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A New Function of Human Haptoglobin: Endogenous Inhibition of Prostaglandin Biosynthesis and its Relation to Haemoglobin Binding

Sheikh Arshad Saeed, Sagheer Ahmed, Ali Imran Amjad,
Asma Azmatullah, Sana Sultan Siddique,
Sumbal Ashraf Janjua, Faria Nasim, Sana Syed,
Sameer Khan, Sumaira Talib Sheikh, Ahmed Jawad,
Rashid Zaheer Syed and Faraz Ali Khan

Human haptoglobin (Hp) is a glycoprotein present in plasma or serum which has the capacity to bind hemoglobin (Hb) specifically and stoichiometrically. Since Hb stimulates prostaglandin (PG) synthase, we investigated the possibility that Hp might affect PG synthesis by combining with Hb. Human Hp was purified by extraction, ammonium sulphate fractionation and DEAE-ion exchange chromatography. The results showed that Hp (50-250 µg protein) selectively inhibited in a concentration-related manner, the biosynthesis of PGs via cyclooxygenase pathway. Hp also inhibited lipoxygenase pathway of arachidonic acid metabolism in a concentration related manner. Additional evidence that Hp inhibited PG biosynthesis was obtained by I) the loss of Hp inhibitory activity upon removal of Hp by affinity chromatography on hemoglobin-sepharose from a sample of purified Hp or plasma, ii) inhibiton by Hp of arachidonic acid or bradykinin-induced bronchoconstriction in the guinea-pig and iii) inhibition of arachidonic acid-induced tone of the rat gastric fundus strip. Hb (a known stimulant of cyclooxygenase) reduced the inhibitory effect of Hp in a concentration-related manner, such

that all its inhibitory property was lost when it was completely bound by Hb. These findings point to a new function of Hp: endogenous regulation of PG synthesis by combining with Hb release during injury (thus inflammatory processes). Therefore, mammals have dual defence system i.e. a specific immune system and non-specific Hp defence system.

Key words: Human haptoglobin, inhibitor, prostaglandin biosynthesis, haemoglobin, cyclooxygenase, haptoglobin function

Introduction

Human blood plasma haptoglobin (Hp) is a well-characterized glycoprotein present in the $\alpha2$-globulin fraction of most mammalian plasma or serum. The most characteristic property of Hp is to bind haemoglobin (Hb) stoichiometrically and irreversibly. Several good reviews (Sutton, 1970) have appeared on the chemistry and physiology of Hp. But its exact physiological function still remains uncertain. It is well known that Hp levels in the serum vary greatly in different conditions. It is usually elevated in inflammatory conditions and acute infections, e.g. respiratory allergy and asthma but depressed in severe chronic liver disease and certain haemolytic conditions (Higginbotham, 1975 and Fumei *et al.*, 2000). Recently, we have been studying the inhibition of prostaglandin (PG) biosynthesis by human blood plasma or serum (Collier *et al.*, 1980 and Saeed *et al.*, 1977). These studies demonstrated that Hp potently inhibits PG synthesis. This finding suggested that the biological function of Hp may be associated with the inhibition of PG synthesis *in vivo*. We have therefore, evaluated the effects of purified Hp on several test systems used to study PG synthase inhibitors.

Materials and Methods
Assay of bovine seminal vesicle PG synthase activity

Two methods were used for the assay of PG synthase activity of bovine seminal vesicle homogenate (BSV). The first was measurement of oxygen uptake during cyclooxygenation of arachidonic acid and the second was bioassay of PGE_2 on hamster fundic strip. The standard assay mixture used for both assays contained 50 m mol l^{-1} sodium phosphate buffer pH 7.4, 6 mg ml^{-1} BSV homogenate protein, 61 μ mol l^{-1} sodium arachidonate as substrate, an appropriate amount of test substance and 1.3 m mol l^{-1} reduced glutathione (GSH) as co-factor.

Preparation of enzyme

Bovine seminal vesicles (BSV) from non-castrated adult bulls, supplied either fresh or frozen from a local abattoir, were freed of fat and connective tissue at room temperature before the homogenate was prepared at 4°C. The vesicles were cut into small pieces and put through a meat mincer. The coarsely ground mixture thus obtained was homogenized in a Kenwood food blender with three volumes of 50 m mol l^{-1} phosphate buffer pH 7.4. The resulting homogenate was clarified first by straining through cheesecloth and then by centrifugation at 600g for 15 min. The supernatant was removed, saturated with oxygen-free nitrogen and stored in aliquots at -20°C for up to 6 months. This preparation containing 24 mg ml^{-1} protein was used as a source of BSV PG synthase.

Preparation of substrate

Arachidonic acid (Grade I) was supplied by Sigma. A 10 mg ampoule (99% pure) was taken up in 1.0 ml of 96% ethanol and diluted to 10.0ml with 0.2% w/v aqueous sodium carbonate. This solution was stored at -20°C and diluted when required in 50 m mol l^{-1} phosphate buffer.

