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IN VITRO AND *IN VIVO* ARACHIDONIC ACID CONVERSIONS INTO BIOLOGICALLY ACTIVE DERIVATIVES ARE ENHANCED BY URIC ACID*

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Abstract—Hypoxia enhances hypotensive effects of i.v. arachidonic acid (AA), in the rabbit; this phenomenon is correlated with the increased uricemia following hypoxia. Sensitization by hypoxia to AA is blocked by allopurinol, which inhibits the formation of uric acid. Injections of purines, in normoxic rabbits, stimulate also AA hypotension, by the way of a conversion into uric acid, since allopurinol blocks the stimulation produced by injection of adenine or hypoxanthine. Purine sensitization to AA is also observed on stomach strips, without sensitization to PGE_2 or PGI_2. Uric acid appears to be a potent activator of *in vitro* PGs biosynthesis, particularly in absence of the antiradicalar couple: hydroquinone plus glutathione. Uric acid may be considered as a hydroxyl radical scavenger; its enhancing action on AA conversions may be due to this property.

Intravascular injections of arachidonic acid (AA) cause a cascade of physiopathological events, from which the fall of arterial blood pressure is easy to measure. These phenomena are produced by the enzymatic conversion of AA into several biologically active substances chiefly prostacyclin and thromboxane A_2 [1], since the AA effects are completely blocked, not only by nonsteroidal antiinflammatory agents (aspirin, indomethacin) but also by TYA (eicosatetraynoic acid) a more specific inhibitor of cyclooxygenase. The hypotensive action of AA may be taken as an indicator of the ability of a mammal to convert this precursor into biologically active prostanoids [2]. To standardize the experimental results, we propose the use of the AA_{50}, the dose of i.v. injected AA needed to induce a fall of the arterial blood pressure equal to 50 per cent of initial blood pressure value. The data published in the literature, for the rabbit's AA_{50}, are generally between 400 and 600 μg/kg [3–5], the lethal dose being higher than 1 mg/kg [5]. These doses realize unphysiological plasma AA concentrations, and doubts were emitted concerning the specificity of the observed results, since, at these high plasma AA concentrations, the soap properties of sodium arachidonate are predominant [6].

Three factors, heparinisation, hypoxia and injections of autologous hemolyzed blood, sensitize the rabbit to i.v. injected AA; the AA_{50} is lowered to 8–15 μg/kg, and the AA lethal dose falls to 60–80 μg/kg [7, 8]. Thus, with this method, the animal becomes about 20 times more sensitive to AA. We have discussed [8] the interest of this finding for human pathology, especially in cardiovascular surgery, where a massive heparinisation is accompanied by frequent hemolysis and tissue anoxia following arterial clamping. We proposed at least one mechanism for the heparine sensitization [9].

In this paper, we are dealing with the mechanisms of sensitization by hypoxia. Many parameters vary in hypoxia, but the dramatic rise of hypoxanthinemia and uricemia described by several authors [10, 11] kept our attention for two reasons: (a) i.v. injected hypoxanthine enhances the hypotensive response of the rabbit to AA [12]; (b) hydrogen peroxide catalyzes, in well defined conditions, the biosynthesis of PGs [13]; hypoxanthine and uric acid (UA) are involved in enzymatic reactions producing H_2O_2 (Fig. 1).

The reactions mediated by xanthine-oxidase are inhibited by allopurinol and the attack of UA by uricase is inhibited by oxonate.

We describe here the results of our research on the role of the purines in the AA conversion into prostanoids. Three methods were used: AA hypotension in the rabbit, stomach strip's contraction with AA and rate of the *in vitro* PGs biosynthesis.

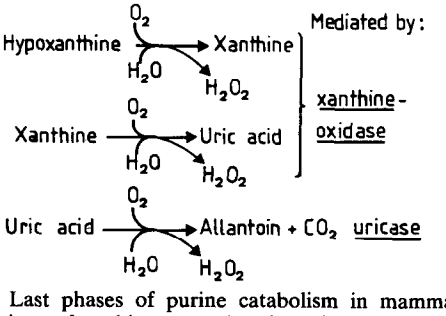

Fig. 1. Last phases of purine catabolism in mammals. Uricase is not found in man: uric acid is the final product.

