Vasocontractile Responsiveness of Perfused Arterial Segments to Platelet-Derived Thromboxane A₂

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Abstract

It remains not entirely accepted that changes in prostanoid metabolism in the blood vessel wall, as well as in whole blood, have a certain influence on vascular responsiveness to vasoactive agents. The aim of the present study is to elucidate whether platelet-derived thromboxane A₂ (TxA₂) participates in enhancement of vasocontractile response to a pressor agent. Platelet aggregation was extraluminally induced by application of collagen to autologous platelet rich plasma (PRP), and then the PRP treated with collagen was infused into the perfusion system by means of a small infusion pump. All the prostanoids in the perfusate were assayed radioimmunologically. Infusion into the perfusion system of PRP treated with collagen, as well as that of untreated PRP, apparently caused pronounced enhancement of vasocontractile response to noradrenaline (NA), accompanied by elevations of both the level of TxB₂, a stable metabolite of TxA₂, and the TxB₂/prostaglandin E (PGE) ratio. In addition, treatment with either OKY-046 (a Tx A₂ synthetase inhibitor) or ketanserin (a selective S₂-serotonergic antagonist) resulted in diminution of the raised vasoconstrictor response to NA induced by application of collagen to PRP.

Thus, it is possible to draw the conclusion that platelet-derived TxA₂ is as potent a vasoactive substance as 5-hydroxytryptamine (5-HT) and at least in part, contributes to the enhancement of vasocontractile response to NA (NA-R) during raised platelet aggregability.

Key words: Thromboxane A₂, Platelet rich plasma, Artery, Noradrenaline, Collagen

Introduction

It is well known that blood flow is regulated by neurogenic, humoral, myogenic and structural factors (Uchida & Bohr, 1969). Total peripheral vascular resistance, related intimately to these factors, seems to be partially influenced by plasma constituents (Moretti & Abraham, 1978; Rothhut, 1983) and blood corpuscle-derived vasoactive substances (Garcia-Szabo, 1988; O'Brien *et al.*, 1988). However, in many reports concerning vascular responsiveness, blood vessel contractility using a normal test solution (Tyrode's solution, Krebs' solution, etc.), containing plasma constituents and/or blood corpuscles has hardly been examined. And so, the results obtained in angiological investigations performed using normal test solutions do not always appear to reflect the genuine blood vessel contractility in the body. Even if an experiment using vascular preparations could be carried out by means of whole blood perfusion,

it would be very difficult to evaluate whether some of the vasoactive substances originate from plasma chemical components, blood cells or blood vessels. It remains, moreover, not fully known as to whether alterations in prostanoid metabolism in the vascular beds have an influence on blood vessel contractility (Cappuccio, 1986 ; Nowak & FitzGerald, 1987).

Thus, the present study was undertaken using perfused arterial segments with the aim of evaluating whether changes in the prostanoid level in the blood vessel wall participate in vascular responsiveness to vasoactive substances when infusing PRP with or without collagen into the perfusion system.

Materials and Methods

Male Japanese white rabbits weighing 2.5 to 3.0 kg were anesthetized with sodium pentobarbital (30 to 60 mg/kg). Following injection of heparin (1,000 U/kg, i.v.) into the marginal ear vein, approximately 80 ml of whole blood was obtained from the femoral artery with needle (21 gauge) cannulation and collected into a beaker containing sodium citrate (3.8% w/v, 1 part+9 parts of blood). PRP (platelet count $40.6 \times 10^4 \pm 14.1 \times 10^4/\text{mm}^3$, mean $\pm$ S.D., $n=4$), obtained by centrifuging the whole blood at 1,000 rpm for 10 min at room temperature, was used within 15 hrs following blood collection. Following this step, the arterial segment was dissected from the proximal portion of the central artery of the rabbit ear. This segment, approximately 2.7 cm long, was separately cannulated at the proximal and distal ends with polyethylene tubing, and it was installed in a nearly 5 ml organ bath apparatus in a horizontal plane according to a slight modification of the procedure of De La Lande (De La Lande *et al.*, 1966). This preparation was then perfused with a modified Krebs solution by means of a roller pump delivering a constant flow of 3 ml/min (Michibayashi, 1984). The modified Krebs solu-

