leucocytes can generate the potent anti-aggregating agent prostacyclin and that platelets are, therefore, likely to behave differently in blood than in PRP. Another related problem is that the preparation of PRP from blood may take 15–30 min and that labile modulators of aggregation such as thromboxane A_2 (Hamberg, Svensson & Samuelsson, 1975) and prostacyclin (Moncada, Gryglewski, Bunting & Vane, 1976) may decay substantially during sample preparation. Recently, some attempts have been made to circumvent this difficulty: Gryglewski, Korbut, Ocetkiewicz & Stachura (1978) have described a bioassay technique for measuring platelet aggregation in an extracorporeal circulation and we now describe experiments designed to evaluate a novel electronic aggregometer which accepts blood as well as PRP. This is described in our demonstration (Cardinal & Flower, 1979).

In an initial series of experiments we measured the aggregation responses of citrated (or heparinised) human and rabbit PRP by the optical and electronic aggregometer in parallel. Both techniques gave dose related responses to collagen (0.1–10 μg/ml), ADP (1–20 μM), arachidonic acid (1–10 μg/ml), thrombin (0.1–1.0 U/ml) and prostaglandin endoperoxides (0.05–1.0 μg/ml) and these were antagonized by prostacyclin (1–5 ng/ml) and (with collagen, ADP and arachidonic acid) indomethacin (1–10 μg/ml). Although very similar, there were differences in the results obtained with the two techniques. The electronic aggregometer gave no 'shape-change' information, but was more sensitive, especially to collagen. Biphasic and reversible responses of human blood to ADP (1–4 μM) could be seen with the electronic aggregometer as well as the optical machine although they were not so well marked in the former. In a second series of experiments citrated (or heparinised) rabbit and human blood was used and the ability of the above agents to induce aggregation was checked. All these stimuli (same concentrations) gave similar dose-related responses to those seen in PRP and these could be antagonized by prostacyclin and (in appropriate cases) indomethacin. The electronic aggregometer gives accurate and precise measurements of platelet aggregation in blood. Quantitative studies of platelets in their natural milieu, a procedure which has never before been possible, should lead to fresh insight into the mechanisms which control clot or thrombus formation.

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A prostacyclin-like substance in rat brain

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Prostaglandin I_2 (PGI_2, prostacyclin) has been shown to have vasodilator and potent platelet antiaggregation activity (Dusting, Moncada & Vane, 1977). PGI_2 is a major product of arachidonic acid metabolism in many tissues but has not thus far been identified in brain. Sun, Chapman & McGuire (1977) studied prostaglandin and thromboxane formation in rat brain homogenates. Following incubation with the prostaglandin endoperoxide [1-^{14}C]-PGH_2 the major product was PGD_2 with lesser amounts of TxB_2 and PGE_2 and $PGF_{2\alpha}$. No PGI_2 was detected as measured by the appearance of [1-^{14}C]-6-keto $F_{1\alpha}$ in the homogenate. This method of detection is relatively insensitive; therefore, PGI_2 occurrence in rat brain has been reassessed using a sensitive biological method of detection. Wistar rats (250 g) were killed by cervical dislocation and the cerebral cortex removed. This was finely chopped and 200–500 mg placed in 1.0 ml Tris buffer pH 7.5 (0.05M) at 22°C. At various times the homogenate was rapidly centrifuged and the supernatant(S) treated in a variety of ways. Platelet aggregation activity of S was measured using an aggregometer and human PRP. 0.1 ml S or standards were added to PRP, alone or 1 min before aggression was induced with ADP (1-2 μM). 2.1 nM PGI_2 and 30nM PGD_2 could be detected.

0.1 ml S from homogenates tested immediately after 1–2 min incubation induced platelet aggregation; this is suggestive of the presence of a thromboxane-like substance which has previously been detected in rat brain (Wolfe, Rostorowski & Marion, 1976; Sun *et al.*, 1977). This activity was not further investigated.