* A preliminary account of part of this work has already been published as a preliminary note (Z. M. Bacq, C. Deby and L. Lavergne, *Brit. J. Pharmac.*, in press).

MATERIALS AND METHODS

(a) *Physiological assays.* Male rabbits "Fauve de Bourgogne", 4–6 months old, weighing from 2 to 2.7 kg, were anaesthetized (40 mg/kg Nembutal) and moderately sensitized to AA, according to a previously described procedure [8]: 50 min after a massive heparinization (10 mg/kg), small quantities of autologous hemolyzed blood are injected i.v., in such a way as to reach a plasma hemoglobin level around 0.2–0.3 mg/ml. The AA_{50} is then lowered about 120–160 μg/kg. The trachea was cannulated and the breathing controlled by the respirator Servo-Ventilator Siemens-Elema 900 B. The mixture of N_2 and O_2 was regulated by Fisher and Porter rotameter valves and the oxygen monitored by a polarographic device OHIO 600. ECG was monitored by an oscilloscope "Physiological Monitor" Tektronix; the blood pressure was electrically recorded, using a gauge LX 600 NS. The partial oxygen pressure was measured in arterial blood, at 37°, by the automatic device Radiometer ABL 2.

(b) In vitro *biological assays.* Arachidonic acid was dissolved in Tyrode, at pH 7.6, at final molar concentrations between 1.10^{-6} and 1.10^{-5} M in the bath. Stomach strips were maintained in Tyrode at 36° and automatically washed every 90 sec. The isotonic contractions were recorded by an optical device [14]. The specificity of the responses to AA was demonstrated by their suppression, either by eicosatetraynoic acid, either by lysine acetylsalicylate. 10^{-4} M to 10^{-3} M concentrations of purines were easily obtained. Contacts between chemicals and strips were maintained during 30 sec in the case of AA or PGE_2, during 90 sec in the case of purines. Their effects on AA and on PGE_2 strip sensitivities were compared, and the responses to AA expressed in PGE_2 nanograms equivalent. The chemicals which alter the strip responses to PGE_2 were discarded as was the case with oxonate, which markedly weakens the PGE_2 contraction.

(c) *Purines estimation in arterial blood plasma.* Two methods were simultaneously used for hypoxanthine and xanthine measurements. (a) Fluorometrically: in a first step, the attack of hypoxanthine and xanthine by xanthineoxidase generates H_2O_2; that latter oxidizes dichlorofluorescin, a non fluorescent compound, into the fluorescent dichlorofluorescein. The time-dependent fluorescence in recorded with an Aminco Bowman fluorometer, giving data from which the H_2O_2 levels, and consequently, the combined hypoxanthine and xanthine concentrations may be calculated [15, 16]. (b) Spectrophotometrically: the plasma level of uric acid is measured before and after the action of xanthine-oxidase on the sample; 0.04 U of xanthineoxidase and 10^{-4} M EDTA are added to the sample, and the mixture is incubated 10 min at 37°; the conversion of hypoxanthine and xanthine to uric acid is complete. The two procedures are applied to each plasma sample and the uncoincording values are rejected.

Uric acid was measured by an enzymatic method [17]: it is transformed in allantoin by uricase, with hydrogen peroxide production; that latter acts on methanol, producing formaldehyde, which reacts with acetylacetone to give a stable compound measured at 410 nanometer.

