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Cadmium-induced calcium release and prostaglandin E_2 production in neonatal mouse calvaria are dependent on cox-2 induction and protein kinase C activation

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Abstract The mechanisms by which cadmium (Cd) causes skeletal impairment have not been fully clarified. Release of calcium from neonatal mouse calvaria in organ culture is stimulated by submicromolar concentrations of Cd, an effect that is associated with increased production of prostaglandin E_2 (PGE_2). The prostaglandin-synthesising enzyme cyclooxygenase (cox) exists in two forms, one constitutive (cox-1) and the other inducible (cox-2). Cox-2 can be induced by mitogenic stimuli and inflammatory cytokines, such as parathyroid hormone (PTH), interleukin-1α and tumour necrosis factor-α. Cd potently activates protein kinase C (PKC), which in turn induces cox-2 production in several cell types. Our aim was to determine whether Cd-induced Ca release and PGE_2 production in neonatal mouse calvaria involve induction of cox-2 and, if so, to ascertain whether that effect is mediated by activation of PKC. Cd dose-dependently stimulated Ca release from cultured neonatal mouse calvaria, with a maximal effect at 0.4–0.8 μM. Different sensitivity was observed to Cd-induced Ca release between two breeds of mice suggesting that the susceptibility to Cd may be genetically determined. Dexamethasone (10 μM) added to the culture medium abolished the Ca releasing effect of Cd, an effect not overcome by addition of arachidonic acid (10 μM). The cox-2-selective inhibitors NS-398 and DFU and the less selective inhibitor meloxicam, potently impeded Cd-induced Ca release (IC_{50} of 1 nM, 41 nM and 7 nM, respectively) and calvarial production of PGE_2. Cd-induced and phorbol 12-myristate 13-acetate (PMA; 20 nM)-induced Ca release was inhibited by the PKC inhibitor calphostin C (0.5 μM) and by NS-398. The effects of PMA and Cd on Ca release were not additive, suggesting that both operated via the PKC pathway. We suggest that Cd-induced Ca release from neonatal mouse calvaria in culture depends on induction of cox-2 that occurs via the PKC signalling pathway.

Abbreviations *DFU* 5,5-Dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl) phenyl-2(5H)-furanone · *NS-398* N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide

Key words Cadmium · Cox-2 · Osteoporosis · Prostaglandin E_2 · Protein kinase C

Introduction

Several experimental and epidemiological studies have indicated that environmental and occupational exposure to cadmium (Cd) is an important risk factor for osteoporosis. The toxicological effect of cadmium was first observed in Japan, where dietary exposure was found to be involved in the aetiology of the Itai-Itai disease (Nogawa 1981). Subsequent reports suggested that skeletal impairment is induced by much lower levels of Cd than was previously assumed (Buchet et al. 1990; Staessen et al. 1991).

The physiological and biochemical mechanisms underlying the effects of Cd on bone are only partly understood. One of several proposed mechanisms (Kjellström 1992) is that Cd-induced osteoporosis is a direct effect of the metal on bone cells, which results in increased bone resorption; decreased bone formation has been noted in rats exposed to Cd (Iguchi and Sano 1982). The stimulatory effect of Cd on bone resorption has been observed in organ cultures of bone (Miyahara

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et al. 1992; Morita et al. 1990; Suzuki et al. 1989) and also after administration in vivo (Bhattacharyya et al. 1988; Wang and Bhattacharyya 1993; Wang et al. 1994). In one of the studies on cultured bone (Suzuki et al. 1989) this effect was associated with increased production of prostaglandin E_2 (PGE_2).

Inhibitors of prostaglandin (PG) synthesis (e.g. indomethacin and ibuprofen) and the protein synthesis inhibitor, cycloheximide, block Cd-induced Ca release from cultured neonatal mouse calvaria (Carlsson and Lundholm 1996). The rate-limiting step in PG synthesis is the enzyme prostaglandin endoperoxide G/H synthase or cyclooxygenase (cox), which converts arachidonic acid to PGs. Two isoforms of this enzyme have been described. Cox-1 is the constitutive 'house-keeping' enzyme, which is localised to the endoplasmic reticulum and present at relatively constant levels in almost all cell types; this isoform is responsible for maintaining several important physiological functions, including gastric cytoprotection, vascular homeostasis, platelet aggregation and renal function (Smith et al. 1994; Funk et al. 1991). Cox-2 is found in the nuclear envelope and is normally present only in the central nervous system, macula densa cells, and testes. Cox-2 can be induced in many cells by mitogenic stimuli and inflammatory cytokines. Cox-1 is not detectable by Northern blotting in human osteoblasts in culture, and under normal circumstances PG production in these cells is low (deBrum-Fernandes et al. 1994). PG production through cox-2 in osteoblasts can be induced by parathyroid hormone (PTH; Kawaguchi et al. 1994; Maciel et al. 1997), interleukin-1 (IL-1α; Akatsu et al. 1991), and tumour necrosis factor (deBrum-Fernandes et al. 1994).

