Effect of Endogenous Prostaglandin E on the Vasoconstrictor Response to Noradrenaline

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Abstract—The effect of the endogenous prostaglandin E (PGE) level in the vascular wall on the vasoconstrictor response to noradrenaline (NA) was examined using the perfused central artery of an isolated rabbit ear. The endogenous PGE level in the ear artery was estimated from that in the venous perfusate, which was measured by radioimmunoassay. Pretreatment with indomethacin, a cyclooxygenase inhibitor, was followed by a significant decrease in the PGE level and a significant increase in the vasoconstrictor response to exogenous NA. In addition, there was a significant correlation between the response to NA and the PGE level in the venous perfusate in combination with the results during perfusion of indomethacin and of PGE1 plus indomethacin, although the former did not correlate with the latter under the basal conditions alone. From these results, it seems possible to draw the conclusion that as far as vasoconstrictor responsiveness is concerned, there is some antagonism between NA and endogenous and/or exogenous PGE in the rabbit ear artery.

It has been proposed that PGs are biosynthesized in vascular walls (1–4) and that they may play a physiological (5–7) as well as a pathophysiological role (8–12) in various vascular beds. In addition, it is generally accepted that exogenous PGE has an inhibitory effect on the vasoconstrictor response to pressor agents such as NA and angiotensin II (13–15). Since PGE, in contrast, is a vasoconstrictor under some conditions (14, 16–18), it seems impossible to draw the conclusion that the endogenous PGE in the vascular wall exerts a constant inhibitory effect on the action of the pressor agents. In the present study, the interrelationship between the response to NA and the endogenous PGE level reflected in the venous perfusate was examined in the rabbit ear artery.

Materials and Methods
Male rabbits weighing 2.0 to 3.0 kg were anesthetized with sodium amobarbital (1.0 mg/kg, i.v.) following injection of heparin (1000 U/kg, i.v.) into the marginal ear vein. Preparation of the isolated perfused rabbit ear artery was carried out according to a slight modification of the procedure of De La Lande et al. (19), as reported previously by us (15). Briefly, these preparations were perfused by means of a roller pump, delivering a constant flow of 3.0 ml/min in almost all preparations or of 1.25 ml/min in a few preparations, with modified Krebs solution. Modified Krebs solution consisted of the following composition (mM): Na+, 137.0; K+, 5.9; Ca++, 1.8; Mg++, 1.2; Cl−, 123.9; HCO3−, 25.0; glucose, 8.3 and sucrose, 20.0. This solution (pH 7.4, 37°C) was equilibrated with a gas mixture of 95% O2+5% CO2, and then 25 µg/ml of ascorbic acid was added. A freshly prepared solution of NA, 10–100 ng in 0.1 ml of normal Krebs solution, was injected intraarterially through a rubber tube connected to the central arterial cannula.

Effects of cyclooxygenase inhibitors on the response to NA and on the PGE levels in the venous perfusate were examined as follows. First, perfusion was carried out with
modified normal Krebs solution containing a suitable concentration of cyclooxygenase inhibitor, indomethacin (0.5 to 3.0 \( \mu g/ml \)) or acetylsalicylic acid (3.0 to 90.0 \( \mu g/ml \)), for 30 min. Subsequently, their effects on the response to NA and the PGE level in the venous perfusate were examined.

In the experiment examining the effect of NA on the PGE level in the venous perfusate, the perfusates were collected for 2 min just before and following a bolus injection of NA. PGE levels in each perfusate were measured by radioimmunoassay.

Each sample of the perfusate obtained in every experiment was stored at \(-35^\circ C\) immediately following collection. Extraction of PGE in the perfusate was undertaken within one week according to a slight modification (20) of the method of Jaffe et al. (21). Alkaline conversion of PGE to PGB was carried out according to the method of Levine (available in kit form, Clinical Assay Inc., Cambridge, MA), followed by a radioimmunoassay for PGB using antiserum with a high specificity for PGB\(_1\) and PGB\(_2\). In the present study, PGE concentrations in all the perfusate samples in the same experiment were determined in a similar manner at the same time. Recovery of PGE\(_1\) through extraction, conversion of PGE\(_1\) into PGB\(_1\), and identification amounted to 72±3\% (mean±S.D.), and the mean intraassay precision for the perfusate sample was 10.5\%.

