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THE EFFECT OF 4-ACETAMIDOPHENOL ON PROSTAGLANDIN SYNTHETASE ACTIVITY IN BOVINE AND RAM SEMINAL VESICLE MICROSOMES

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Abstract—The effect of paracetamol (4-acetamidophenol) on PG synthetase activity was tested in bull (BSVM) and ram (RSVM) seminal vesicle microsomes. In the presence of glutathione (165 μM) and hydroquinone (45 μM) in BSVM paracetamol (up to 67 μM) had no effect or inhibited (IC_{50} = 1500 μM) the enzymic activity. In the absence of cofactors paracetamol (67–667 μM) stimulated the generation of PGs in BSVM. Also in RSVM paracetamol (33–333 μM) stimulated PG and malondialdehyde generation as well as oxygen consumption, provided that the exogenous cofactors were omitted from the incubation mixture. Hydroquinone (150 μM) was able to abolish the stimulatory effect of paracetamol on the enzymic activity. We conclude that paracetamol can replace hydroquinone and other cofactors for cyclo-oxygenation of arachidonic acid in BSVM and RSVM. The inhibitory effect of paracetamol on PG synthetase activity occurs only in the abundance of exogenous cofactors in the microsomal preparation.

Paracetamol (4-acetamidophenol) is supposed to be a more active inhibitor of prostaglandin (PG) synthetase in brain microsomes [1] than in spleen microsomes [2], although paracetamol hardly inhibits PG generation in brain slices and in brain homogenates [3]. On the other hand *p*-aminophenol, a compound chemically related to paracetamol, stimulates the conversion of arachidonic acid to PGs in bovine seminal vesicle microsomes [4] and in myocardial microsomes [5]. Here we report that paracetamol has either stimulatory or inhibitory effect on PG synthetase *in vitro*, depending on the presence of cofactors in the incubation mixture.

MATERIALS AND METHODS

Lyophilyzed seminal vesicle microsomes of rams (RSVM) [6] or bulls (BSVM) [4] were used as the source of PG synthetase. Microsomes were suspended (2 mg protein/ml) in 0.1 M Tris–HCl buffer pH 8.2 and incubated with arachidonic acid (33 μM) in the presence or in the absence of glutathione (165 or 500 μM) and hydroquinone (45 or 150 μM). Incubation was carried out at 30° or at 37° during 5 or 20 min with RSVM or BSVM, respectively. Paracetamol (33–3330 μM) was preincubated 5 min with the microsomal suspension before arachidonic acid was added. PG synthetase activity was determined by three methods:

(a) Bioassay on a rat fundic strip [7] of PGE_2 which was formed during the incubation. We have reported [8] that over 80 per cent of biologically active metabolites formed by BSVM from arachidonic acid (33 μM) in the presence of glutathione (165 μM) behaves chromatographically as PGE_2.

(b) Bioassay of malondialdehyde formation by the thiobarbituric acid method [9]. This method was suggested by Flower *et al.* [10] as the alternative route for estimation of PG synthetase activity.

(c) Measurement of the oxygen uptake during cyclo-oxygenation of arachidonic acid by RSVM [11]. The consumption of oxygen was measured using Clarke's electrode (Oxygen monitor Yellow Spring Instruments model 53).

The enzymic activity was expressed in nmoles of PGE_2, malondialdehyde or oxygen which were generated or consumed by 1 mg of the microsomal protein. The following reagents were used:
Malondialdehyde tetraethyl acetal from K and K Laboratories Inc., Plainview, U.S.A., Arachidonic acid from Sigma Chemical Company, U.S.A., Glutathione from "biomed", Poland, Hydroquinone from Malinckrodt, U.S.A., PGE_2 was kindly donated by Dr J. Pike from the Upjohn Co., Kalamazoo, Michigan, U.S.A.

