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Endogenous biosynthesis of prostaglandin I_2 and thromboxane A_2 by isolated rat dental pulp

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Recent studies have revealed the heterogenous distribution of prostaglandin (PG) and thromboxane (TX) biosynthetic pathways in different tissues and cells. Dental pulp is a soft connective tissue encased in dentine and responsible for the formation and maintenance of dentine. PGE has been identified in homogenates of rat dental pulp [1]. Also PGE is released from isolated rat dental pulp into the incubation medium [1, 2], and from perfused tooth pulp of the dog by electrical stimulation of the dental nerves [3]. These releases of PGE are bloked by indomethacin, a cyclooxygenase inhibitor [4]. Although these findings suggest the presence of the cyclooxygenase pathway of arachidonic acid metabolism in the dental pulp, the PG and TX biosynthetic profile has been not determined. Attempts to measure biosynthetic capacity of pulp tissue homogenates by incubation with labelled precursor arachidonic acid showed no detectable conversion to PGs, probably due to exceedingly low capacity [1]. In the present study, therefore, the endogenous biosynthetic capacity of isolated dental pulp tissue to form PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 was investigated by radioimmunoassay.

Materials and methods

Materials. [^{3}H]PGE_2, [^{3}H]$PGF_{2\alpha}$ and [^{3}H]6-keto-$PGF_{1\alpha}$ were obtained from Radiochemical Center, Amersham, and [^{3}H]TXB_2 from New England Nuclear, Boston. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2 and anti-$PGF_{2\alpha}$ and anti-TXB_2 rabbit serum were gifts from Ono Pharmaceutical Co., Osaka. Anti-6-keto-$PGF_{1\alpha}$ rabbit serum was purchased from Seragen Inc., Boston, and anti-PGE_2 rabbit serum from Pasteur Institut, Paris. Indomethacin and tranylcypromine were obtained from Sigma Chemical Co., St. Louis, and thin layer silica gel plates (Kieselgel 60 F_{254}) from Merck, Darmstadt. Ionophore A23187 was a gift from Dr. R. Hamill, Eli Lilly and Co., Indianapolis, and OKY-046, (E)-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride, from Kissei Pharmaceutical Co., Nagano to Dr. Yusuo Endo.

Preparation and incubation of dental pulp. Adult male Wistar rats weighing 200–250 g were sacrificed by decapitation, and the mandibles and maxilla were excised. The dental pulp tissues were carefully removed in one piece from pulp cavities of incisors as described previously [2] and maintained in ice-cold physiological saline until use (30–60 min). In some expt., in order to remove blood the dental pulp was perfused *in situ* via carotid arteries with 4° Krebs–Henseleit bicarbonate buffer (pH 7.4) under pentobarbital anaesthesia. The isolated pulp tissues from one rat (approx 50 mg) were first preincubated for 30 min in 2 ml of Krebs buffer containing 1 mg/ml glucose, and then further incubated for 30 min in 2 ml of fresh Krebs buffer in the presence or the absence of test agents. When A23187 was used, dental pulp tissues were treated with A23187 during the preincubation period, and then incubated in fresh Krebs buffer not containing the ionophore. All incubations were carried out at 37° under a gas phase of 95% O_2 and 5% CO_2.

Extraction and assay of PGs and TXB_2. At the end of incubation, the medium was withdrawn, acidified with 0.5 M citric acid to pH 3.0–3.5, and extracted twice with 3 vol. of ethylacetate by collecting the organic phase by Pasteur pipette. The combined organic phase was evaporated to dryness under vacuum. Recoveries with this extraction procedure were constantly more than 90% as determined by expt. with tritium labelled PGs and TXB_2. Dried extract was redissolved in an appropriate volume of 0.1 M sodium phosphate buffer (pH 7.4) containing 0.9% (w/v) NaCl, 0.1% (w/v) NaN_3 and 0.1% (w/v) gelatin. Samples of 0.1 ml were subjected to radioimmunoassay (RIA) for PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2. These metabolites were also measured after further separation by TLC according to the method of Salmon (5). Briefly, an aliquot of

Table 1. Release of PGs and TXB_2 from isolated rat dental pulp*

Conditions	N	ng/g tissue (mean ± S.E.)			
		PGE_2	$PGF_{2\alpha}$	6-Keto-$PGF_{1\alpha}$	TXB_2
Without perfusion	6	6.4 ± 0.4	21.9 ± 1.9	97.4 ± 4.6	47.0 ± 5.1
		(5.4 ± 0.6)†	(25.0 ± 5.6)	(119.1 ± 14.0)	(41.1 ± 6.7)
With perfusion‡	6	6.5 ± 0.8	29.3 ± 4.9	107.0 ± 15.1	41.0 ± 4.0

* PGs and TXB_2 were determined by radioimmunoassay.

