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PRELIMINARY COMMUNICATION

ARACHIDONATE INDUCED HYPOTENSION AND HAPTOGLOBIN PLASMA LEVEL IN THE RABBIT

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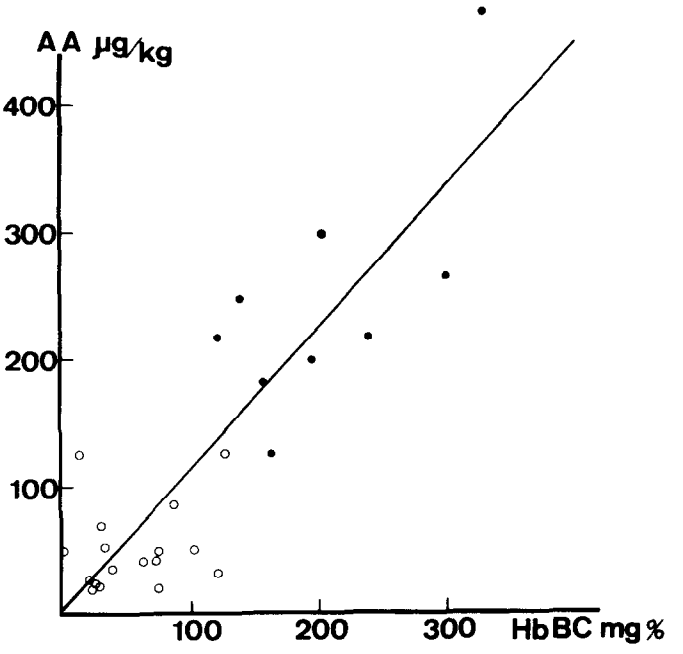
We have observed that the fall of blood pressure and the eventual sudden death induced by injection of arachidonic acid (AA) in the rabbit (60 to 250 μg/kg) (1-3) varies considerably. Much of our work has been undertaken in order to discover the reasons for this variability. We have shown that heparine, which lowers blood lipids (2), and tryptophan (4) increase the response of the rabbit to i.v. AA injection. One hour after the start of heparin infusion (2 mg/kg/hour), one generally obtains a 50 % decrease of arterial pressure after i.v. injection of 25-35 μg/kg of AA. Certain animals are particularly resistant and one must increase the dose of AA to 400 μg or more, in order to induce that fall of blood pressure. Often they belong to the same litter and are affected by skin or gastrointestinal problems, they behave exactly as if they had been treated by a nonsteroidal antiinflammatory substance. The existence of endogenous antiinflammatory proteins is now well documented (5 and 6). We have felt that the level of haptoglobin (Hp) in the blood might be the controlling factor as for several decades it has been known that the concentration of this globin is increased in inflammation (7) and that by its binding to hemoglobin (Hb) the peroxidasic activity of Hb is enhanced (8). Hp is the main component of the "acute phase" proteins (9). Thus we have measured the Hp level of the plasma of rabbits before AA injections and at the end of the experiment. We also induced an increased synthesis of Hp by a classical procedure : intramuscular injections of turpentine. In 26 animals we found a good correlation between levels of Hp and resistance to AA (Fig. 1).

Normally fed male rabbits (Fauve de Bourgogne) 1.8-2.4 kg, anaesthetized by urethane (20 % in water) 2.5-3.5 g/kg were heparinized as follows : heparine Roche 5 mg/kg i.v. in one minute, than slow perfusion of 1 mg/kg during one hour, and finally a second shot of 5 mg/kg. Carotid blood pressure was recorded (N.S. transducer LX 1.600); rectal temperature and E.C.G. monitored. AA (Sigma) 5 mg, stored in chloroform-hexane (1:1) was evapored under N_2, dissolved in 0.5 ml 1N NaOH and 10 ml NaCl 0.9 % ; final volume 20 ml, buffered at pH 7.7 (tris 0.2 M HCl); lipoperoxide level never exceeded 1.2 nEq/ml. 0.6 ml of turpentine were injected in the muscles of each thigh; i.v. injections of AA and haptoglobin assay were performed 48 hrs later.

Hp was measured according to the spectrophotometric method of Tarukoski (10). Results are expressed in terms of hemoglobin binding capacity (HbBC), i.e. in mg of

Hb which can be bound by Hp in 100 ml of plasma or serum. Standardization was achieved by adding increasing amounts of serum rich in Hp, with known quantities of hemoglobin isolated according to Drabkin (11) and measured according to Richterich (12).



Legend to fig. 1

In ordinates, amounts of i.v. arachidonic acid (AA) necessary to induce a 50 % decrease of carotid blood pressure in heparinized anaesthetized rabbits. In abscissae, haptoglobin levels expressed as hemoglobin binding capacity in mg per 100 ml of plasma O: normal rabbits; ● : 48 hours after intramuscular injections of turpentine. The regression line has been calculated according to the least squares method (r = 0.8621).

The correlation between Hp level and response to AA might be interpreted by the assumption that Hp (alone or in combination with Hb) in the presence of suitable cofactors inactivates part of the endoperoxides resulting from the action of the cyclooxygenase on AA. Indeed, other peroxidasic systems inhibit the biosynthesis of prostaglandins (PGs) by reduction of the endoperoxides (13 and 14). The following preliminary observations indicate that this working hypothesis must be carefully considered. Two hydroperoxides are good substrates for rat serum peroxidase at pH 7.0 in the presence of GSH. When the rat serum is poor in Hp the peroxidasic activity is moderate ; a serum of the same species rich in Hp is much more active . Addition of Hb does not increase the activity. Hb alone has no activity (Table 1).

The results of our experiments performed according to a different working hypothesis firmly support the suggestion of Saeed et al (6) that haptoglobin might be the main protein which inhibits PG synthesis in vitro and the reaction of the rat paw to carrageenin in their experiments.

Table 1. Peroxidasic activity of rat blood sera.

Hydroperoxides (64 mEq/l)	Sera (2 µl)[+]	Activity[x]
Cumyl	A	667 ± 42
Cumyl	A, 56°C, 1 h	37
Cumyl	A, + Hb 24 mg/l	615
Cumyl	B	1797 ± 175
Cumyl	B, + Hb 24 mg/l	2034
Ethyllinoleate hydroperoxides	B	2201

[x] : expressed in µM of $NADPH_2$ oxidized min/ml

[+] : Serum A : Hemoglobin binding capacity/100 ml = 43
Serum B : Hemoglobin binding capacity/100 ml = 112

The peroxidasic activity of rat sera kept for several weeks at -18°C has been tested according to Prohaska and Ganther (15) in a phosphate buffer pH 7.0 containing sodium azide (0.04 M) and EDTA (5 mMol instead of 1 mMol). Reduced glutathion acted as hydrogen donor. The peroxide is dissolved in 10 µl ethanol; 10 µl of serum diluted 1/5; total volume 300 µl. Reaction was automatically registered with UNICAM spectrophotometer double beam at 25°C, 340 nm, for 4 min. Peroxidasic activity disappears by heating at 56°C during 1 h. Ethyllinoleate hydroperoxyde is as good a substrate as cumyl hydroperoxyde. Absence of GSH or replacement by guaiacol inhibit the reaction although another hydrogen donor $NADPH_2$ is present.

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