Phorbol 12-myristate 13-acetate (PMA) is a potent activator of protein kinase C (PKC), and PKC induces cox-2 expression in many cell types (Herschman 1996). Cd is also a potent activator of PKC in several kinds of cell (Beyersmann et al. 1994; Bagchi et al. 1997), including the osteoblastic cell line ROS 17/2.8 (rat osteosarcoma cells; Long 1997a, b). A previous investigation in our laboratory (Carlsson and Lundholm 1996) revealed that Cd-induced Ca release from calvarial bones in culture involves stimulation of PGE_2 synthesis and is inhibited by cycloheximide. In the light of that finding, the present study was performed to determine whether Cd-induced Ca release from neonatal mouse calvaria involves induction of cox-2, and, if so, whether such induction is mediated by PKC.

Materials and methods

Reagents

Stock solutions of the following were used: interleukin-1-alpha, arachidonic acid, dexamethasone, calphostin C, PGE_2, $CdCl_2$, indomethacin, PMA, all from Sigma (Stockholm, Sweden); N-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulphonamide (NS-398) from Cayman Chemical (USA) and 5,5-dimethyl-3-(3-fluorophenyl)-4-(4-methylsulphonyl)phenyl-2(5H)-furanone (DFU), kindly provided by Merck Frosst (Canada). The solutions were prepared as follows: interleukin-1-alpha, 1 μg/ml, in sterile-filtered buffered saline containing 0.5% albumin [radioimmunoassay (RIA) grade, Sigma]; arachidonic acid (90% purity) 100 mM in 95% ethanol; dexamethasone and NS-398, 1 mM in 95% ethanol; DFU, 10 mM in 95% ethanol; calphostin C, 0.1 mM in dimethyl sulphoxide (DMSO); PMA, 0.1 mM in DMSO, stored at −80 °C; meloxicam (Mobic®, Boehringer Ingelheim), 10 mg/ml, stored at +4 °C; PGE_2, 0.01 mM in 95% ethanol; $CdCl_2$, 0.01 M in double-distilled water stored at −20 °C; indomethacin, 2 mM in 95% ethanol, stored at −20 °C; synthetic bovine parathyroid hormone (PTH 1-34; Bachem, Basle) dissolved in 1 mM HCl containing 0.1% albumin (bovine, RIA grade, Sigma) stored at −80 °C. CMRL 1066 culture medium was obtained as a ×10 stock solution was supplemented with 0.27 mM L-glutamine, 0.25 μM iron(III)nitrate, 0.57 mM ascorbic acid, 100 IU/ml penicillin and 100 μg/ml streptomycin; all constituents of the culture medium, except albumin (bovine), were from Gibco BRL (Stockholm, Sweden).

Bone resorption assay

The bone resorption assay was performed as described by Lerner (1987). New-born (1- to 3-day-old) mice of the BALBc strain, bred in our laboratory, were injected subcutaneously with 1.5 μCi $^{45}CaCl_2$ dissolved in 15 μl of sterile 0.9% NaCl. Four days later, the calvarial bones were carefully and aseptically dissected out, rinsed in Tyrode's solution, and divided into four pieces with a sharp scalpel (Ljunggren et al. 1991). To reduce the rate of basal bone resorption, and thus increase the sensitivity of the assay, the bone specimens were preincubated in petri dishes with 1 μM indomethacin (20 bones/10 ml of medium) for 18–24 h. The bones, still in the petri dishes, were then rinsed twice with 10 ml Tyrode's solution (prewarmed to +37 °C) and incubated for an additional 3 hours in fresh culture medium to remove all indomethacin. Subsequently, the bones were transferred to 24-well multidishes. The specimens were placed in separate wells, each containing 2 ml of culture medium and different drugs and incubated for an additional 48 h. After the 48-h incubation period, the bones were removed and placed in 1 ml of 6 M HCl for at least 4 h. A 5-ml portion of scintillation medium (Ultima Gold, Packard) was added to an aliquot (200 μl) of the medium and to an aliquot (100 μl) of the dissolved bones, and the samples were counted (with quench correction) for 5 min in a liquid scintillation counter (LKB Wallac). The release of calcium was calculated as follows: dpm (medium) × 100/dpm (medium) + dpm (bone); (dpm = disintegrations per minute).