RESULTS

The activity of PG synthetase in BSVM is strongly dependent on the presence of cofactors in the incubation mixture (Fig. 1). In the presence of glutathione (165 μM) and hydroquinone (45 μM) the enzymic activity was 5.8 $\pm$ 0.9 nmoles of PGE_2 equivalents/mg of protein, whereas in the absence of these cofactors the enzymic activity dropped to 0.84 $\pm$ 0.08 nmoles of PGE_2 equivalents/mg of protein (n = 4, $P < 0.01$). When cofactors were omitted from the incubation mixture, paracetamol (67–667 μM) stimulated the enzymic activity. However, in the presence of cofactors, paracetamol (200–3330 μM turned out to be an enzymic inhibitor with IC_{50} = 1500 μM (r = 0.859) (Fig. 1).

PG synthetase in RSVM was not stimulated by glutathione (165 μM) and hydroquinone (45 μM). The enzymic stimulation was observed when concen-

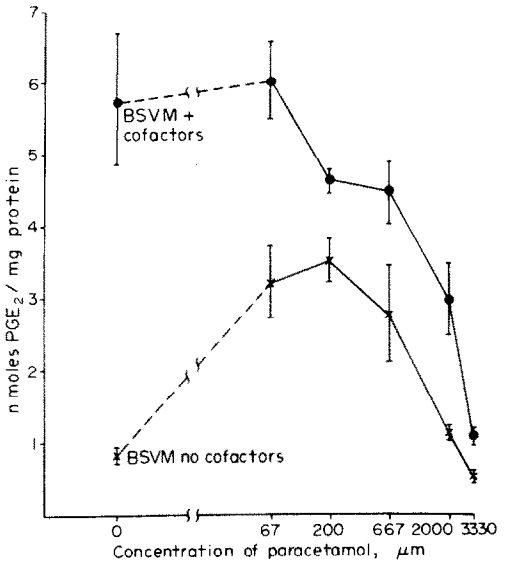

Fig. 1. Effects of paracetamol on prostaglandin synthetase activity in bovine seminal vesicle microsomes (BSVM) in the absence (crosses) or in the presence (dots) of glutathione (165 μM) and hydroquinone (45 μM). Ordinate: nmoles of PGE_2 per mg of microsomal protein (±S.E.) which was formed during 20 min of the incubation. Abscissa: final concentration (μM) of paracetamol in the incubation mixture.

trations of these cofactors were increased up to 500 μM and 150 μM, respectively. The presence of both cofactors at high concentrations is required to produce a distinct activation of the enzyme (Fig. 2).

In the absence of cofactors paracetamol (33–333 μM) stimulated the cyclo-oxygenation of arachidonic acid, the generation of PGE_2 and the formation of malondialdehyde by RSVM (Fig. 3). Low concentrations of glutathione (165 μM) and hydroquinone (45 μM) suppressed the stimulatory effect of paracetamol (Figs 3 and 4). High concentrations of the cofactors abolished the stimulatory effect of paracetamol (Fig. 4) however glutathione (500 μM) alone

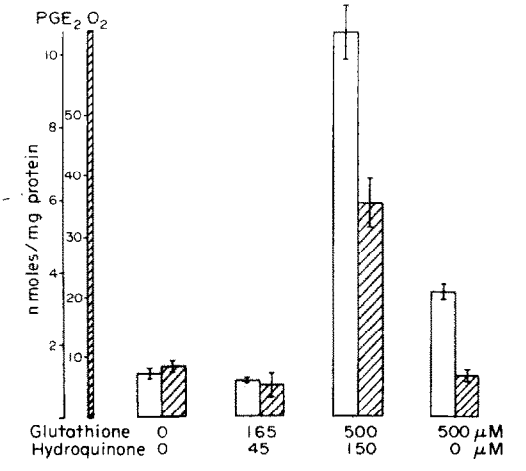

Fig. 2. Influence of cofactors on the PG synthetase activity in ram seminal vesicle microsomes (RSVM), measured as PGE_2-like activity (white columns) or oxygen consumption (hatched columns).

was unable to abolish the stimulatory effect of paracetamol on PG synthetase in RSVM.