† Values in parentheses are those obtained by determination of same samples after further purification by TLC.

‡ Dental pulp was perfused *in situ* via carotid arteries with 4° Krebs buffer under pentobarbital anaesthesia.

incubation medium containing tritium labelled PGs and TXB_2 (approx 2,000 cpm, respectively) was acidified and extracted with ethylacetate. The extracts were applied to silica gel plates. The plates were developed in the organic layer of the mixture; ethylacetate: acetic acid: trimethyl pentane: water (110:20:50:100). The areas corresponding to standard PGs and TXB_2 were extracted with ethylacetate. The dried extracts were reconstituted to an appropriate volume of 0.1 M phosphate buffer to determine the PGs and TXB_2 contents and the recoveries.

RIA for PGE_2 was carried out as described previously (2). RIA methods for $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2 were essentially identical to that for PGE_2. The PGE_2 antibody cross-reacts 13.2% with 15-keto-PGE_2, 10.7% with PGE_1, 2.1% with dihydro PGE_2, 0.11% with $PGF_{2\alpha}$ and less than 0.01% with 6-keto-$PGF_{1\alpha}$ and TXB_2. The $PGF_{2\alpha}$ antibody cross-reacts 41.7% with $PGF_{1\alpha}$, 2.7% with PGE_2, 1.5% with 6-keto-$PGF_{1\alpha}$ and less than 0.2% with TXB_2. The 6-keto-$PGF_{1\alpha}$ antibody cross-reacts 10.7% with $PGF_{1\alpha}$, 2.3% with $PGF_{2\alpha}$, 1.5% with 6-keto-PGE_1, 0.4% with PGE_1 and less than 0.1% with PGE_2 and TXB_2. The TXB_2 antibody cross-reacts less than 0.15% with PGE_2, $PGF_{2\alpha}$ 6-keto-$PGF_{1\alpha}$ and others. The values were expressed as nanogram PGs and TXB_2 per gram wet tissue weight (ng/g tissue).

Statistical analysis was made by Student's *t*-test.

Results and discussion

When rat dental pulp was incubated in Krebs solution, PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) and TXB_2 (a stable metabolite of TXA_2) were released into the medium as measured by RIA. As shown in Table 1, 6-keto-$PGF_{1\alpha}$ was a predominant PG (about 56% of total), followed by TXB_2, $PGF_{2\alpha}$ and PGE_2 (24%, 16% and 4%, respectively). The values obtained by direct assay of the crude extracts from the incubation medium did not differ significantly from those obtained by determination of these same samples after further separation of PGs and TXB_2 by TLC. Further, a similar PG and TX profile was observed with the pulp tissue which was perfused *in situ* before its isolation. Therefore, in all subsequent expt. the dental pulp was excised without the perfusion, and the crude extracts from incubation medium were directly subjected to RIA.

PGs and TXB_2 in the medium during initial 30 min preincubation were also determined. The amounts of PGs and TXB_2 were 3–4 times higher than those during the subsequent 30 min incubation, although the PG and TX biosynthetic pattern did not differ between the two incubation periods (data not shown). This may be due to the traumatic stimuli during preparation of the tissue [6]. In the present study, in order to reduce the variable contribution of these stimuli, the PG and TX biosynthetic capacity was evaluated under standarized incubation conditions as described in Methods.

Figure 1 shows the effects of some drugs known to inhibit

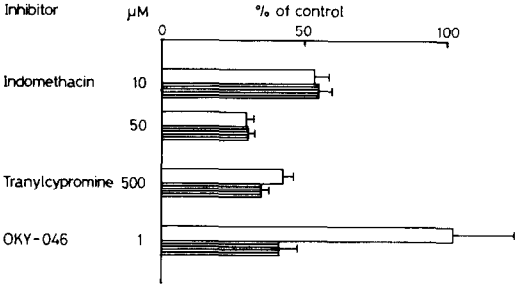

Fig. 1. Effects of some inhibitors on 6-keto-$PGF_{1\alpha}$ (open column) and TXB_2 (striped column) release from isolated rat dental pulp. After preincubation, the dental pulp was incubated for 30 min in 2 ml of Krebs buffer in the presence or the absence of inhibitors. 6-Keto-$PGF_{1\alpha}$ and TXB_2 in the incubation medium were determined by radioimmunoassay. Each column represents mean ± S.E. (N = 5).