The amount of PGE\(_1\) escaping from inactivation and/or trapping in a single passage through an isolated perfused rabbit ear was examined in order to estimate the disappearance rate of PGE on the arterial side both in the presence and in the absence of angiotensin II. In this experiment, the PGE level in the venous perfusate was expressed as \( \text{ng/min/10 g of a rabbit ear} \), and PGE\(_1\) was used in a range of 2.0 to 9.0 \( \mu g/ml \).

Data analysis and statistics: In all the experiments except one, the vasoconstrictor response to NA was expressed as the change in the perfusion pressure in mmHg. In the experiment examining the relationship between the vasoconstrictor response to NA and the PGE level in the perfusate, the response to NA was expressed as a relative value (% control) of the response to NA, 10 to 100 ng, on the basis of a dose-response curve in which the dose and the response indicate ng of NA/g of wet wt. of a rabbit ear and change in vascular resistance (PVR), mmHg/ml-min\(^{-1}\)-g\(^{-1}\), respectively. All the data were analyzed using Student’s t-test, and a difference of \( P<0.05 \) was considered to be significant.

The drugs used were: noradrenaline (Fluca, A.G.), indomethacin (Banyu Pharmaceutical), prostaglandin E\(_1\) (Ono Pharmaceutical), \(^3\)H prostaglandin B\(_1\) (New England Nuclear), acetylsalicylic acid (Tanabe Pharmaceutical), and angiotensin II (Ciba-Geigy Pharmaceutical).

Indomethacin and acetylsalicylic acid were dissolved with 50 mM \( \text{Na}_2\text{CO}_3 \). Subsequently, pH was adjusted to approximately 7.4 with 100 mM \( \text{NaH}_2\text{PO}_4 \). Thereafter, these drugs were diluted with modified normal Krebs solution and were used.

Results

Perfusion pressure significantly (\( P<0.001 \)) increased from 10.5±2.3 (mmHg, mean±S.D., \( n=15 \)) to 22.2±3.9 by pretreatment with indomethacin, 0.5 to 3.0 \( \mu g/ml \), in response to an exogenously injected bolus of NA, 10 to 100 ng. Pretreatment with acetylsalicylic acid, 3.0 to 90.0 \( \mu g/ml \), also resulted in a significant (\( P<0.001 \)) increase in the response to NA from 12.3±2.5 (mmHg, \( n=6 \)) to 18.9±2.6. These pretreatments, however, elicited hardly any rise in basal perfusion pressure, although the latter was slightly and significantly (\( P<0.05 \), \( n=6 \)) increased by indomethacin, 3.0 \( \mu g/ml \), alone. In addition, the PGE level in the venous perfusate was 0.331±0.065 ng/ml under the basal conditions, but significantly (\( P<0.01 \), \( n=10 \)) decreased to 0.176±0.044 ng/ml 30 min following the perfusion of indomethacin (Fig. 1).

Next, we considered whether or not there was any correlation between the vasoconstrictor response to NA and the PGE level in the perfusate. We found that there was a significant correlation (\( P<0.025 \), \( r=-0.395 \), \( n=33 \)) between the response to NA and the PGE level in the venous perfusate in combination with the results during
perfusion of indomethacin and of PGE, plus indomethacin, although the former did not correlate with the latter under the basal conditions alone (Fig. 2).

Subsequently, the effect of NA on the PGE level in the venous perfusate was explored. In consequence, it was clarified that there was a significantly negative correlation (P<0.05, r=−0.543, n=14) between the dose of the applied NA and the change in PGE level in the venous perfusate (Fig. 3). In this experiment, the change in PGE level (ΔPGE, ng/ml) was obtained by the subtraction of the PGE level just before the application of NA from that following its application.