Fig. 1. Changes in perfusion pressure. Control: perfusion of a modified normal Krebs solution, PRP: perfusion during infusion of collagen-untreated PRP, PRP+Collagen: perfusion during infusion of collagen-treated PRP. Changes in basal perfusion pressure ($\square$, $n=8$) and in vasoconstrictive response to noradrenaline ($\blacksquare$, $n=7$) were represented in this figure. Each value and vertical bar indicate the means and standard deviations of the mean, respectively. Noradrenaline, 1.25 to 5.0 ng/0.1 ml of a modified normal Krebs solution, was injected as a single bolus.

tion used in the present study had the following composition (mM): Na^+ , 137.0; K^+ , 5.9; Ca^{++} , 1.8; Mg^{++} , 1.2; Cl^- , 123.9; HCO_3^- , 25.0; glucose, 8.3 and sucrose, 20.0. This solution (pH 7.4, 37°C) was equilibrated with a gas mixture of 95% O_2 + 5% CO_2 , and then 20 to 40 $\mu\text{g}/\text{ml}$ of ascorbic acid was added. A suitable concentration of 0.1 ml of NA, freshly prepared in normal Krebs solution, was injected as a bolus into a rubber tube connected to the central arterial cannula, followed by perfusion pressure change (mmHg). The perfusates were collected for 2 min 2 hr after beginning of perfusion of a modified normal Krebs solution alone (the control sample), 30 min after infusion of PRP (the PRP sample), and 8 min after infusion of PRP treated with collagen (the PRP•collagen sample). Each sample of perfusate from every experiment was stored at -20°C immediately following collection. Extraction of prostanoids in the perfusate was carried out within one week after perfusate collection, as reported previously (Michibayashi, 1989), followed by further purification handling according to the method of Inagawa (Inagawa *et al.*, 1972) to eliminate remaining lipids. And then, the concentrations of PGE ($\text{PGE}_1 + \text{PGE}_2$), TxB_2 and 6-keto- $\text{PGF}_{1\alpha}$ in the perfusate were measured by radioimmunoassay. In the experiment examining the effect of PRP with or without collagen on NA-R, autologous PRP was infused into the perfusion system at the rate of 3 ml/hr with a Micro

Fig. 2A. Changes in TxB_2 level in perfusate. Control: perfusion of a modified normal Krebs solution, PRP: perfusion during infusion of collagen-untreated PRP, PRP•Collagen: perfusion during infusion of collagen-treated PRP. Thick line indicates mean value ($n=6$).

Fig. 2B. Changes in PGE level in perfusate. Control: perfusion of a modified normal Krebs solution, PRP: perfusion during infusion of collagen-untreated PRP, PRP•Collagen: perfusion during infusion of collagen-treated PRP. Thick line indicates mean value ($n=11$).

Fig. 3. Changes in TxB_2/PGE ratio in perfusate. Control: perfusion of a modified normal Krebs solution, PRP: perfusion during infusion of collagen-untreated PRP, PRP+Collagen: perfusion during infusion of collagen-treated PRP. Thick line indicates mean value ($n=6$).

Infusion Pump (SP-5, Nipro, Japan). Under this condition, the flow rate was increased about one sixtieth of that during perfusion of the modified normal Krebs solution alone (about 0.05 ml/min) more than that of preinfusion state, while this infusion rate of normal test solution without PRP did not elicit any elevation of basal perfusion pressure. Platelet aggregation was induced by the addition of collagen (0.4 to 4.0 $\mu\text{g}/\text{ml}$) into the syringe containing PRP. This step was followed by the observation of NA-R represented on the smoked drum as perfusion pressure change, and then, by the perfusate sampling. Furthermore, OKY-046 (a thromboxane synthetase inhibitor, 0.1 to 1.0 $\mu\text{g}/\text{ml}$) was applied to autologous PRP for the purpose of inhibiting TxA_2 biosynthesis in platelets. Also, ketanserin tartrate (a selective 5-HT₂ receptor antagonist, 45 to 450 ng/ml) was applied to the perfusion system in order to suppress the effect of platelet — derived 5-HT on the blood vessel contractility.

The drugs used were sodium citrate (Midorijyuzi); collagen (Worthington Biochemical Co.); noradrenaline (SIGMA); authentic prostanoids of PGE_1 , TxB_2 and 6-keto-PGF_{1 α} (Ono Pharmaceutical Co.); ^3H -labelled prostanoids of ^3H -PGB₁, ^3H - TxB_2 and ^3H -6-keto-PGF_{1 α} (New England Nuclear); antisera for PGB (Ono Pharmaceutical Co.), TxB_2 (bio yeda) and 6-keto-PGF_{1 α} (bio yeda); indomethacin (SIGMA); OKY-046 (Ono pharmaceutical Co.) and ketanserin tartrate (Kyowahakko).