0.1 ml S from homogenates tested immediately after 20 min incubation caused significant inhibition of ADP induced aggregation. Adjustment of S after 20 min incubation at pH 12 stabilized inhibitory activity for at least 4 h. This property is shared by pure PGI_2 but the platelet aggregation inhibitory activity of PGD_2 is destroyed at pH 12 in less than 20 min; this would also destroy TxA_2 and PGE_2 activity. By adjusting S to pH 12 for 2 h and neutralizing an estimate of PGI_2-like activity could be obtained without interference from PGD_2 or TxA_2. After 25 min incubation 2 ng PGI_2-like activity/100 mg wet-weight tissue was measured (n =3). The half-life of PGI_2-like inhibitor activity in supernatant was about 10–15 min at 22°C. Maintaining S at room temperature for 1 h or at 37°C for 45 min destroyed 80–90% of the inhibitory activity (n = 20). These properties were also shared by PGI_2 in parallel investigations.

Indomethacin (2 μg/ml) added to brain homogenates had no significant effect on PGI_2-like activity but treatment of rats for 3 days (10 mg kg^{-1} day^{-1}) and addition of 50 μg/ml to incubates abolished PGI_2-like activity. Tranylcypromine (12.5 mg $kg^{-1}day^{-1}$) plus *in vitro* addition of 500 μg/ml failed to modify PGI_2-like activity.

We conclude that a PGI_2-like substance is synthesized in chopped rat brain incubates.

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Prostacyclin-release by bradykinin *in vivo*

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Some vascular actions of bradykinin (Bk) have been attributed to prostaglandin generation (see Vane & Ferreira, 1976; McGiff, Terragno, Terragno, Colina & Nasjletti, 1976). The discovery of prostacyclin (PGI_2) and its generation by the vascular wall (Moncada & Vane, 1977) suggests that this substance, rather than PGE_2 is the potential modulator of vascular tone (Dusting, Moncada, Mullane & Vane, 1978). This study was carried out to determine whether Bk releases prostacyclin *in vivo*.

Prostaglandins were detected by the blood-bathed organ technique (Vane, 1964). Blood was continuously withdrawn from catheters placed in the femoral artery or renal vein of chloralose anaesthetized dogs, reoxygenated, and then used to superfuse a bank of assay tissues including bovine coronary artery (BCA), rabbit coeliac (RbCA) or mesenteric artery (RbMA), rabbit aorta (RbA), rat stomach strip (RSS) and rat colon (RC). Propranolol (2 μg/ml), phenoxybenzamine (0.1 μg/ml) and Sar^1-Ile^8)-angiotensin II (0.025 μg/ml) were continuously infused into the blood bathing the tissues to antagonize the effects of catecholamines and angiotensin.

Bk (2–10 μg $kg^{-1}min^{-1}$) infused intravenously or intra-arterially produced dose-related decreases in systemic arterial pressure, the magnitude of the response being independent of the route of administration. The higher doses (5–10 μg $kg^{-1}min^{-1}$) also produced a relaxation of RbCA, RbMA and BCA and contractions of RSS and RbA. Moreover, there was a reduction in the spontaneous activity of RC. Most of the effects on the bioassay tissues could be reproduced by direct infusions of PGI_2 (5–20 ng/ml) into the blood bathing the tissues, while similar infusions of Bk (100–500 ng/ml) contracted the RbA and RSS, and reduced the spontaneity of RC without affecting the other bioassay tissues.

Indomethacin (5 mg/kg, intravenously) abolished the release of the PGI_2-like substance but did not modify the peak hypotensive response to intravenous Bk, but reduced the sustained fall in blood pressure. In contrast, inhibition of converting enzyme with SQ14,225 (0.1–0.2 mg/kg) led to an enhanced release of a PGI_2-like substance to subsequent infusions of Bk (0.1–0.5 μg $kg^{-1}min^{-1}$) (3 experiments).

When infused into the kidney Bk (1–5 μg $kg^{-1}min^{-1}$) produced an increase in renal blood flow concomitant with the appearance in renal venous blood of a substance with the same bioassay profile as PGI_2 (2 experiments). In one experiment, acute nephrectomy prevented prostacyclin release due to intravenous bradykinin.

These results suggest that bradykinin selectively increases PGI_2 release; the kidney is the main organ involved, and finally that the released prostacyclin contributes to the sustained hypotension produced by intravenous Bk in the dog.

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