DISCUSSION

Microsomal PG synthetase is stimulated by thermostabile factors from high-speed supernatant of homogenates [12]. These cofactors can be replaced by glutathione [10, 13, 14] and several other agents e.g. hydroquinone [13], *p*-aminophenol [4, 5], adrenaline [10] or tryptophane [14]. The role of glutathione is to facilitate the breakdown of cyclic endoperoxide to PGE_2 [11, 14]. Tryptophane (similarly to hydroquinone and adrenaline) is supposed to be a cofactor for cyclo-oxygenation of the substrate [14]. We have found that paracetamol can partially replace hydroquinone as the cofactor in PG biosynthesis in BSVM and RSVM. Paracetamol stimulated not only the generation of PGE_2 and the formation of malondialdehyde from arachidonic acid but also the consumption of oxygen by microsomes was increased in parallel to the accelerated formation of the end-products. Therefore we believe that in the native microsomal preparation paracetamol stimulates PG biosynthesis at the stage of cyclo-oxygenation of arachidonic acid.

Our results support the assumption [11, 14] that glutathione facilitates the isomerisation of cyclic endoperoxides to PGE_2 since in RSVM glutathione (500 μM) stimulates preferentially PGE_2 generation over the oxygen consumption (Fig. 2).

We have also shown an essential difference between RSVM and BSVM in their requirements for exogenous cofactors. The optimal concentrations of cofactors (hydroquinone and glutathione) for BSVM [15] are unable to stimulate the enzymic activity in RSVM. An increase in concentrations of glutathione and hydroquinone by a factor of 3 is necessary to activate RSVM and then paracetamol is no more a stimulator of cyclo-oxygenase. Moreover, in RSVM the suboptimal concentrations of cofactors are sufficient to depress the stimulatory effect of paracetamol. In the activated BSVM paracetamol behaves like a typical PG synthetase inhibitor with IC_{50} = 1500 μM (Fig. 1). This inhibitory potency of paracetamol is not far from the value IC_{50} = 662 μM which was reported [2] for dog spleen microsomes in the presence of glutathione (165 μM) and hydroquinone (45 μM).

Does paracetamol stimulate or does it inhibit PG biosynthesis *in vivo*? After ingestion of a therapeutic dose of paracetamol its plasma levels are at a range of 66–330 μM [16]. In our experiments these concentrations of the drug were ineffective (in the presence of cofactors) or stimulatory (in the absence of cofactors) on the enzymic activity in RSVM or in BSVM. However in the feverish cats paracetamol normalizes the elevated PG levels in cerebro-spinal fluid in parallel to the hypothermic effect of the drug [17]. It may well be that the brain synthetase is more sensitive to the inhibitory action of paracetamol than PG synthetase in RSVM (IC_{50} > 3000 μM), in BSVM (IC_{50} = 1500 μM) (this paper), in dog spleen microsomes (IC_{50} = 662 μM) [2] or in rat skin homogenates (IC_{20} = 500 μM) [18]. Indeed, Flower and Vane [1] reported that paracetamol inhibited PG synthetase in rabbit brain microsomes at IC_{50} = 93 μM. In these experiments the incubation

