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PROSTAGLANDIN D_2 IS THE PREVAILING PROSTAGLANDIN IN THE ACUTE INFLAMMATORY EXUDATE OF URATE ARTHRITIS IN THE CHICKEN

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An acute inflammation was elicited in intertarsal joints of chicken by injection of urate crystals. Inflammatory exudates recovered at different times were assayed for prostaglandin D_2(PGD_2) E_2 and $F_{2\alpha}$ and thromboxane B_2 content by specific radioimmunoassays. We found that PGD_2 was the prevailing prostaglandin reaching concentrations up to 10 times in excess of PGE_2. This finding was confirmed by gas chromatography-mass spectrometry. It is concluded that PGD_2 should be considered as a possible mediator of acute inflammation.

Introduction Prostaglandin D_2 (PGD_2) levels in inflamed tissue have not been reported to date. This lack of information on endogenous levels of PGD_2 is surprising because exogenous PGD_2 produces proinflammatory effects similar to PGE_2. Intradermal injection of PGD_2 causes an increase of vascular permeability in rat skin and induces long lasting erythema on the human forearm (Flower, Harvey & Kingston, 1976). Moreover, like PGE_2, PGD_2 in low doses enhances the inflammatory response to carrageenan or histamine (Flower *et al.*, 1976). Hence it should be considered a potential mediator of inflammation. We have now measured the concentration of PGD_2 in inflammatory exudates, by means of specific radioimmunoassays and combined gas chromatography–mass spectrometry. We found unequivocally that PGD_2 reaches much higher concentrations in the inflammatory exudate than either PGE_2, $PGF_{2\alpha}$ or thromboxane B_2 (TXB_2).

Methods Acute inflammation was elicited in white Leghorn chickens (2 kg body weight) by the injection of urate crystals (0.2 ml of a 4% (w/v) suspension) into the intertarsal joints as described previously (Brune & Glatt, 1974). After various time intervals the joints were washed with 0.5 ml of a 1% sodium edetate solution, pH 7.4. The washes were centrifuged (3000 *g*, 20 min) at 4°C. PGD_2 was determined in the cell-free supernatants by a specific radioimmunoassay using an anti-PGD_2 antiplasma, the specificity of which has been described previously (Anhut, Peskar, Wachter, Gräbling & Peskar, 1978) and PGD_2 coupled covalently onto ^{125}I-bovine serum albumin (Anhut, Peskar & Bernauer, 1978) as tracer.

Levels of PGD_2 and PGE_2 from a typical experiment were also measured by gas chromatography-mass spectrometry. For this, deuterated PGE_2 and deuterated PGD_2 were added to the sample to serve as internal standard along with small amounts of radioactive analogues of these prostaglandins. Extraction, chromatography, derivatization and mass spectrometric analysis have previously been described (Frölich, 1976).

Results The results are given in the table. They clearly show the increase of all prostaglandins measured following the injection of urate crystals. All prostaglandins reached a maximum 2 h after urate injection. At all times assayed, PGD_2 was quantitatively the prevailing prostaglandin reaching a concentration up to 10 times higher than that of PGE_2. The mass spectrometric determinations showed that PGD_2 and PGE_2 levels were high and similar to those obtained by radioimmunoassay.

Discussion Our results show the presence of large amounts of PGD_2 in inflammatory exudates of the urate crystal-induced arthritis of chicken. The high levels of PGD_2 measured in joint exudate by radioimmunoassay have been confirmed by gas chromatographic-mass spectrometric analysis. This observation supports the view that PGD_2 is an important mediator of acute inflammation as suggested by Flower *et al.* (1976). These authors found that PGD_2 acted as a proinflammatory agent, like PGE_2 but that it was less potent. Since our results clearly demonstrate that the concentrations of PGD_2 in inflamed tissue might be much higher than those of PGE_2, $PGF_{2\alpha}$ and

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Table 1 Concentration of prostaglandins (ng/ml) in exudates of inflamed joints at different times after injection of urate crystals

	30 min	*60 min*	*120 min*	*180 min*
PGD_2	< 5.9	19.4 ± 6.6	148.8 ± 40.6 (164)	126.2 ± 38.7
PGE_2	< 1.6	9.3 ± 2.4	19.8 ± 2.0 (26)	10.2 ± 1.7
$PGF_{2\alpha}$	< 0.3	2.7 ± 0.5	5.5 ± 0.4	3.0 ± 0.3
TXB_2	< 0.3	1.0 ± 0.2	1.2 ± 0.2	0.4 ± 0.1

Means and s.e. mean of 8 or more experiments. The values in parentheses are from gas chromatography-mass spectrometry evaluation of one representative exudate.

TXB_2 (Glatt, Peskar & Brune, 1974; Peskar, Glatt, Anhut & Brune, 1978) we believe that the contribution of PGD_2 to the development of the symptoms of acute inflammation deserves further attention. This is especially so, because PGD_2 and PGE_2 differ in their effects on other mediators released in inflammation. For example, PGD_2 does not enhance the effect of bradykinin on vascular permeability (Flower *et al.*, 1976) as does PGE_2 but PGD_2 (Smith, Silver, Ingerman & Kocsis, 1974; Nishizawa, Miller, Gorman, Bundy, Svensson & Hamberg, 1975) unlike PGE_2 (Kloeze, 1969) is a powerful inhibitor of platelet aggregation in some species and may thus help to preserve microcirculation in the inflamed tissue. The relative amounts of PGD_2 and PGE_2 formed in inflammation may thus provide a mechanism by which the action of other mediators may be effectively modulated. For these reasons it would be interesting to know how the formation of PGD_2 can be regulated. The mechanism of PGD_2 formation in acute inflammation is unknown. However, it is known that PGD_2 can be formed by enzymatic or non-enzymatic isomerization from the prostaglandin endoperoxide PGH_2 (Nugteren & Hazelhof, 1973; Hamberg & Samuelsson, 1973; Abdel-Halim, Hamberg, Sjöquist & Anggard, 1977).

It has been shown (Hamberg & Fredholm, 1976) that serum albumin from a number of species can promote isomerization of PGH_2 to PGD_2. The presence of large amounts of PGD_2 in the inflammatory exudates might be taken as indication of the formation and probably also activity of large amounts of prostaglandin-endoperoxides. The possible pivotal role of PGG_2 in inflammatory processes has been repeatedly emphasized (see e.g. Kuehl, Humes, Egan, Ham, Beveridge & Van Arman, 1977). Our results showing large quantities of PGD_2 in the exudate of inflamed joints of the chicken suggest that PGD_2 in addition to other prostaglandins may contribute significantly to the development of inflammatory symptoms, at least in this specific type of inflammation.

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