(d) In vitro *PGs biosynthesis.* Biosynthesis was performed following a previously described method [18]. ^{14}C-labelled and unlabelled AA were mixed to realize a final concentration of $1.25 \cdot 10^{-5}$ M of AA in the assays. Glutathione (5.10^{-4} M) and hydroquinone (9.10^{-4} M) were added in certain experiments. PGs were extracted with diethylether, chromatographed on silicagel in the solvent system chloroform/methanol/CH_3COOH/H_2O (90:8:1:0.75). Autoradiographs of the developed TLC plates were performed, to facilitate the localization of the labelled spots, using X-Omat XRP Kodak films (contacts maintained during 120 hours). The PGs were then eluted by an automatic device (Eluchrom Camag), and counted by liquid scintillation (Nuclear Chicago, Mark II).

(e) *Purine-free O_2^- generating system during PGs in vitro biosynthesis.* A procedure of Kellog and Fridovich was adapted to our studies [19]. Acetaldehyde (6 mM) and xanthine-oxidase (0.13 μM), with or without EDTA (3.10^{-6} M), generate superoxide anion and H_2O_2 in the vesicular microsomal suspension, uric acid being present or absent. Acetaldehyde, EDTA and uric acid were added together in the suspension and preincubated during 15 min at 37°. Xanthine-oxidase and arachidonic acid were then added.

(f) *Xanthine-oxidase and uricase assays on microsomes.* 8-^{14}C-hypoxanthine was incubated with bull seminal microsomes preparation at 37° for 10 min. After ice cooling and addition of unlabelled hypoxanthine and uric acid, the whole volume is ultrafiltrated on CF25 cones (Amicon) for protein elimination. Hypoxanthine was separated from its metabolite (^{14}C-uric acid) by TLC on PEI-cellulose F, in Tris–HCl buffer at pH 7.6. After u.v. revelation and autoradiography of the plates, ^{14}C-hypoxanthine and ^{14}C-uric acid are eluted with 0.05 M Na tetraborate and counted by scintillation method.

(g) *Chemical reagents.* Hypoxanthine and uric acid Merck; Heparine Roche, Na salt; oxonate K salt and allantoin Aldrich; Allopurinol Wellcome; AA Sigma (99 per cent); Xanthine-oxidase, Uricase and Horseradish peroxidase Boehringer; Urica-Quant Boehringer (for uric acid estimation); all solvents (pro analysi) and TLC plates were purchased from Merck. Autoradiography was made using X-Omat XRP-1 plates from Kodak. Allopurinol being poorly soluble in biological liquids, the following method was employed: 40 mg of allopurinol are dissolved in 2 ml of polyethylene glycol 400 Baker; after addition of 0.5 ml of dimethyl formamide, heating at 50° and cooling again at 37°, 2 ml of autologous plasma are added before injection to the rabbit. Dissolution of allopurinol must be careful regarding pH and temperature because this molecule may be transformed and induce unexpected effects. Labelled compounds were ^{14}C-arachidonic acid (Amersham) and 8-^{14}C-hypoxanthine (CERN-Saclay).

(h) *Hemoglobin estimation.* Hemoglobin estimation in the plasma was performed according to Drabkin [20].

RESULTS

1. Arachidonic hypotension, hypoxia and uricemia

Figure 2 shows the correlation between the increase of uricemia (which begins few minutes after the reduction of the oxygen percentage in the gas mixture: 12 per cent O_2 in N_2) and the decrease of AA_{50}.

These data concern 8 rabbits, heparinized 1 hr before 15 min. hypoxia (12 per cent O_2), which received no other preliminary treatment. Before and after hypoxia, the gas mixture contained 21 per cent O_2 in N_2. Five minutes after the onset of hypoxia, the AA_{50} decreased from 140–180 μg/kg to 60–100 μg/kg, while the uric acid in the arterial plasma increased from 15–20 μmoles/l to 150–180 μmoles/l. Immediately after the return to normoxia, a new fall of the AA_{50} was registered, and a high sensitivity to AA persisted during 50–80 min. Simultaneously, uricemia was maintained at values at least 5 fold higher than before the hypoxic treatment. Hypoxanthine levels were not so closely linked to the evolution of AA_{50} (see figure 1 in Ref. [29]).

