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SYNERGISTIC ANTIPLATELET ACTION OF NITRIC OXIDE (NO) WITH PGD_2 AND ITS METABOLITE PGJ_2 — RELEVANCE FOR CEREBRAL CIRCULATION?

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The PGI_2/NO axis is well accepted for its central regulatory role in maintaining haemostatic balance in large arteries. Earlier findings suggest that PGD_2 may also play a role in haemostatic regulation of human cerebral circulation. We therefore wondered whether PGD_2 and its metabolite PGJ_2 synergise *in-vitro* with NO. We approached this question using platelets of ten healthy donors and ADP as aggregation-inducing stimulus.
Both PGD_2 and PGJ_2 do inhibit ADP-induced platelet aggregation in a dose-dependent manner. Platelet aggregation findings demonstrate that PGD_2 and NO synergise, as does the metabolite PGJ_2. Our data are indicative that the PGD_2/NO and, in less extent, PGJ_2/NO synergism might be of special importance for the cerebrovascular haemostatic control.

Key words: *PGD_2, PGJ_2, nitric oxide (NO), cerebral vessels, cerebrovascular disease, platelet aggregation, haemostasis.*

INTRODUCTION

The regulatory role of prostaglandin (PG) I_2 in maintaining haemostatic balance in large vessels (1) and its synergism with nitric oxide (NO), the active compound of endothelium derived relaxing factor (EDRF), (2), is well known (3). Experimental animal data, however, indicate that PGD_2 may be of even equal biological relevance for cerebral circulation than PGI_2 (4).

PGD_2 is produced, among others, by the cerebral capillary and microvascular endothelium from endogenous and exogenous substrate as well (5). Although PGD_2 has been shown to interact with the vessel wall causing vasoconstriction and/or vasodilation (5) and also to inhibit platelet aggregation

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(6) via specific receptors on the surface of platelets (7, 8), its physiological role is not elucidated yet. A decrease in platelet sensitivity to PGD_2 has been described in myeloproliferative disorders (9), acute thrombosis (10) and peripheral vascular disease (11). Similarly, evidence has been put forward that PGD_2 sensitivity might be altered in patients with cerebrovascular disease (5, 12).

A compound claimed to be 9-deoxy-Δ9-PGD_2 named also PGJ_2 (13), is readily formed from PGD_2 in aqueous solution. This compound is active in inhibiting aggregation induced by ADP in citrated human platelet rich plasma (PRP) (14).

It was thus the goal of this study to examine whether PGD_2 is able to exert a comparable synergism with NO found for PGI_2 (3) and PGE_1 (15) before, and whether it can be demonstrated for its metabolite PGJ_2 as well.

MATERIAL AND METHODS

Volunteers

Blood was drawn from 10 healthy volunteers (6 males, 4 females; aged 24—43 years) without any risk factor for the development of atherosclerosis. They had not taken any medication since at least four weeks prior to blood withdrawal.

PRP — preparation

20 ml blood drawn from a non-occluded cubital vein were anticoagulated (1:9) using 3.8% sodium citrate (Heilmittelwerke, Vienna, Austria). Thereafter, blood was sedimented for 15 minutes at 22°C. Platelet rich plasma (PRP) was prepared by centrifugation (150 × g, 7 minutes, 22°C). After the careful removal of PRP, a further 15 minutes centrifugation at 1500 × g at 22°C to obtain platelet poor plasma (PPP) was performed.

ADP — induced platelet aggregation

Aggregation was induced in 600 µl PRP — samples by addition of 1 µM ADP/ml) in a Born — aggregometer (16). Aggregation response was quantified using the angle α (slope of the aggregation curve after the addition of the aggregation inducing agent) and the maximal amplitude (T_{max}) of the response curve. PRP was adjusted with PPP to a constant platelet count of 2.5×10^5 cells/µl. In addition, a PRP — sample was recorded for 10 minutes to monitor spontaneous aggregation.

PGJ_2 preparation

PGJ_2 was prepared from PGD_2 as described by Mahmud et al. (14). It was used within one week and stored at −70°C.

NO preparation

A glass vial was filled with 10 ml Tris-buffer (pH 7.4). This Tris-buffer was bubbled for 15 minutes with Argon gas and then with NO-gas for 10 minutes. The gas — bulb was sealed with a rubber stopper. 1 ml was removed with a syringe and injected into another gas bulb which was filled with 9 ml Tris-buffer. This buffer was also bubbled for 10 minutes with Argon gas. The final concentration of NO therefore was 0.25%. 1 ml of this 0.25% NO-solution was added to 10 ml PRP, to obtain 10 μM NO.

Testing of the sensitivity of platelets against PGD_2 and PGJ_2 and addition of PGD_2/NO or PGJ_2/NO

For testing the platelet sensitivity in the presence of PGD_2 and PGJ_2, the aggregation response was suppressed using at least three different doses of PGD_2 (The Upjohn Company, Kalamazoo, Michigan, USA) or PGJ_2 and NO, respectively, added prior to induction of aggregation with ADP. 100 μl Tris-buffer (pH 7.5) for control or 100 μl of the PG-solution (PGD_2, PGJ_2) were added to the aggregation vial. After 30 seconds 10 μl Tris-buffer or 10 μl NO (10 μM) and after further 30 seconds 100 μl ADP (1 μM) were added to the PRP into the aggregometer-vial, for the determination of synergism between PGD_2 or PGJ_2 and NO. The temperature was constantly kept at 37°C *via* a heating block.