Measurement of oxygen uptake by BSV homogenate (cyclo-oxygenase activity)

The uptake of oxygen into arachidonic acid by BSV homogenate was determined by difference from measurements at 37°C of oxygen saturation of the incubates with a Yellow Springs Instrument Co. Model 53 oxygen monitor and a Toshima electron flat-bed recorder. The instrument and recorder were calibrated with air saturated distilled water at 37°C. A standard incubation volume of 4 ml was used and the reaction initiated by the addition of 1ml of BSV homogenate at 37°C after a 3 min pre-incubation of all other components, allowing them also to reach 37°C. the addition of enzyme was followed immediately by insertion of the oxygen electrode and the change in oxygen saturation of the incubate recorded for 15min. The proportion of total oxygen uptake that could be attributed to cyclo-oxygenase activity in this test system was determined with indomethacin. The maximal inhibition of oxygen uptake by indomethacin (28 μ mol l^{-1}) was 51.9±1.3% whereas inhibition of PGE_2, was complete. Since indomethacin is a known cyclo-oxygenase inhibitor it was assumed that this proportion of the oxygen consumption was directed towards the production of prostaglandins (cyclo-oxygenase). To calculate inhibition of cyclo-oxygenase activity, test samples were incubated both with and without indomethacin at 28 μ mol l^{-1}; the negative control was an incubate containing substrate, co-factor, buffer but no enzyme.

Bioassay of PGE_2 on hamster fundic strip

PG activity was measured as described previously (Collier *et al.*, 1976), using a standard assay mixture of 2 ml. The negative control was an incubate containing boiled (10 min) BSV homogenate. The reaction was started by addition of 61 μ mol l^{-1} sodium arachidonate and the mixture was incubated aerobically with gentle shaking for 15min at 37°C. PGs were extracted in acidified ethyl acetate and assayed on hamster stomach strip, as described elsewhere(Collier *et al.*, 1976 and Ubatuba, 1973). Total PG activity was expressed in terms of a reference standard of PGE_2.

Measurement of haptoglobin

Hp concentration in plasma, serum or purified Hp preparations was determined according to the peroxidase method of Connell and Smithies (Connell and Smithies, 1958) and expressed as haemoglobin-binding capacity (HbBC). Briefly, this method depends on the peroxidase activity of haemoglobin-haptoglobin complexes. Hydrogen peroxide is the oxidizing substrate and guaiacol the hydrogen donor. The reaction conditions are chosen so that the peroxidase activity of free haemoglobin (Hb) is essentially zero. The formation of tetra-guaiacol during the reaction was followed spectrophotometrically at 470 nm. In the present work the assay was calibrated with human plasma Cohn fraction IV_4 as a source of Hp and lyophilized human methaemoglobin (Sigma).

It should be noted that the peroxidase acidity of the complexes is independent of Hp phenotype (Nyman, 1959).

Tone of rat gastric fundus strip

Since the intrinsic tone of the isolated rat gastric fundus strip is due to intramural generation of prostaglandins (Eckenfels and Vane, 1972), this preparation can be used as an index of PG synthesis (Collier *et al.*, 1976). A strip of rat gastric fundus was suspended from a Harvard isotonic transducer (H386) under a tension of 1 g in a 4 ml organ bath at 37°C, in Kreb solution bubbled with 5% CO_2 in oxygen and the response was recorded. After setting up, the preparation was washed repeatedly with Kreb solution for about 1 h and, when the tone was constant, small volumes (0.005-0.2 ml) of sodium arachidonate, dissolved in ethanol, 10% in 0.2% Na_2CO_3 in water, were added in increasing concentration every 5 min until the tone was maximal (usual dose range, 4 to 657 μ mol l^{-1} arachidonate). The concentration of arachidonate required to increase tone to 25% of maximum (SC25) under control conditions was determined graphically. To measure the effects of Hp the above procedure was repeated in the presence of test substance after 20 min pre-incubation with the test substance before addition of arachidonate and the SC25 values were determined as before.