2. Enhancement of arachidonic hypotension by purine injections

Fifty two normoxic rabbits, sensitized to AA 1 hr previously by heparinization and injections of autologous hemolyzed blood (to reach 0.3–0.4 mg hemoglobin per ml of plasma), were injected i.v. with purines of the catabolic series (adenine, hypoxanthine, xanthine and uric acid), and the ultimate metabolite: allantoin. In order to compare the resulting AA_{50}, we used a sensitization coefficient, i.e. the number by which the AA_{50} measured before the application of the tested substance must be divided to obtain the new AA_{50} observed 5 min after the test (Fig. 3).

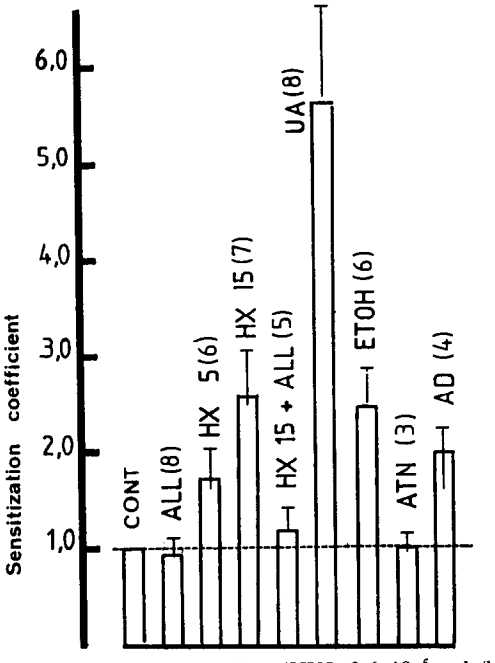

Fig. 3. Effects of hypoxanthine (HX5: $3.6 \cdot 10^{-5}$ mole/kg; HX 15: $1.1 \cdot 10^{-4}$ mole/kg), allopurinol (ALL: 20 mg/kg), uric acid (UA: $1.1 \cdot 10^{-4}$ mole/kg), allantoin (ATN: $9.4 \cdot 10^{-5}$ mole/kg), and adenine (AD: $7.3 \cdot 10^{-5}$ mole/kg), on the sensitivity of rabbits to the AA hypotension. Ordinates: sensitization coefficient (number by which the previous AA_{50} must be divided to obtain the new AA_{50} observed after the application of the sensitizer). In brackets: number of animals tested. CONT: controls. The sensitizing effect of UA may be explained by the hydroxyl scavenging property of this purine [13]. The sensitizing effect of a well-known hydroxyl scavenger, ethanol (ETOH: $1.3 \cdot 10^{-4}$ mole/kg), is represented here as reference.

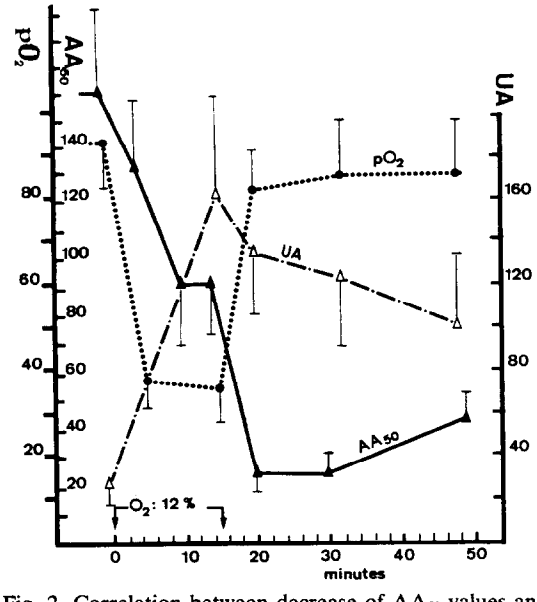

Fig. 2. Correlation between decrease of AA_{50} values and increase of uricemia, during and after hypoxia, in the rabbit. Mean values in 8 animals. Left ordinates: pO_2 partial pressure of oxygen in arterial blood, in mm Hg (●·····●); AA_{50} expressed in μg/kg (▲———▲): AA_{50} is the AA dose necessary to obtain a 50 per cent fall in systolic pressure. Right ordinates: arterial concentrations of uric acid (UA), in μm/l (△·—·—·△. Abscissae: time in min, measured from the beginning of hypoxia (12 per cent O_2 in N_2).