The inhibitory concentration was calculated in ng PG/ml PRP for PGD_2 as well as PGJ_2 and in μM NO/ml PRP for NO.

RESULTS

Platelet aggregation response

ADP-induced platelet aggregation resulted in a slope α of $73.6 \pm 3.7°$ and a ΔT_{max} of the response curve of $62.7 \pm 4.1\%$.

Inhibition of ADP-induced platelet aggregation

The suppression of ADP-induced platelet aggregation was dependent on the dose of PGD_2 and PGJ_2 (*Fig. 1*). However, the amount of PGJ_2 necessary to get a similar platelet aggregation inhibitory effect was higher than that of PGD_2.

The IC-50 for ADP-induced platelet aggregation amounted 6.17 ± 0.87 ng/ml PRP for PGD_2, 20.84 ± 2.63 ng/ml PRP for PGJ_2 and 0.94 ± 0.17 μM/ml PRP for NO.

Synergistic effects

Platelet aggregation findings demonstrate that PGD_2 and NO synergise, as does the metabolite PGJ_2 (*Table 1*).

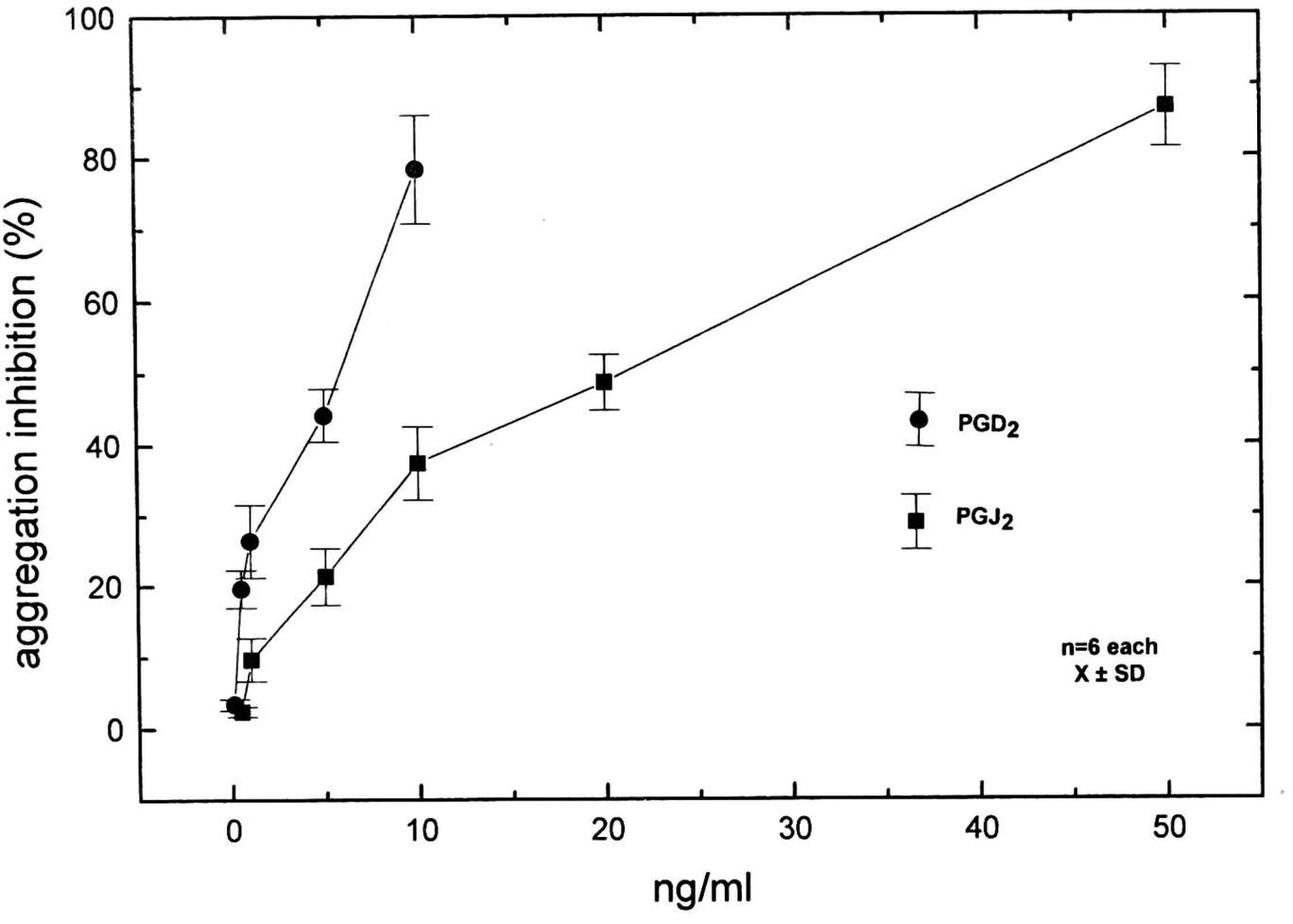

Fig. 1. Inhibition of ADP — induced platelet aggregation by PGD_2 and PGJ_2.