Results obtained in the present study were expressed as mean $\pm$ S.D.. All data were analyzed using the F-test and a difference of $P < 0.05$ was considered to be significant.

Results

1. Blood vessel contractility and platelet aggregation

At first, the effect of PRP on the basal perfusion pressure was examined. During infusion of untreated PRP, the basal perfusion pressure was hardly altered, though it was raised a little

Fig. 4. Inhibitory effect of OKY-046 on the enhanced vasoconstrictor response to noradrenaline during infusion of collagen-treated PRP.

Three experiments were carried out. Of these experiments, a typical case undertaken in the presence of OKY-046, 100 ng/ml, is shown in this figure.

Fig. 5. Inhibitory effect of ketanserin on the enhanced vasocontractile response to noradrenaline during infusion of collagen-treated PRP.

Four experiments were carried out. Of these experiments, a typical case undertaken in the presence of ketanserin, 45 ng/ml, is shown in this figure.

in a few preparations, whereas NA-R was significantly augmented (11.4 ± 4.4 mmHg, $n=7$, $P < 0.05$) in comparison with the control response (4.6 ± 3.1 mmHg, $n=7$, Fig. 1). Subsequently, the effect of collagen-induced platelet aggregation on NA-R was examined. The basal perfusion pressure was transiently unstable, after which it was gradually stabilized several minutes after collagen application. Changes in the basal perfusion pressure during infusion with collagen (6.8 ± 6.1 mmHg, $n=8$, $P < 0.05$) was shown to be significantly more increased than those of PRP without collagen (1.8 ± 2.0 mmHg, $n=8$). Following this, NA-R was examined. As can be seen in Fig. 1, 1.25 to 5.0 ng of NA injected as a bolus resulted in a significantly raised response

(32.1 ± 16.9 mmHg, $n=7$, $P < 0.025$) in comparison with that during infusion of untreated PRP.

2. *Changes in prostanoid level in perfusates*

As can be seen in Fig. 2A, no TxB_2 level was detected in any perfusates during perfusion of a modified normal Krebs solution, whereas this level was elevated to assayable values in all the preparations during infusion of PRP. Both levels of TxB_2 during infusion of PRP alone (16.8 ± 8.2 pg/ml, $n=6$, $P < 0.01$) and of PRP treated with collagen (40.3 ± 23.9 pg/ml, $n=6$, $P < 0.05$) were significantly higher than the control (undetectable amount, $n=6$). Furthermore, there was a significant ($P < 0.05$) difference between both levels of TxB_2 during infusions of PRP with and without collagen. Also, with regard to PGE (Fig. 2B), both levels of PGE during infusion of PRP alone (0.295 ± 0.284 ng/ml, $n=11$, $P < 0.05$) and of PRP treated with collagen (0.320 ± 0.324 ng/ml, $n=9$, $P < 0.05$) were significantly higher than the control (0.172 ± 0.156 ng/ml, $n=11$), although the PGE level deviated greatly and there was no significant difference between either level of PGE during infusions of PRP with and without collagen. As regards 6-keto-PGF $_{1\alpha}$, there was no significant difference between levels of 6-keto-PGF $_{1\alpha}$ in any perfusate (normal Krebs solution: 0.166 ± 0.161 ng/ml, $n=8$, PRP alone: 0.172 ± 0.135 ng/ml, $n=6$, and PRP treated with collagen: 0.170 ± 0.190 ng/ml, $n=6$). Likewise, alterations in the TxB_2 /PGE ratio in the perfusates were evaluated (Fig. 3). Consequently, it was shown that the TxB_2 /PGE ratio during infusion of untreated PRP (0.07 ± 0.04 , $n=6$, $P < 0.02$) was significantly higher than the control (approximately zero, $n=6$) and as expected, was significantly lower than that of PRP treated with collagen (0.130 ± 0.051 , $n=6$, $P < 0.02$).

3. *Effect of the thromboxane synthetase inhibitor on vasoconstrictor response*

Firstly, OKY-046 (0.1 to 1.0 $\mu\text{g/ml}$) was added to the syringe containing PRP in order to depress TxA_2 synthetase activity. About 30 min after OKY-046 treatment, platelet aggregation was elicited by application of collagen (0.4 to 4.0 $\mu\text{g/ml}$) to the syringe. Subsequently, this PRP was infused into the perfusion system using a MICRO INFUSION PUMP, after which the comparison between the responses to NA in the absence and presence of OKY-046 was made. In consequence, it was clarified that collagen — induced enhancement of NA-R was diminished by OKY-046 (Fig. 4).