Arachidonate or bradykinin induced bronchoconstriction in the guinea pig

Male albino guinea pigs weighing 400-600g each (Dunkin-Hartley strain) were anaesthetized by administration of sodium pentobarbitone, 60 mg kg^{-1} intraperitoneally. The trachea was cannulated for artificial ventilation by a Starling pump (5-8 ml) at 72 strokes min^{-1} via a Fleisch pneumatochograph. The difference in presence in pressure across this device was measured with a Grass differential pressure transducer. This gives a flow signal which was fed into a Hewlett-Packard 8816A analogue comuter. Transpulmonary pressure (TPP) was measured with another differential pressure transducer via side arms to the thoracic cavity and the tracheal cannula. The signals for low and TPP were applied to the analogue comuter so that there was approximately 180° phase difference. The computer derived the dynamic compliance and resistance from the ingoing signals. Test substances were administered intravenously via the external jugular vein. Sodium arachidonate doses of 0.1-0.5 mg kg^{-1} or bradykinin as triacetate from Sigma at 1-5 mg kg^{-1} were given via the cannulated left external jugular vein and 'washed in' with 0.5ml of 0.85% w/v sodium chloride solution (saline) containing 10 units ml^{-1} of heparin. These doses were given at 30 min intervals to produce a large but submaximal bronchoconstriction. After 2 or 3 similar responses to the same dose of challenge substance had been obtained, the test substance in saline (0.1-1 ml) was injected intravenously and 45s after another dose of challenge substance is given.

Measurement of platelet aggregation

Human blood obtained from healthy donors who had not taken any non-steroidal anti-inflammatory drugs for 1 week was immediately mixed with 10 units ml^{-1} of heparin and centrifuged at 200 g for 15 min. The platelet-rich plasma thus obtained was centrifuged at

600 g for 15 min and the resulting platelet pellet washed with saline. The pellet of washed platelets was then suspended in 0.5 volumes of 0.1 mol l^{-1} Tris-HCl buffer, pH 7.4. Platelet aggregation was monitored with a Chronolog Dual channel aggregometer. Haptoglobin or Cohn IV$_4$ was added to 0.5 ml of platelet suspension at 37°C, the volume was made up to 0.7 ml with saline and the mixture pre-incubated at 37°C for 5 min, prior to the addition of sodium arachidonate (aggregating agent). The percentage inhibition due to test substance compared with control (arachidonate alone) was calculated at 1, 2, 3 and 4 min after addition of arachidonate.

Results
Purification of haptoglobin
The procedure was based on extraction with 60 m mol l^{-1} Tris-HCl buffer, pH 7.3 ammonium sulphate precipitation followed by ion-exchange chromatography on DE-52 cellulose. All steps were performed at 0-4°C and centrifugation at 600 g for 30 min unless otherwise specified (Table 1).

Extraction with Tris-HCl buffer
3.74 g of human plasma Cohn fraction IV$_4$ containing 2.95g protein) prepared from normal pooled plasma was extracted with 60 m mol l^{-1} Tris-HCl buffer, pH 7.3 and centrifuged to remove any insoluble matter. The supernatant was subsequently and extensively dialyzed against deionized water and lyophilized. The powder obtained was stored at -20°C until used.

Ammonium sulphate precipitation
To distilled water containing 10 mg ml^{-1} of human plasma Cohn IV$_4$ powder from step 1, solid pulverized ammonium sulphate was added in small portions with stirring each time, to 50% saturation, which precipitated most of the haptoglobin (Hp). After standing for 2 h at 4°C the suspension was centrifuged dissolved in a small volume of distilled water, dialysed for 24 h against frequent changes of distilled water and lyophilized.

Ion-exchange chromatography on DE-52 cellulose
The lyophilized powder from step 2 containing 370 mg protein was taken up in 15 ml of 0.01 mol l^{-1} sodium acetate buffer pH 5.0 and centrifuged to remove any insoluble matter. The supernatant was layered onto a 2.7 x 70 cm DE-52 cellulose column equilibrated with 0.01 mol 1^{-1} acetate buffer. The elution was carried out a flow rate of 60 m1 h^{-1} with a linear gradient of 0.01-0.5 mol 1^{-1} acetate buffer over 48h. The fraction collector was operated on a time basis and set to collect 15 ml fractions. Protein concentration of the eluate fractions was followed by measuring absorption at 280 nm. Fractions containing protein were then assayed for inhibition of PG synthase and Hp content.

Results of fractionation
The elution profile showed that the major protein peak coincided with the peaks of haptoglobin and its inhibition of PGE$_2$ biosynthesis. The fractions which contained Hp were

Table 1: Purification of haptoglobin from human plasma Cohn IV_4

	Total protein (mg)	Recovery of protein (%)	Hp content (% w/w)	Recovery of Hp (%)	ID_{50}^* ($\mu g\ ml^{-1}$)
Cohn IV_4	2947	100.0	15.1±0.2	100.0	475±30
Extraction with 60 m mol/Tris-HCl buffer, pH 7.3	2510	85.1	18.7±0.4	100.0	372±46
Ammonium sulphate precipitation	370	12.5	47.0±3.8	43.7	240±25
Ion exchange chromatography on DEAE cellulose	133	4.5	99.0±0.2	23.9	110±70.3

*The amount of material required to inhibit PGE_2 biosynthesis, by 50% as measured by bioassay

Table 2: Inhibition of increased airway resistance and decreased lung compliance induced by sodium arachidonate or bradykinin