PG and TX synthesis on 6-keto-$PGF_{1\alpha}$ and TXB_2 release from incubated rat dental pulp. Indomethacin, a cyclooxygenase inhibitor [4], dose-dependently decreased the release of these metabolites. Tranylcypromine has been reported to be a specific inhibitor of PGI_2 synthetase [7], and reduced the release of 6-keto-$PGF_{1\alpha}$ by 58% at 500 μM. However, it also inhibited TXB_2 release by 65%, suggesting the lack of specificity of the inhibitor as reported in MDCK cells in culture by Hassid [8]. OKY-046, reported to be a specific inhibitor of TX synthetase [9], inhibited TXB_2 release by 59% at 1 μM, but did not influence 6-keto-$PGF_{1\alpha}$ release. These results suggest that these immunoreactive products measured in our system are derived from endogenous arachidonic acid via PG endoperoxides. Since there are no detectable enzymatic degradations of PG in rat dental pulp [1], the release of these products may directly reflect these biosynthetic capacities. PGD_2 is also formed from the PG endoperoxides, but this is not measured in this study.

The abilities of divalent cation ionophore A23187 and norepinephrine to stimulate PG and TX production in rat dental pulp were also investigated. As shown in Table 2, the treatment with A23187 (10 μM) was found to significantly increase the production of both 6-keto-$PGF_{1\alpha}$ and TXB_2. Norepinephrine has been reported to stimulate PG and TX release from various tissues [6]. Norepinephrine at 0.1 mM also caused significant increases in 6-keto-$PGF_{1\alpha}$ and TXB_2 formation by the dental pulp. In either case, PGI_2 and TXA_2 measured as 6-keto-$PGF_{1\alpha}$ and TXB_2 were the major metabolites formed by the dental pulp.

The dental pulp is a highly specialized soft connective tissue and abundantly vascularized. Since the vascular endothelial and smooth muscle cells are a rich source of

Table 2. Effects of ionophore A23187 and norepinephrine on 6-Keto-$PGF_{1\alpha}$ and TXB_2 release from isolated rat dental pulp†

Incubation conditions	N	ng/g tissue (mean ± S.E.) 6-Keto-$PGF_{1\alpha}$	TXB_2
Control	8	103.7 ± 9.3	44.3 ± 4.5
Ionophore A23187 (10 μM)	6	223.7 ± 19.5*	117.6 ± 7.3*
Norepinephrine (0.1 mM)	6	147.4 ± 12.4‡	76.1 ± 4.7*

† 6-Keto-$PGF_{1\alpha}$ and TXB_2 were determined by radioimmunoassay.
* $P < 0.01$, ‡ $P < 0.05$.

PGI_2 [10, 11], the large amount of PGI_2 released from incubated dental pulp may be mainly vascular in origin. However, the origin of TXA_2 is unclear. The possibility that it derives from platelets [12] may be ruled out, since the perfusion *in situ* of dental pulp before its isolation does not alter the PG and TX profile (Table 1). Not only platelets but some types of fibroblasts [13, 14] produce mainly TXA_2. Dental pulp is a tissue containing not only fibroblasts and undifferentiated mesenchymal cells, but also specialized types of cells such as histiocytes and odontoblasts. Whatever the origin of TXA_2, our results suggest that TXA_2 is formed in dental pulp tissue. The production of TXA_2 has been also demonstrated in various tissues such as the lung [15], brain [16], heart [17] and kidney [18].

In summary, the present study demonstrated that the major metabolites of endogenous arachidonic acid formed via the cyclooxygenase pathway in the dental pulp are PGI_2 and TXA_2. However, the role of these metabolites in the dental pulp is still unclear. The demonstration of an active PGI_2 production, possibly mainly in the pulp vascular system, raises the important question as to its role in the regulation of the pulp microcirculation. PGs also may be involved in certain stages of heat-induced pulp inflammation [19]. Our studies provide a biochemical basis for further studies on the roles of PGs and TXA_2 in physiological functions or pathological conditions of the dental pulp.

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