Potential endogenous inhibitor of prostaglandin synthetase in plasma: failure to inhibit cyclo-oxygenase in platelets and the gastric mucosa

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A proposed endogenous inhibitor of prostaglandin biosynthesis has recently been reported to occur in plasma (Saeed, McDonald-Gibson & others, 1977). Certain plasma fractions were shown to inhibit the conversion of arachidonic acid to prostaglandins E_2 and $F_{2\alpha}$ by homogenates of bovine seminal vesicles. Several experiments have now been carried out to study the actions of the plasma fraction *in vitro* on intact cellular structures using platelets, and also *in vivo* on the formation of prostacyclin (PGI_2) by rat gastric mucosa.

Platelet aggregation induced by arachidonic acid in human citrated platelet-rich plasma (PRP) can readily be inhibited (Vargaftig & Zirinis, 1973) by known cyclo-oxygenase inhibitors such as indomethacin or aspirin (Vane, 1971) and thus can be a rapid and sensitive test for cyclo-oxygenase activity in this intact-cell system. The actions of human Cohn fraction IV-4, reported to be the most active of the plasma fractions, have now been investigated and compared with those of aspirin and indomethacin.

Human blood was collected into tri-sodium citrate (3·15%, 0·1 volume) and centrifuged (200 *g* × 15 min at 22°). Portions of PRP were incubated at 37° for up to 15 min in an aggregometer cuvette (Born, 1962) with human Cohn fraction IV-4 (Miles Labs., Inc.), using as controls the Cohn fraction IV-1 or egg albumin (all 1–10 mg ml^{-1} final concentration). In experiments using PRP from 3 different donors, no inhibition of arachidonate-induced (0·75–1·5 mM) aggregation (i.e. no delay in the onset of aggregation) was observed (Table 1) whereas indomethacin (1 μg ml^{-1}) or aspirin (10 μg ml^{-1}) caused complete inhibition of aggregation after 5 min incubation. Although in some experiments, high concentrations of Cohn IV-4 (10 mg ml^{-1}) caused a small reduction in the amplitude of the aggregation-trace, this is not considered a typical inhibition with arachidonate-induced aggregation, and probably represented changes in the optical density of the PRP in the aggregometer since the control Cohn IV-1 or egg albumin produced similar effects.

To investigate whether prolonged incubation of PRP with the plasma fraction would inhibit platelet cyclo-oxygenase, samples of PRP from three donors were incubated at room temperature (22°), and samples tested after 1·5, 3 and 6 h. Again, there was no significant ($P > 0.05$) inhibition of arachidonate-induced aggregation with Cohn IV-4 (1–10 mg ml^{-1}) compared with the controls in the 3 experiments. In another 3 experiments, Cohn IV-4 (10 mg ml^{-1}) had no effect on the interval (1–2 min) between addition of low concentrations of arachidonic acid (0·5 mM) and the initiation of platelet aggregation. In contrast, incubation of plasma with low concentrations of aspirin (1 μg ml^{-1}) for 1 h led to complete inhibition of platelet aggregation (Table 1).

A further three experiments were carried out with rabbit PRP, in which platelet aggregation can be induced by lower doses of arachidonate (75–250 μ M). Cohn fraction IV-4 (1–10 mg ml^{-1}) incubated with the PRP for up to 3h failed to inhibit the platelet aggregation, whereas both aspirin (6 μg ml^{-1}) or indomethacin (0·6 μg ml^{-1}) caused complete inhibition after 5 min.

The ability of Cohn fraction IV-4 to inhibit cyclo-oxygenase activity in the rat gastric mucosa *in vivo* has also been studied. Prostacyclin generation by the rat gastric mucosa *in vitro* can be inhibited by administration of indomethacin (5 mg kg^{-1}, s.c.) (Moncada, Salmon & others, 1978) or aspirin (100 mg kg^{-1}, s.c.) to rats 1–3 h before removal and incubation of the mucosa, as shown in Table 2. Thus, cyclo-oxygenase inhibition *in vivo* prevents subsequent prostacyclin generation *in vitro*. In the present study, Cohn IV-4 (50 mg kg^{-1}) was administered by intravenous or intraperitoneal injection in a dose used in the original paper to reduce arachidonate-induced bronchoconstriction in guinea-pigs (Saeed & others, 1977).