Treatment	Dose ($mg\ kg^{-1}$ i.v.)	Challenge Substance	Mean % inhibition + SE of response to challenge	
			Resistance	Compliance
Haptoglobin	5	arachidonate sodium	31.8±4.7	24.4±5.3
	20		44.3±6.7	34.3±7.4
	50		67.7±0.3	49.0±3.2
	100		92.0±7.0	90.0±6
Haptoglobin	5	Bradykinin	34.7±1.7	19.5±5.5
	20		48.3±4.4	33.4±11.8
	50		67.0±0	73.2±19.8
	100		94.0±6.0	86.5±13.5
Acetyl salicylate	2	arachidonate sodium	85.5±3.5	71.5±6.5
	2	Bradykinin	82.0±9.6	80.7±9.9
Indomethacin	0.03	arachidonate sodium	47.0±15.8	58.7±15.7
	0.1		90.5±9.5	85.5±14.5
	0.01	Bradykinin	35.5±9.9	25.3±8.2
	0.03		58.0±14.0	53.0±3.0
	0.1		83.5±11.5	86.0±6.0

Table 3: Effect of human plasma Cohn fraction IV_4 and Hp on platelet aggregation induced by arachidonic acid

Plasma fraction	Concentration ($mg\ ml^{-1}$ protein)	Percentage inhibition of aggregation; measured at various times (min) after addition of arachidonate			
		1	2	3	4
Cohn IV_4	1.13	60.0±4.7	53.3±5.6	51.4±4.3	52.4±2.8
Cohn IV_4	2.81	90.8±2.2	87.2±4.9	84.8±4.7	84.7±3.8
Hp	3.6	34.0±5.5	29.1±10.0	32.2±12.8	36.6±12.3
Hp	7.2	54.8±8.8	56.9±10.5	56.7±11.8	59.5±10.9

Washed human platelets were incubated for 5 min with test proteins before the addition of sodium arachidonate (28 $\mu g\ ml^{-1}$). Platelet aggregation was measured as decreased in the Methods section. The percentage inhibition due to test protein compared with control (arachidonate alone) was calculated at 1,2,3 and 4 min after the addition of arachidonate. Results are mean values ± SE of three determinations
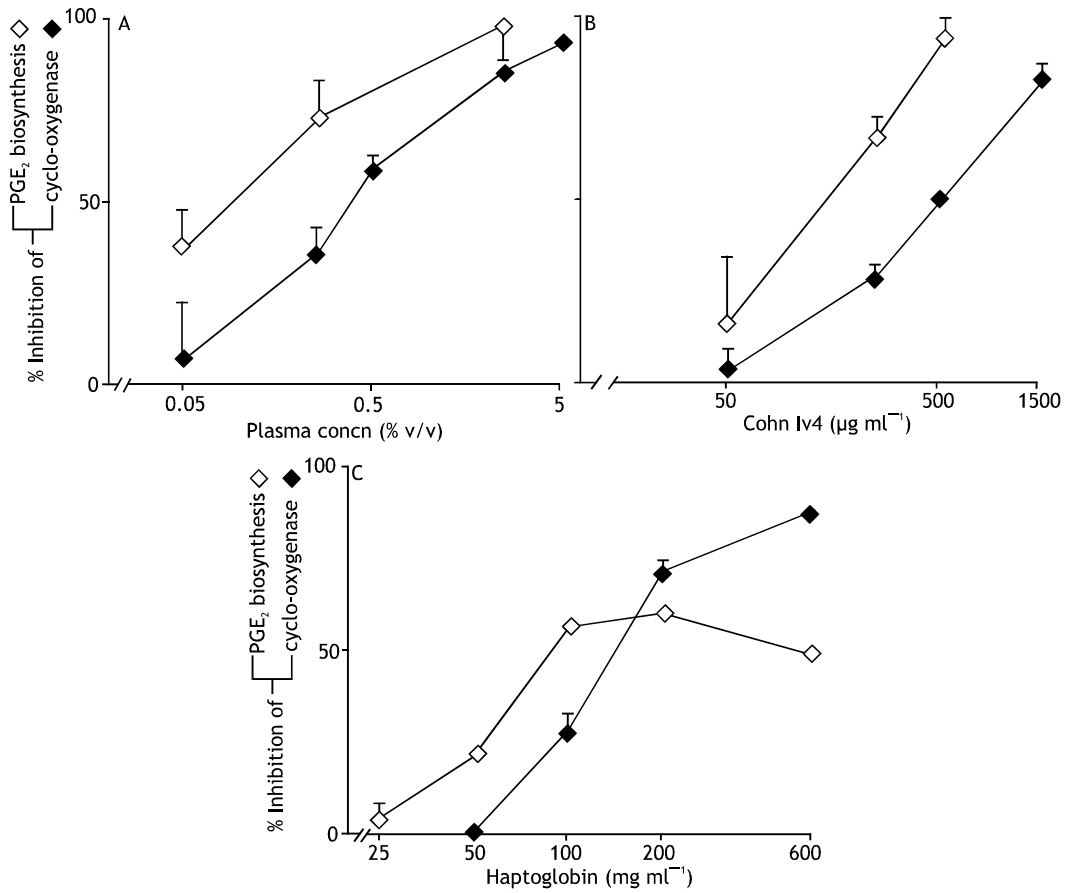
Fig.1: Inhibition of PGE$_2$ tiosynthesis (closed symbols) and of cyclo-oxygenase activity (open symbols) by BSV homogenate. (A) Human plasma: (B) plasma Cohn IV$_4$: © haptoglobin. Points represent the mean of at least three observations and vertical bars represent the standard error of the mean. Where no bar is shown, the error falls within the symbol