All the tested purines sensitized to AA, but the ultimate product of the purine catabolism, allantoin, did not. Uric acid appeared to be the best sensitizer. All these effects were suppressed by indomethacin (2 mg/kg) and by TYA (150 μg/kg mixed ith AA). The hypotensive effect of PGE_2 or PGI_2 was not altered by hypoxia and purine injections. These observations show that it is the metabolism of AA which is modified and not the reaction of the animal to its metabolites.

All the tested purines sensitized to AA, but the ultimate product of the purine catabolism, allantoin, did not. Uric acid appeared to be the best sensitizer. After uric acid intraveinous administration, the AA_{50} decreased to 16 μg/kg (Table 1), and death occurred after i.v. AA doses of 50–60 μg/kg.

All these effects were suppressed by i.v. indomethacin (2 mg/kg), by i.v. lysin acetylsalicylate (25 mg/kg) or by TYA (150 μg/kg, mixed with AA). After indomethacin or lysin acetylsalicylate, rabbits showed no blood pressure reactions, even at

500 μg/kg; but after a huge dose of AA, 1 mg/kg, 25–40 per cent arterial blood pressure falls were observed, followed by a rapid recovery; these effects are explainable by the detergent properties of sodium arachidonate. The inhibitory effects of indomethacin were observed even 8 hr after its injection; total AA hypotension recovery was observed 4 hr after acetylsalicylate injection, but the lethal dosis of AA was significantly higher than before this injection (400 μg/kg AA).

3. Inhibition of purines catabolism and arachidonic hypotension

As shown in Table 1, allopurinol decreases the AA hypotension only in rabbits sensitized to AA by hypoxia or by injection of an uric acid precursor, such as hypoxanthine. This phenomenon appeared clearly on heparinized rabbits moderately sensitized to AA by a previous injection of small dose of hemolyzed blood, the AA_{50} being lowered around 50–70 μg/kg. The role played by hypoxia or by purine injections became more evident, since the AA_{50} was reduced by a factor of 2.5–3.0. Allopurinol did not modify the hypersensitivity to AA afforded either by a supplement of hemolyzed blood, or by an injection of UA. Allopurinol did not change the vascular responses to PGE_2 or to PGI_2. Thus, the desensitization to AA hypotension by allopurinol was related to its inhibitory effect on uric acid synthesis. It was not possible to use oxonate *in vivo* (an uricase inhibitor which enhances uricemia) because it was toxic and reduced the vascular responses to PGE_2 and PGI_2.

4. Stomach strips responses to AA and purines

AA, at final concentrations $5 \cdot 10^{-6}$ M, generally produced the same contractions of stomach strip than those obtained with $1 \cdot 10^{-9}$ M of PGE_2. The AA effects were not potentiated in the same manner by hypoxanthine or uric acid, as shown in Fig. 4. The enhancement of strip contractions was proportional to the molarity of uric acid; but in the case of hypoxanthine, above an optimal concentration, the AA responses were depressed by hypoxanthine. Allantoin was ineffective. In all our assays, the responses to PGE_2 or PGI_2 were not affected by the tested

