endings and augments vasocontractile response to NA at the postjunctional sites (Rump et al., 1995; Storgaard et al., 1997; Guimaraes et al., 1998; Jensen et al., 1999; Boehm et al., 2002), the restrictive effects of Hypo-PGs were compared between vasocontractile responses to VSNS and to NA in the presence of AII. There was little difference between the inhibitory actions of Hypo-PGs in the absence or presence of AII. The hypotensive action of i.v. infused Hypo-PGs in EHT was estimated on the basis of the

Fig. 4. Comparison between inhibitory actions of hypotensive PGs on the vasocontractile responses to electrical stimulation of perivascular sympathetic nerve and exogenous noradrenaline in the presence of angiotensin II. Each value and vertical bar indicates the mean and standard error of the mean (n=5 to 9), respectively. ※, P<0.005, NS not significant. Vasocontractile responses to VSNS and NA are expressed as peripheral vascular resistance (mmHg/ml.min⁻¹.g⁻¹).
results of in vitro experiments conducted in isolated perfused rabbit ear artery preparations. However, the effects of Hypo-PGs on vasocontractility differ for individual animals (Strong et al., 1967; Malik et al., 1975; Malik et al., 1976) and vary widely between experiments that use various kinds of vasculatures originating from tissues or organs in the body (Strong et al., 1967). In addition, species differences should be taken into consideration. Thus, it should not be assumed that the hypotensive mechanism of Hypo-PGs in patients with EHT is entirely equivalent to that deduced from the rabbit ear artery preparation. According to the proposals concerning the inhibitory action of Hypo-PGs on neurotransmitter release from the peripheral adrenergic nerve terminals in experiments with human blood vessels (Stjärne et al., 1973; Stjärne et al., 1977), there seems to be little difference between the results of human preparations and those of the present study used rabbit ear artery preparations. Thus, it is not unreasonable to speculate that the hypotensive action of i.v. infused Hypo-PGs in patients with EHT is mainly dependent on depression of NA release from prejunctional perivascular adrenergic nerve endings.

Calcium ions play a primary role in the process of neurally evoked neurotransmitter release at adrenergic nerve junctions (Stjärne, 1973) and the process is influenced by the concentration of extracellular calcium ions. Surprisingly, as far as the interrelation between extracellular calcium levels and the inhibitory action of Hypo-PGs on the excitability of excitable cell membranes is concerned, the neurotransmitter release mechanism at adrenergic nerve terminals is nearly similar to that involved in smooth muscle contraction (Michibayashi, 1978). The hypotensive action of Hypo-PGs is probably linked to their intensive inhibitory effect on NA release from adrenergic nerve terminals and/or vasocontractile responses to pressor agents, both of which require extracellular calcium ions.

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