pooled, concentrated with carbowax 20 M and dialysed against 2 L of water which was changed every 15 h for 2 days. The dialysed solutions were then lyophilized and finally stored at -20°C until used for various biochemical studies. The material prepared by this method achieved purification both of haptoglobin and its inhibitory potency of PG synthase. In Table 1, values of Hp content and its inhibitory activity on PG synthase increased at each stage of the purification procedure, the final product regularly containing >99% Hp and having four times the inhibitory activity against PG synthase of the starting material.

Inhibition of bovine seminal vesicle PG synthase by Hp

Figure 1 A, B and C illustrate the concentration/response relationship for inhibition of cyclo-oxygenase activity and PGE$_2$ biosynthesis by human plasma, Cohn fraction IV$_4$ and purified Hp,
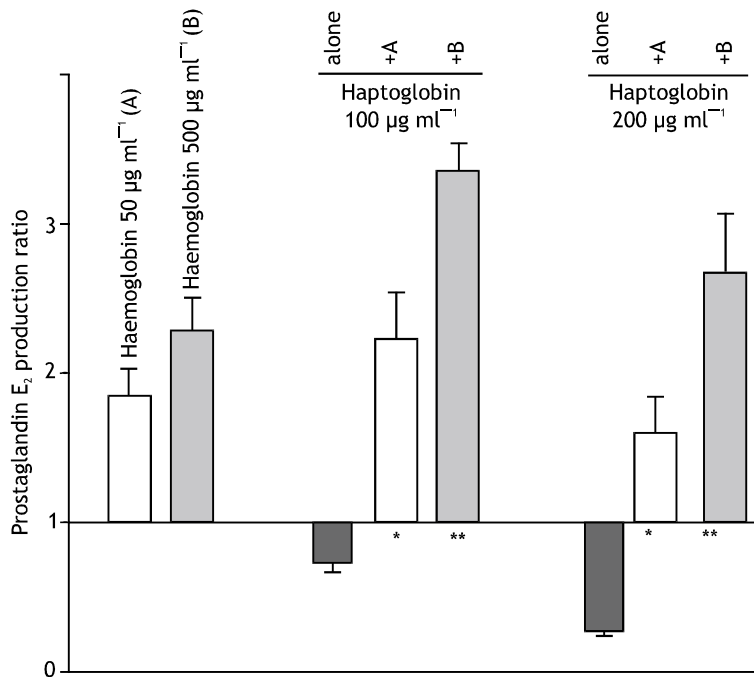
Fig. 2: Reversal by haemoglobin of the inhibition of PGE_2 biosynthesis by human haptoglobin.
(A) Human haemoglobin 50 μg ml^{-1} and (B) Human haemoglobin. 500 μg ml^{-1}. Results are
expressed in terms of the prostaglandin E_2 biosynthesis ratio which was taken as 1 in the
controls with no haemoglobin or haptoglobin. *$P<0.005$; **$P<0.0005$ for difference from
the corresponding sample containing haptoglobin only

respectively. It is evident that Hp was less potent against PGE_2 biosynthesis than against cyclo-
oxygenase, but its inhibitory effect on cyclo-oxygenase was incomplete. With Cohn IV$_4$, however,
virtually complete inhibition of cyclo-oxygenase was achieved.

In other experiments the time course of inhibition of PGE_2 by Hp was compared with that
of Cohn fraction IV$_4$, using two concentrations of each inhibitor which compared favourably with
the time course of acetyl salicylate, reported previously (Saeed *et al.*, 1977).