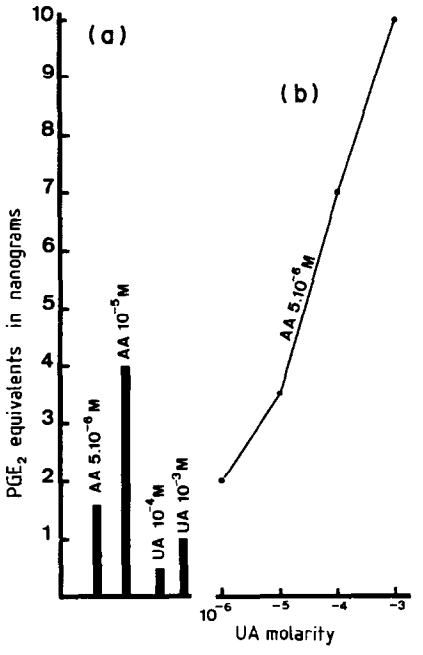

Fig. 4. Potentiation of AA-induced stomach strip contractions by uric acid. Strips are standardized with a PGE_2 concentration scale, and response intensities expressed in PGE_2 equivalents. (a) Effects of AA and UA, separately assayed. (b) AA is assayed immediately after a contact of the strip with uric acid, diluted in the biological bath, during 90 sec.

Table 1. Effects of allopurinol on the sensitization to arachidonic acid, induced by hypoxia or by injections of hypoxanthine and uric acid, in rabbits*

Factor	number of animals	AA_{50} (μg/kg) before factor	AA_{50} (μg/kg) after factor	AA_{50} (μg/kg) after factor + allopurinol¶
Hypoxia†	6	55 ±10.5	22 ± 3.3	62 ± 1
Hypoxanthine‡	5	48 ± 7.5	18 ± 3	54 ± 6
NaCl§	6	58 ± 10	58 ± 10	60 ± 9.7
Hemolysate‖	5	52 ± 6	23 ± 2.3	23 ± 2.8
Uric acid*†	3	68 ± 17	16 ± 4	18 ± 4.3

* The rabbits were, one hour before the assays, heparinized and injected with moderated doses of hemolyzed blood (to realize a plasma hemoglobin concentration of 0.2–0.3 mg/ml).

† Hypoxia: 12 per cent O_2 in breathed air, during 15 min, the mean arterial pO_2 being 38 mm Hg.

‡ Hypoxanthine (i.v.): 15 mg/kg.

§ NaCl (i.v.): solution 0.9 per cent, 2 ml/kg.

‖ A new amount of autologous hemolyzed blood is injected, plasma hemoglobin reaching 0.5–0.6 mg/ml.

¶ Allopurinol (i.v): 20 mg/kg, 6 min before the test.

*† Uric acid (i.v): 10 mg/kg

Table 2, Rate of *in vitro* biosynthesis by bull seminal vesicle microsomal preparations, in per cent of controls

(A) Influence of the addition of the couple GSH* + HQ†				
Uric acid	10^{-6} M (per cent	10^{-5} M (per cent)	10^{-4} M (per cent	10^{-3} M (per cent)
GSH* + HQ†	107 ± 7	115 ± 9.6	99 ± 8.9	120 ± 8.4
No GSH or HQ	105 ± 8.4	111 ± 9.8	159 ± 11	293 ± 26
(B) Role of H_2O_2 generating system, uricase + uric acid, in absence of GSH + HQ				
Uric acid	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
+ uricase‡	115 ± 14.9	105 ± 8.3	98 ± 13.1	190 ± 20.5
+ uricase + oxonate§	116 ± 16.3	96 ± 11	145 ± 6.1	254 ± 28
(C) Role of oxonate alone, in absence of GSH + HQ				
Oxonate	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M
	101 ± 6.1	97 ± 7.7	99 ± 8.7	81 ± 4.2

* Glutathione: $5 \cdot 10^{-4}$ M.
† Hydroquinone: $9 \cdot 10^{-4}$ M.
‡ 0.2 U per assay of 2 ml.
§ Oxonate was added at the same concentrations as uric acid.

purines. All the AA-induced contractions were suppressed by indomethacin (10^{-4} M), lysine acetylsalicylate (10^{-5} M) or TYA (10^{-5} M). Allopurinol could not be tested; it is poorly soluble in tyrode, and if dissolved in albumin, O_2 bubbling is impossible. Oxonate was toxic for the strip; it decreased markedly the reaction to PGE_2 and PGI_2.