4. *Effect of the 5-hydroxytryptamine receptor antagonist on vasoconstrictor response*

Platelet aggregation is well known to cause the release of several vasoactive agents from platelets. Among the vasoactive agents originating from platelets, 5-HT is as potent a vasoactive substance as TxA_2 . And so, finally, the effect of ketanserin on NA-R during infusion of PRP treated collagen was examined. As can be seen in Fig. 5, ketanserin, 45 ng/ml, was firstly infused into the perfusion system, then 30 min later 5 ng of NA was applied as a bolus. Consequently, ketanserin was revealed to abolish the enhancement of NA-R during infusion of PRP with collagen.

Discussion

As is well known, many clinical signs of cardiovascular disorders are clearly related to

changes in blood vessel contractility and/or blood flow. Recently, it has been demonstrated that alterations in prostanoid metabolism in vascular beds have an influence on the vasocontractile responsiveness to vasoactive agents (Michibayashi, 1984; Nowak & FitzGerald, 1987; Neri Serneri *et al.*, 1990). It now seems that prostanoid biosynthesis of the vascular beds in the body appears to be more accurately reflected in results obtained by perfusing normal test solution containing blood chemical components and/or blood cells rather than by normal test solution alone. However, reports concerning the relationship between prostanoid metabolism in blood vessel walls and vascular responsiveness in the presence of plasma constituents and blood cells have been fewer (Moretti & Abraham, 1978; Smith *et al.*, 1980). And so, in the present study, the interaction of blood vessel contractility with platelet aggregation was evaluated by infusing PRP into the perfusion system. It was impossible to detect TxB_2 in the perfusate collected during perfusion of normal test solution, although it became detectable following infusion of PRP in all preparations (Fig. 2A). This result may suggest that TxA_2 mainly originates from PRP, primarily platelets. Also, PRP infusion-related enhancement of NA-R was accompanied by a significant elevation of the TxB_2 level (Fig. 2A) and the TxB_2/PGE ratio (Fig. 3). As regards vasodilator prostanoids, the PGE level was significantly elevated during infusion of PRP with and without collagen in comparison with that of control, though application of collagen to PRP did not cause any significant elevation of the PGE level (Fig. 2B). In addition, PRP introduction did not elicit any significant difference in the 6-keto- $\text{PGF}_{1\alpha}$ level in any preparation. Therefore, TxA_2 appears to be, at least partially, involved in PRP-induced augmentation of NA-R. This estimation seems to be supported by the fact that pretreatment with either OKY-046 (Fig. 4) or indomethacin (Michibayashi, 1991), applied to PRP with the aim of suppressing prostanoid biosynthesis, attenuates the enhancement of NA-R following application of collagen to PRP. Moreover, since ketanserin almost entirely abolishes NA-R during collagen-induced platelet aggregation (Fig. 5), it is speculated that TxA_2 may have an accelerating action on the augmented NA-R with the infusion of collagen-treated PRP. As regards platelet-derived 5-HT, in order to understand the powerful action of ketanserin, it appears necessary to take into consideration that, in addition to a selective 5-HT₂ receptor antagonistic action, this blocker possesses a moderate α_1 -adrenoreceptor antagonistic effect (Amstein *et al.*, 1988; Ramage, 1988). Thus, it seems possible to propose that 5-HT, as well as TxA_2 , is involved in augmentation of NA-R during infusion of collagen-treated PRP.

As is generally accepted, ischemic heart disease, cerebral thrombosis, essential hypertension, diabetes mellitus and the classical cardiovascular disorders are readily accompanied by platelet aggregation and their clinical signs are greatly affected by an interaction of vascular responsiveness with platelet aggregability, the main subject in the present study. Recently, in addition to prostanoid and 5-HT, many investigators have presented a great amount of data concerning endothelium-derived relaxing factor (Ignarro *et al.*, 1987a; Ignarro *et al.*, 1987b), endothelin (Yanagisawa *et al.*, 1988; Salom *et al.*, 1991), platelet-derived growth factor (Araki *et al.*, 1990) and leukotriene (Wittman *et al.*, 1987; Hillyard *et al.*, 1991). Therefore, it may be necessary, in order to elucidate their physiological and/or pathophysiological roles in the body, to study these vasoactive substances by using blood vessel preparations, blood cells and plasma constituents in the future.

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