Reversal by haemoglobin of inhibition of PGE_2 biosynthesis

Figure 2 shows that human Hb, at 50 or 500 μg ml^{-1}, stimulates PGE_2 biosynthesis by BSV
homogenate and almost completely reverses the inhibitory effect of Hp. It is evident that Hb (50
μg ml^{-1}) a concentration that completely bound Hp (100 μg ml^{-1}), abolished its inhibitory effect
on PGE_2 biosynthesis. Neither noradrenaline nor reduced glutathione which, as co-factors of PG
synthase, stimulate PGE_2 biosynthesis (Saeed and Cuthbert, 1977), antagonized the inhibitory
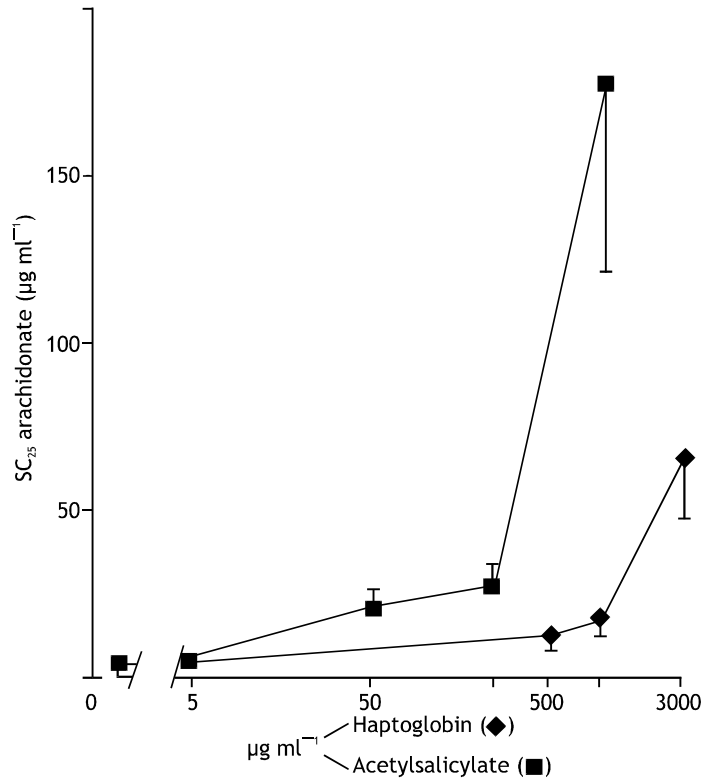activity of Hp.

Fig. 3: Inhibition by acetyl salicylate (■) and haptoglobin (◆)of increase in tone of rat gastric fundus strip induced by sodium arachidonate (4-657 μ mol l^{-1}). The SC25 is the concentration of arachidonate required to increase tone to 25% of the maximal increase obtained in the preparation used

Inhibition of tone of rat fundus strip

Both acetylsalicylate and Hp inhibited sodium arachidonate (4-657 mol 1^{-1}) induced increase in tone of the isolated rat gastric fundus strip in a dose-related manner, but on a weight for weight basis acetylsalicylate was more active than haptoglobin (Fig. 3).

Inhibition of bronchoconstriction

Purified Hp when given intravenously (i.v.) to anaesthetized guinea pigs, in doses of 5.20, 50 or 100 mg kg^{-1}, inhibited the increase of airway resistance and the decrease of lung compliance induced by i.v. injection of either 0.1-α 0.5 mg kg^{-1} of arachidonate or 1-5 mg kg^{-1} of bradykinin (Table 2). At doses effective in inhibiting bronchoconstriction induced by arachidonate or bradykinin, Hp did not inhibit bronchoconstriction induced by submaximal doses of $PGF_{2\alpha}$. Like Hp, indomethacin and acetylsalicylate, two well-known inhibitors of PG synthase, also inhibited the arachidonate or bradykinin-induced bronchoconstriction in the guinea pig (Table 2).
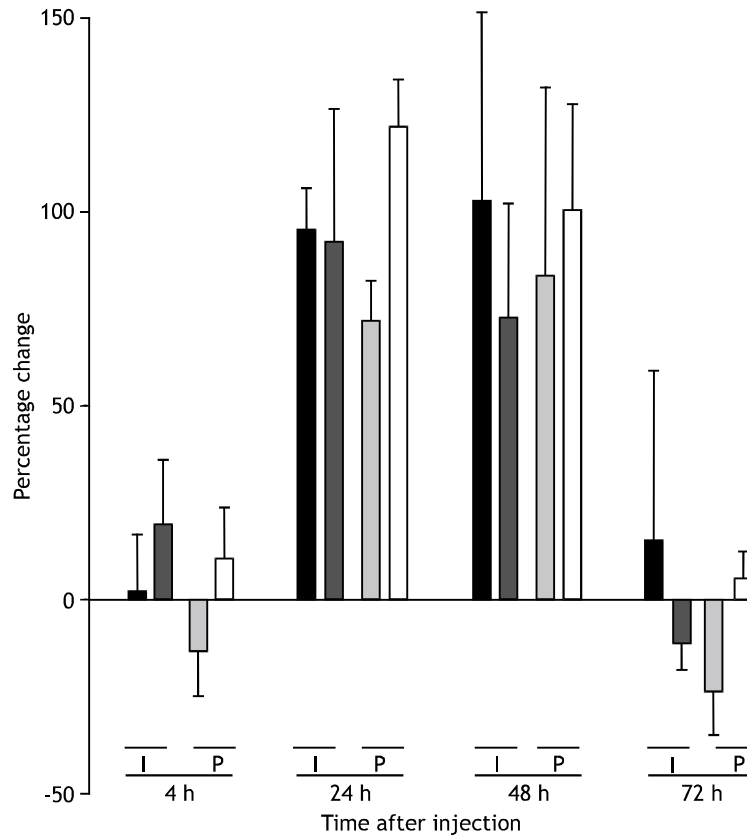
Fig. 4: Time course of the effect of hydrocortisone and dexamethasone on rat blood plasma PG synthase inhibitory activity (1) and peroxidase activity (P), as a measure of haptoglobin concentration. Hydrocortisone sodium succinate 360 mg kg^{-1} (cross-hatched columns) and dexamethasone sodium phosphate 24 mg kg^{-1} (striped columns). Results are expressed as percentage changes in PG synthase inhibitory activity as estimated by bioassay of PGE_2 and peroxidase activity, compared with saline injected controls. $^*P=0.05$; $^{**}P=<0.0025$