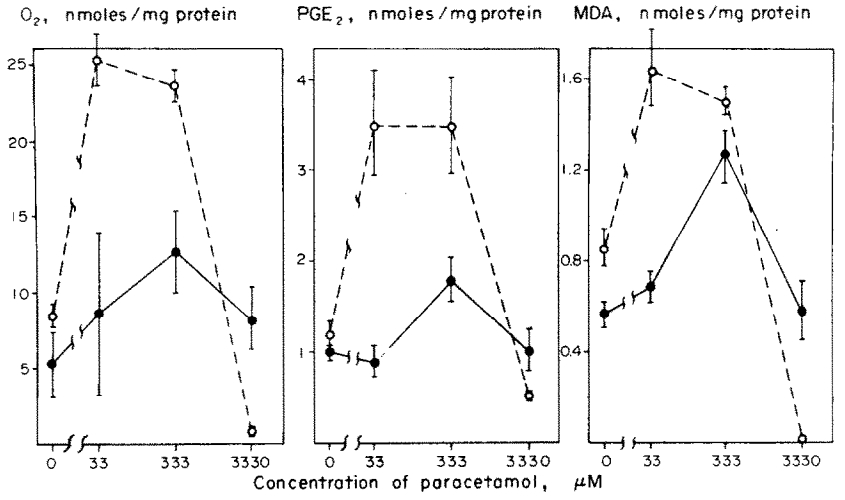

Fig. 3. Influence of paracetamol on oxygen consumption, PGE_2 generation and malondialdehyde (MDA) formation in ram seminal vesicle microsomes in the absence (open symbols) and in the presence (closed symbols) of glutathione (165 μM) and hydroquinone (45 μM). Ordinate: nmoles of measured products per mg of microsomal protein. Abscissa: concentration of paracetamol (μM) in the incubation mixture.

mixture comprised glutathione (165 μM), hydroquinone (45 μM) and exogenous arachidonic acid (33 μM). On the other hand in the rat brain homogenates paracetamol hardly inhibited PG generation (IC_{50} > 1000 μM) from endogenous arachidonic acid in absence of exogenous cofactors [3]. Thereby the selective inhibitory action of paracetamol on the brain PG synthetase *in vitro* has been questioned [3]. Further experiments are needed to explain the mode of action of paracetamol on the brain PG synthetase *in vivo*. We conclude that *in vitro* the exogenous cofactors have a profound effect on the mode and potency of action of so called "PG synthetase inhibitors". Our preliminary data have shown that aminopyrine and dipyrone (both antipyretic and analgesis drugs) behave exactly like paracetamol, namely these drugs stimulate or inhibit PG synthetase in BSVM or RSVM depending on the presence of glutathione and hydroquinone in the incubation mixture.

We feel that the tissue slices or tissue homogenates have an advantage over microsomal preparations when looking for the unknown effects of drugs on the enzymic biotransformation of arachidonic acid. Microsomes are nearly deprived of natural cytoplasmic cofactors, or at least their concentrations are insufficient. The artificial composition of exogenous cofactors, activating microsomal preparations has a dramatic effect on the mode of action of tested drugs. The final effect of a drug of the PG generating system in slices or in homogenates is closer to that observed *in vivo* than the effect of a drug on microsomes which are either deficient in the essential cofactors or artificially stimulated by exogenous cofactors. The danger of metabolizing of PGs by cytoplasmic enzymes can be diminished by shortening of the period of incubation of homogenates [19].

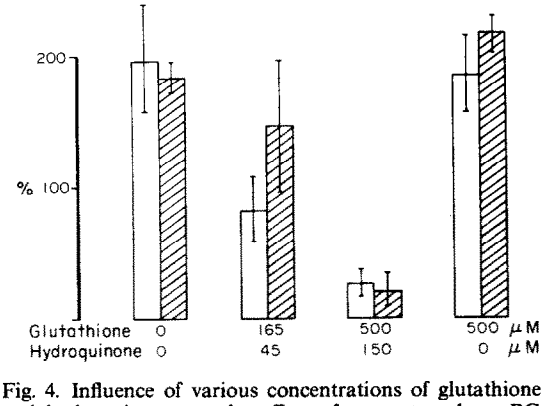

Fig. 4. Influence of various concentrations of glutathione and hydroquinone on the effect of paracetamol on PG synthetase activity in ram seminal vesicle microsomes. This activity was measured as the amount of generated PGE_2 (white columns) and oxygen consumption (hatched columns). In each case a 100 per cent of enzymic acitivy refers to the activity the control incubation mixture which did not contain paracetamol.

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