Finally, the escape rate of PGE₁ in a single passage through an isolated perfused rabbit ear was examined both in the presence and in the absence of angiotensin II, having the pressor effect under physiological and pathophysiological conditions. The escaping amount of PGE₁ increased with a rise in the infused PGE₁ concentration, and there was a significant positive correlation (P<0.001, r=0.974, n=15) of the former with the latter in the combination of the results in the presence of angiotensin II, 1.0 ng/ml, and those in its absence (Fig. 4). In addition, since 61.7±11.8% (mean±S.D., n=10) of the amount of PGE₁ infused intraarterially at a range of 2.0 to 9.0 ng/ml was subjected to inactivation and/or trapping and the escaping rate of PGE₁ was approximately 40%, it seems that the PGE level in the venous perfusate reflects the PGE level originating on the arterial side as well as on...
the venous side.

Discussion

It is well known that a cascade of arachidonate mainly consisting of cyclooxygenase and lipoxygenase pathways exists in the vascular walls (1–4). Among the metabolites of arachidonate, PGE$_2$ and PGI$_2$, as vasodilators, and PGF$_{2\alpha}$, thromboxane A$_2$ and leukotrienes as vasoconstrictors, occur mainly
in the vascular beds. Since there is a sex difference in the PGs levels in the vascular beds (12), the interrelationship between endogenous PGE as a vasoconstrictor and exogenous NA as a vasoconstrictor was examined using male rabbit ears alone in the present study. It is generally accepted that PGE directly and indirectly dilates vascular smooth muscles, the latter being done through a mechanism inhibiting the release of NA from the adrenergic nerve terminals (14, 22-24). Approximately 0.3 ng/ml of PGE was detected in the venous perfusate. This concentration of PGE reflects the summation of the amount released from the arterial and venous sides, and this value would correspond to that in the venous perfusate, if approximately 0.75 ng/ml of PGE₁ would be infused into the arterial side of a rabbit ear, because the escape rate of PGE₁ from inactivation and/or trapping in a single passage through a rabbit ear is about 40% (Fig. 4). Therefore, the indomethacin-induced reduction in the PGE level in the venous perfusate (Fig. 1) reflects that in the endogenous PGE level in the arterial wall as well as that in the venous wall and results in an increase in the response to NA. Moreover, this conclusion is, at least in part, supported by a significantly negative correlation between the response to NA and the PGE level in the venous perfusate, although the former did not correlate with the latter under the basal conditions alone (Fig. 2). Accordingly, it is possible to speculate that a rising and falling of the endogenous PGE level in the vascular walls influences the response to NA. In the experiment examining the effect of NA on the PGE level in the venous perfusate, it was clarified that there was a significant negative correlation between the dose of NA and the alteration in the PGE level (JPGE) and that NA had biphasic effects on the PGE level; i.e., the higher dose of NA decreasing and, in contrast, the lower dose of NA increasing the PGE level (Fig. 3). Based on several reports that PG synthesis in some tissues is elevated under appropriately hypoxic conditions, but if oxygen deprivation is so severe as to limit the availability of oxygen for synthesis, PG synthesis is, in contrast, inhibited (25-28), the above mentioned biphasic effects of NA may relate to the grade of hypoxia in the isolated rabbit ear. Also, the decrease in the PGE level in the venous perfusate following the augmentation in PVR by the higher dose of NA may probably be influenced by the other mechanisms; i.e., the trapping in the blood vessel walls, the leakage out of the intravascular space and the inactivation by 15-hydroxy PG dehydrogenase in the vascular beds.

Vascular damage due to mobilization of pressor substances has been observed in many diseases. The interaction between PGE and NA in the vascular wall appears to be pathophysiological valuable in elucidating the clinical background of many diseases with vascular damage, particularly those with hypertensive vascular disturbances. However, to confirm this, further investigation regarding the other polyunsaturated fatty acids metabolites (i.e., PGs and leukotrienes) in the vascular beds will be necessary in future.

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References