5. Role of purines on in vitro *PGs biosynthesis*

The results are summarized in Table 2. The activating properties of uric acid were observed in *in vitro* experiments, only when the antioxidant and antiradicalar system, glutathione + hydroquinone, was absent. The addition of uricase to uric acid, forming together an H_2O_2 generator system, suppressed the increment of biosynthesis induced by uric acid. Oxonate by itself was a mild inhibitor of PGs biosynthesis. In these experiments, all the eluted products corresponding to the different prostanoids were pooled, to calculate the total conversion of ^{14}C-AA to PGs.

6. Mechanism of hypoxanthine, xanthine and uric acid actions

In order to understand the mechanism of hypoxanthine, xanthine and uric acid actions, during the PGs biosynthesis by seminal vesicles microsomes, it was necessary to know if xanthine-oxidase and uricase activities occurred in this preparation, by comparing the results obtained with boiled and crude microsomes. We observed that seminal microsomes can metabolize hypoxanthine into uric acid, with a significative inhibition by allopurinol, but only a very poor uricase activity was detected. Thus, if hypoxanthine was rapidly metabolized, uric acid remained practically unaltered during the time required for a PGs biosynthesis by seminal microsomes (10 min).

DISCUSSION

Our purpose, in this study, was only to have a general view of the impact of purine metabolism on AA conversions into biologically active derivatives, without specifications of the chemical nature of these metabolites. We have decided, in a first step, not to try estimate by radio-immuno-assays one or two metabolites, because the prostanoid catabolites of AA are numerous, appear in variable proportion, and cannot give a global idea of the biological actions instantly recorded of the active derivatives. Further studies are conducted in our laboratory. There is little doubt that the AA derivatives detected by our methods, in this work, are cyclo-oxygenase products, since all the physiological tests employed are inhibited by acetylsalicylate; lipooxygenase, which generates HETE and leucotrienes, is not inhibited by aspirin.

Uric acid appears to be the most active purine, in the increase of AA hypotension in the whole rabbit or AA-induced smooth muscle contractions. The *in vitro* experiments can explain, at least partially, the biochemical mechanism by which UA enhances AA conversion in PGs, in the absence of glutathione and hydroquinone. These UA activations may be due to intrinsic properties of the purine or related to enzymic conversions of UA in H_2O_2 and allantoin, by uricase. H_2O_2 enhances PGs biosynthesis, in well defined conditions, as observed by us [13, 21]. Our experiments discard the first possibility, since our seminal microsomes preparations show only a weak uricase activity. Moreover, the addition of uricase markedly decreases the enhancing action of UA, probably by consumption of the purine; this effect was restored, despite the presence of added uricase, by oxonate, an inhibitor of this enzyme; oxonate is

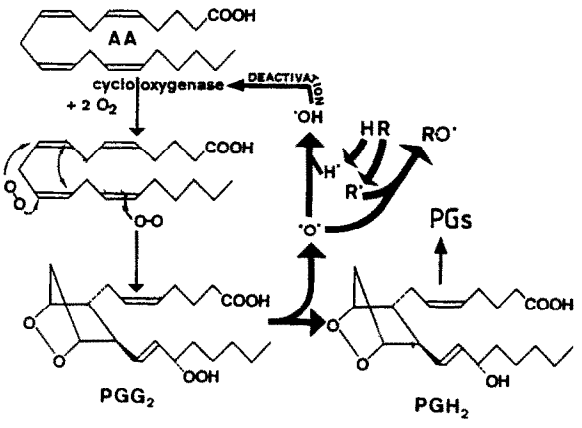

Fig. 5. The mechanism of cyclo-oxygenase self-activation, following Egan *et al.* [21]. One oxygen atom is released, during the conversion of endoperoxide G_2 (PGG_2) into endoperoxide H_2 (PGH_2). This atom may capture a hydrogen atom, forming hydroxyl radical ·OH, or react with an organic chain RH, forming an alkoxy radical RO·[33], which may be a potent agent of enzyme inactivation.