Gastric mucosal tissue (0·3–0·5 g) was rapidly stripped from underlying muscle, washed in buffer (50 mM tris; pH 8·4, 0°) chopped, re-washed (0·5 ml buffer) and incubated in buffer (1 ml) by vortex mixing for 1 min at 22°. After centrifugation (15 s at 22°) in a

Table 1. *Comparison of the effects of aspirin and indomethacin with the plasma fraction Cohn IV-4 on human platelet aggregation induced by arachidonic acid.*

	μg ml^{-1}	(min)	% Inhibition	(n)
Indomethacin	0·5	5	55 ± 18	4 **
,,	1·0	5	100 ± 0	6 **
Aspirin	5	5	26 ± 9	4 *
,,	10	5	100 ± 0	5 **
,,	0·5	60	84 ± 15	4 **
,,	1·0	60	100 ± 0	3 **
Cohn IV-4	1 000	5	0	3
,,	10 000	5	0	3
,,	10 000	180	0	3
,,	10 000	180	0	3
,,	10 000	360	0	3

Human PRP was incubated for the period shown with indomethacin, aspirin or Cohn IV-4 and platelet aggregation was subsequently induced by arachidonic acid (100–500 μg ml^{-1}). Results are expressed as % inhibition of the control aggregation mean ± s.e. mean of (n) values. *$P < 0.05$; **$P < 0.001$.

fixed-speed Eppendorf bench centrifuge (model 5412) the supernatant was immediately tested for its ability to inhibit adenosine diphosphate-induced platelet aggregation, and this prostacyclin-like activity, characterized as previously described (Moncada & others, 1978) was assayed against authentic prostacyclin. In three replicate experiments, using three rats in each group, there was no significant decrease ($P > 0.05$) in the prostacyclin generation by gastric mucosa from rats pretreated for 3 h with Cohn fraction IV-4 compared to the controls (94 ± 8 ng g^{-1} tissue, mean $\pm$ s.e. mean; $n = 9$) as shown in Table 2.

The reported ability of steroid anti-inflammatory drugs to enhance the "endogenous inhibitor" activity *in vivo* (Saeed & others, 1977) has also been investigated. In these experiments, dexamethasone sodium phosphate (24 mg kg^{-1}) was administered in the very high dose used in the original paper, and the mucosal prostacyclin generation was measured 24 h later, at a time when the endogenous inhibitor concentrations were reported to have reached maximum after such treatment. In three experiments there was no significant change in prostacyclin generation by strips of gastric mucosa compared with the control concentrations (Table 2). Since prostacyclin generation reflects cyclo-oxygenase activity, dexamethasone does not appear to promote the inhibition of prostaglandin synthesis *in vivo* in the rat gastric mucosa.

It thus appears that the inhibition of formation of cyclo-oxygenase products by plasma fraction Cohn IV-4, demonstrated by Saeed & others (1977) using bovine seminal vesicle homogenates, does not occur in human or rabbit platelets, even after prolonged incubation of up to 6 h. This may reflect failure of the substance (of high molecular weight, associated with haptoglobins) to readily penetrate cellular membranes. It is possible that an even more prolonged exposure to the plasma fraction is required to demonstrate cyclo-oxygenase inhibition. It also appears that this factor, either administered systematically or induced by administration of corticosteroids does not affect cyclo-oxygenase activity in the gastric mucosa *in vivo*. It seems unlikely that changes in the endogenous concentrations would greatly exceed those used in the present experiments (Cohn IV-4, 10 mg ml^{-1}, or 50 mg kg^{-1}) since normal concentrations of haptoglobins are reported to be only 0·4–2·3 mg ml^{-1} of human serum (Putnam, 1975). Interestingly, prostaglandins themselves have been shown to elevate serum haptoglobin levels (Shim, 1976). It is possible that the plasma fraction could act at a stage beyond cyclo-oxygenase activity, perhaps by diverting the pathway of endoperoxide breakdown or metabolism, as has been shown to occur with other plasma proteins (Christ-Hazelhof, Nugteren & Van Dorp, 1976). In the present study with platelets, however, thromboxane A_2 synthesis from low concentrations of arachidonic acid (as monitored by platelet aggregation) did not appear to be altered or elevated. If a diversion of endoperoxide transformation occurs *in vivo*, then the endogenous inhibitor of the synthesis of a specific prostaglandin may also act as an endogenous stimulator of the synthesis of another prostaglandin by making more substrate available.

Table 2. *Effects of aspirin, indomethacin, dexamethasone and Cohn IV-4 on prostacyclin generation by rat gastric mucosa.*

	mg kg^{-1}	h	% Inhibition	(n)
Indomethacin	2·5	3	86 ± 6	5**
,,	5·0	3	98 ± 1	16**
Aspirin	50	3	73 ± 8	5**
,,	200	3	95 ± 8	5**
Dexamethasone	24	24	9 ± 23	3
Cohn IV-4	50	3	2 ± 9	9

Prostacyclin generation was determined 3–24 h after administration of aspirin, indomethacin or dexamethasone (all by s.c. route) or Cohn IV-4 (i.v. or i.p.). Results are expressed as % inhibition of the control levels, mean ± s.e. mean of (n) values. **$P < 0.001$.

The present experiments do not preclude the attractive hypothesis of an endogenous prostaglandin biosynthesis inhibitor, especially since it is known that the synthetase-complex from different tissues has different sensitivities to inhibitors (Flower & Vane, 1972). However, the results do suggest that caution should be exercised before extrapolating the original findings with Cohn IV-4 fraction to all other systems, or using it as a pharmacological tool to study prostaglandin biosynthesis.

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