Table 1. PGD_2/NO and PGJ_2/NO synergism

	PGD_2/NO			PGJ_2/NO		
	IC 60/IC 40	IC 50/IC 50	IC 40/IC 60	IC 60/IC 40	IC 50/IC 50	IC 40/IC 60
T_{max} (%)	20.50 ± 2.8	21.60 ± 3.7	20.12 ± 2.2	17.2 ± 4.3	18.8 ± 3.6	18.4 ± 3.0

T_{max} — maximal amplitude of the response curve; IC — inhibitory concentration for ADP — induced aggregation

DISCUSSION

Among other thromboregulatory compounds, arterial walls generate antiaggregatory PGs (1, 17) and EDRF (17). NO is synthesized by the vascular endothelium from the terminal guanido nitrogen atom (18) of the aminoacid L-arginine (19).

PGI_2 and PGE_1 are inhibiting platelet activity by increasing intracellular concentrations of platelet cyclic adenosine monophosphate (cAMP) (20,21). Both EDRF and NO cause a relaxation of vascular strips, inhibit platelet

aggregation, induce disaggregation of aggregated platelets and inhibit platelet adhesion (15, 22, 23) through the activation of soluble guanylate cyclase in the cardiovascular and nervous systems, resulting in increased levels of cyclic guanosine monophosphate (cGMP) in platelets.

PGD_2 was detected in various tissues, including brain, in various animal species and man (4). Its presence in the medium obtained from cultured capillary and microvascular endothelium of human brain was recently demonstrated (5). PGD_2 can be formed by either nonenzymatic degradation (6) or enzymatic conversion of PGH_2 by PGD synthetase (24). There is evidence that the actions of PGD_2 on cerebral microvasculature are mediated via specific binding sites coupled to the adenylate cyclase system (6). The existence of an adenylate cyclase coupled receptor for this particular PG, together with the findings that platelet sensitivity to PGD_2 (and not only to PGI_2 and PGE_1) in atherosclerosis is reduced possibly due to an involvement of the cerebrovascular region, provide striking evidence for the hypothesis that PGD_2 is an important factor concerning the local self-regulation of cerebral microvascular blood perfusion (12). Merely, PGD_2 might also act as feedback inhibitor to prevent aggregation and interrupt vicious circle (6). PGJ_2 is a dehydratation product of PGD_2, occuring after spontaneous degradation in plasma, most certainly by means of albumin catalysis. It has been demonstrated to inhibit platelet aggregation as PGD_2 does, being, however, only 10%—25% as active as PGD_2 (13, 14).

Synergistic effects between stimulators of adenylate cyclase and substances that act via cGMP have been extensively described (for example PGI_2 and NO (3), PGI_2 and SIN-1, and exogenous NO-donor, (25), PGE_1 and NO (15), PGE_1 and isosorbide dinitrate (26), PGE_1-metabolites and NO (27), iloprost and NO-donors (28), iloprost and sodium nitroprusside (29). Although the PGI_2/NO — axis is well accepted for its central role in haemostatic regulation in large arteries (1, 3), there is no information available concerning the interaction of either PGD_2 or PGJ_2 and NO yet. The synergistic effects referred and the fact that PGD_2 similarly inhibited the platelet aggregation via cAMP (13, 14) stimulated us to examine the potential additive effect between PGD_2 (and its metabolite PGJ_2 as well) and NO. This study showed that both PGD_2 and PGJ_2 do inhibit ADP-induced platelet aggregation in a dose-dependent manner. The antiaggregatory action of the PGD_2 on blood platelets was confirmed by our findings as being stronger than PGJ_2. These two compounds share the antiplatelet synergism with NO, suggesting that the local synergism of PGD_2/NO (and in less extense PGJ_2/NO) might be of central importance in haemostatic regulation of cerebral circulation. On the contrary to platelets, since the interaction of PGD_2 with the vessel causes vasoconstriction (5) and/or vasodilatation (5, 30), which are mediated by different receptors, antagonist activity of PGD_2 with NO-donors (associated with vasodilatation) (15, 22) in the vascular smooth muscle cells can even be expected.

Prostaglandins, especially PGI_2 and PGE_1 (31), have been successfully used as therapeutic agents for atherosclerotic vascular disease for years. PGD_2 is thought to be involved in controlling local cerebral circulation (5) and there are findings that platelet sensitivity to PGD_2 is reduced (12) and the formation of endogenous NO is impaired in atherosclerotic human vessels (32). One therefore could speculate that PGD_2 as well as its metabolite PGJ_2, that synergise with compounds such as NO-donors, may possibly be used to modulate platelet function in atherosclerotic cerebrovascular disease in the future.

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