however a mild inhibitor of PGs biosynthesis. Allantoin, produced by uricase activity, does not affect biosynthesis. Thus, UA seems to act by itself, in AA conversions activation. Its role is masked by the presence of glutathione+hydroquinone, a couple which exhibits antioxidant and antiradicalar properties, and is implicated in the protection of cyclo-oxygenase against self-deactivation. It is indeed admitted now by some scientists that highly reactive oxygen species are released from PGG_2 during its conversion into PGH_2 [21], as shown on Fig. 5. That species should be the hydroxyl radical ·OH [22]. Hydroxyl scavengers are potent activators of PGs biosynthesis, for instance ethanol [23], benzoate [24], methional [21], phenols [21], and chlorpromazine [25]. These compounds delay the cyclo-oxygenase inactivation by scavenging ·OH generated during the activity of the enzyme [21].

Can UA replace glutathione + hydroquinone in their antiradicalar activity? The response is probably yes. UA may act, indeed, as a radical scanvenger. Already in 1964, UA was proposed as a protector against oxidations [26]. Kellogg and Fridovich [19] have postulated that UA may be considered as an activated oxygen scavenger, but without giving any demonstration of this hypothesis. Recently [27], we observed that uric acid concentrations higher than 1.10^{-4} M inhibit the ethylene production due to the action of ·OH, generated by a Fenton reaction, on ketomethylthiobutyric acid [28]. This zone of active concentrations is the same as needed for significant stimulations of PGs production, during *in vitro* biosynthesis.

If an antiradicalar mixture, such as glutathione+hydroquinone, is present where PGs synthesis occurs, the protecting effect of uric acid becomes unnecessary; the impoverishment in purine by uricase does not induce any decrease in PGs production. Thus, in the presence of glutathione+hydroquinone, the resulting effect of uricase addition to uric acid, consuming this latter, is a stimulation.

These *in vitro* observations are in accordance with the *in vivo* experiments and with the *in vitro* biological assays. Uric acid is the sole purine to stimulate by itself the AA hypotension, when it is injected intraveinously some minutes before AA. The sensitization by hypoxanthine is abolished by allopurinol (a blocker of xanthineoxidase, inhibiting the conversion of hypoxanthine into uric acid). On the other hand, the sensitization to AA induced by hypoxia is well correlated with the rising uricemia, resulting from enhanced release of hypoxanthine, which is rapidly metabolished by the lung xanthineoxidase [29]. In this case, hypoxanthine does not play a role by itself, since injection of allopurinol suppresses the sensitization increment afforded by hypoxia. This experiment, in other respects, strongly suggests that hypoxia sensitizes to AA only through increased uricemia. At equal concentrations, uric acid potentiates AA-induced contractions of stomach strips more efficiently than hypoxanthine. As for the *in vitro* PGs biosynthesis, uric acid may also act, in biological experiments, by its ·OH scavenging property, since the ·OH scavengers activate AA-induced strips contractions as well as AA hypotension [13, 30]. In Fig. 3, UA sensitizing effect on AA hypotension is compared to that obtained with ethanol, a well known hydroxyl scavenger [23].

In summary, all these facts, observed by means of three different techniques, converge to prove that uric acid is probably the direct agent by which purine metabolism stimulates PGs formation.

The role played by the purine catbolism, generator of uric acid, in the prostanoid biosynthesis, provides new explanations for some unexplained symptoms observed in gout. It is well known that, in hyperuricemia, deposition of urates occurs near the metacarpial and metatarsial bones (tophi) and that limited osteolysis may take place in the adjacent bone tissue (geode). Since the prostaglandins are implicated in the activity of the osteoclasts [31], gout osteolysis should be explained by the enhanced PGs biosynthesis occurring around the neighbouring tophi. The beneficial role of allopurinol, in the post-ischemic syndrome, studied by Bruekner *et al.* [32], should be explained in a more satisfactory manner, by the decrease of uric acid, activator of vaso-active compounds biosynthesis. From this point of view, the relation between uric acid and cardio-vascular diseases must be re-investigated.

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