Inhibition of arachidonate-induced platelet aggregation

Aggregation induced by sodium arachidonate is known to be inhibited by acetyl salicylate, an inhibitor of cyclo-oxygenase (Roth and Majerus, 1975). It was found that Cohn fraction IV_4 and Hp which inhibited cyclo-oxygenase also inhibited arachidonate induced platelet aggregation in the absence of plasma (Table 3). Of the two protein fractions, however, Cohn fraction IV_4, was more potent in inhibiting aggregation.

Effect of glucocorticoids on plasma Hp levels and PG synthase inhibitor activity

We have previously demonstrated (Saeed *et al.*, 1977) that glucocorticoids increase the levels of inhibitor of PG synthase in plasma. To determine whether these changes in inhibitory activity

are associated with plasma changes in Hp levels, we studied in rats the effects of intraperitoneal injections of dexamethasone sodium phosphate (24 mg kg^{-1}) and hydrocortisone sodium succinate (360 mg kg^{-1}) on plasma Hp levels and inhibitory activity. Groups of 4 or 5 rats were each given the drug or saline intraperitoneally in an injection volume of not more than 200 μl. After treatment, rats were bled individually (at 4, 24, 48 and 72 intervals) into plastic tubes containing 500 μl of 1% (w/v) EDTA as an anti-coagulant. Plasma was separated by centrifugation at 600 g for 15 min and tested for inhibitory activity and Hp concentration by methods described already.

The results obtained show that administration of dexamethasone or hydrocortisone in rats causes an increase in plasma inhibitory activity as compared with saline-treated control rats. This increase in inhibitory activity and the corresponding increase in Hp concentrations are shown in Fig. 4. It is evident that both glucocorticoids raised inhibitory activity and Hp concentrations to similar extent and with comparable time courses.

Discussion

The α_2-glycoprotein, Hp is present in normal plasma at a concentration (Sutton, 1970) of approximately 1 mg ml^{-1}. Since Hp binds selectively and firmly to Hb both in *vivo* and *in vitro*, it has been proposed that the function of Hp is concerned with the prevention of loss of iron through the kidney (Higginbotham, 1975). This, however, is not its only property. Hp possesses significant inhibitory activity against cathepsin B (Snellman and Sylven, 1967) and also inhibits the haemagglutinating ability of influenza virus for chicken erythrocytes (Dobryszycka and Lisowska, 1966). It is also a potent antioxidant (Asleh *et al.*, 2003), used in the hepatic clearing of Hb from plasma and in inhibition of glomerular filtration of Hb (Lim *et al.*,1998). Recently we have shown that human plasma or serum powerfully inhibits PG biosynthesis (Saeed *et al.*, 1977). The results of these and subsequent studies in this report suggest yet another function for haptoglobin, i.e. as an inhibitor of PG synthase.

By employing a variety of test systems commonly used to study PG synthase inhibitors, we found Hp to be capable of inhibiting the cyclo-oxygenation of arachidonic acid leading to PG formation mediated by bovine seminal vesicle PG synthase (Fig. 1C). The inhibitory effect of Hp on PGE$_2$ biosynthesis was completely reversed by Hb (Fig. 2), a known stimulant of PG synthase. Like acetyl salicylate, Hp also inhibited the arachidonic acid-induced tone increases in the rat gastric fundic strip (Fig. 3) and arachidonic acid and bradykinin induced bronchoconstriction in the guinea pig (Table 2). Finally, plasma fraction Cohn IV$_4$ and Hp also inhibited the arachidonic acid induced aggregation of washed human blood platelets (Table 3). It is probable that the presence of plasma in PRP would protect platelets against aggregation by arachidonic acid. This explanation gains support from the work of Tsao and Holly (Tsao and Holly, 1979), showing that plasma prevents arachidonic acid induced platelet lysis. Further evidence that the PG synthase inhibitory activity of Hp was a property of Hp was obtained by affinity chromatography, since depletion of Hp by affinity chromatography on Hb-Sepharose, failed to inhibit PGE$_2$ biosynthesis. That Cohn IV$_4$, was more effective than haptoglobin inhibiting cyclo-oxygenase (Fig 1, B and C) indicates, however, the possible presence of another inhibitor in Cohn IV$_4$.

Other workers have also provided evidence that Hp inhibits PG synthase. Shim (Shim, 1978) for example, reported that human Hp inhibits PG biosynthesis in the presence of reduced glutathione, but in the absence of tryptophan *in vitro* (Deby *et al.*, 1978) found a direct correlation between Hp levels and response to arachidonic induced hypotension in rabbits. Previously, we have reported (Saeed *et al.*, 1978) that fetal calf serum, which does not contain Hp, was completely devoid of PG synthase inhibitory activity. This and other evidence with glucocorticoids, which increase Hp levels and plasma PG synthase inhibitory activity to similar extents and with comparable time courses, provide further support that Hp inhibits PG synthase.

This unique property of Hp indicates that the function of Hp may be concerned with the endogenous control of PG production. The circumstances in which Hp may act in this way in the body remains to be determined; but it probably forms part of a haemostatic control system that could help to curtail the progress of certain aggregatory or inflammatory reactions. We believe that further work in this area will provide useful information about natural regulatory mechanisms in disease associated with imbalanced PG production and also lead to the development of suitable methods for detecting types of anti-inflammatory activity more relevant to human disease.

Further studies on the role of haptoglobin in the human body has shown that human haptoglobin (Hp) is synthesized at hepatic and extrahepatic sites as an acute-phase reactant protein (APP). The chemotaxis granulocytes and differentiated HL-60 cells has shown to be inhibited in the presence of a physiological concentration of Hp. Phagocytosis of viable *Escherichia coli*, as well as fluorescienated nonviable *E. coli* is also inhibited. Hp also reduces granulocyte intracellular bactericidal activity against E. coli. The observed inhibitory effects of Hp on granulocyte function are similar to those reported for C-reactive protein and suggest that APPs dampen the acute inflammatory response (Rossbacher *et al.*, 1999).

Haptoglobin has popularly been shown to be a major antioxidant, having its role in protecting the vascular system. It completely inhibits the cross-linking of apo B and oxidation of LDLs (Miller *et al.*, 1997).

Haptoglobin of two different phenotypes stimulates the formation of prostaglandin E_2 (PGE_2) in osteoblast-like cells isolated from neonatal mouse calvarial bones and potentiates the stimulatory effect of bradykinin and thrombin on PGE_2 formation (Frohlander *et al.*, 1991)

Hp is expressed at a high level in lung cells, yet its protective role(s) in the lung is not known. Hp-mediated Hb catabolism process exists in aloveolar macrophages. This process is likely coupled to an iron mobilization pathway and may be an efficient mechanism to reduce oxidative damage associated with hemolysis (Yang *et al.*, 2003). Haptoglobin is expressed at high levels in specific cells, including alveolar macrophages and eosinophils in diseased or inflamed human lung tissues, but not in the normal lung, suggesting that Hp is involved, in a variety of lung inflammatory diseases, including respiratory allergy and asthma (Yang *et al.*, 2000).

Expression of human Hp in lung cells was up-regulated when the transgenic mice were treated with endotoxin (Fumei *et al.*, 2000). This protects the lungs from Hb induced lung injury (Yang *et al.*, 2003).

Furthermore, haptoglobin serves as an antioxidant by virtue of this ability to prevent hemoglobin-driven oxidative tissue damage. It is known that an allelic polymorphism in the haptoglobin gene is predictive of the risk for numerous microvascular and macrovascular diabetic complications. Identification of the biochemical basis for differences among haptoglobin types may lead to the rational development of new pharmacologic agents to avert the development of diabetic vascular complications. (Wang *et al.*, 2001 and Melamed-Frank *et al.*, 2001).

High levels of Hp mRNA are found to be transcribed by adipocytes in addition to liver cells in mice. After inflammation had been induced *in vivo*, expression of the haptoglobin gene rises six-fold in adipose tissue, an increase compatible with that observed in the normal mouse liver. The expression of Hp by adipocytes presents new directions in which Hp's role as an antioxidant or as an angiogenic factor can be investigated. (Friedrichs *et al.*, 1995).

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