PGE_2 assay

The content of PGE_2 in the incubation medium was determined by radioimmunoassay according to the protocol included in the ^{125}I-RIA kit (Dupont NEN). A 20-μl aliquot of the incubation medium was used for each determination.

Statistics

The data were analysed using Graph Pad Prism™ (GraphPad Software, San Diego, Calif., USA) and statistical evaluation of the data was done with Student's *t*-test for unpaired samples.

Results

Effects of Cd, PTH, IL-1α and PGE_2 on Ca release from cultured calvaria

Basal Ca release during a 48-h incubation period, without any stimulators or inhibitors in the culture medium,

was 15 ± 0.8%. Addition of Cd to the culture medium dose-dependently increased the release of Ca from the calvaria (Fig. 1). A significant effect was noted at a Cd concentration of 0.2 μM (21 ± 1.6%), and maximal effect was noted between 0.4 and 0.8 μM (27.7 ± 2.2 and 25 ± 3.1%, respectively). Higher Cd concentrations inhibited Ca release. Addition of PTH (10 nM), interleukin-1α (10 ng/ml) and PGE_2 (10 μM) to the incubation medium also stimulated Ca release (27.8 ± 1.7, 25.3 ± 1.4 and 28.4 ± 1.3%, respectively; Fig. 1).

Effects of dexamethasone and arachidonic acid on Cd-induced Ca release

Dexamethasone (10 μM) completely blocked Cd-induced Ca release (Fig. 2) but had no effect on basal Ca release. Addition of 10 μM arachidonic acid (AA) to the culture medium induced a slight but significant increase in Ca release (22.5 ± 1.1 versus 19.2 ± 0.5%), which was not enhanced by inclusion of 0.8 μM Cd plus 10 μM dexamethasone. The fact that this large excess of free AA failed to overcome inhibition of Cd-induced Ca release by dexamethasone indicates that dexamethasone prevents Ca release by blocking the expression of cox-2 and not by inhibition of phospholipase A_2, which releases AA from membrane phospholipids.

Effect of cox-2 inhibitors on Cd-induced Ca release

Two compounds that are claimed to be cox-2-selective inhibitors (NS-398 and DFU) and one less selective (meloxicam) were also tested regarding their effects on Cd-induced Ca release from calvarial bones. The results of these experiments are presented in Fig. 3A–C. All

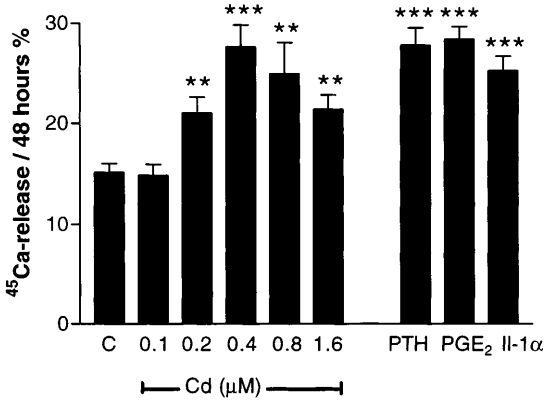

Fig. 1 Dose-response effect of Cd on Ca release from cultured neonatal mouse calvaria. Cd was present in the culture medium at the indicated concentrations (μM) during 48 h incubation. The effects of PTH (10^{-8} M), PGE_2 (10^{-5} M) and IL-1α (10 ng/ml) are also shown. Values represent means ±SEM, $n = 12$. Statistical significance: $^{**}P < 0.01$, $^{***}P < 0.001$. (*C* control · *PTH* parathyroid hormone · *IL-1α* interleukin-1 · *PGE_2* prostaglandin E_2)

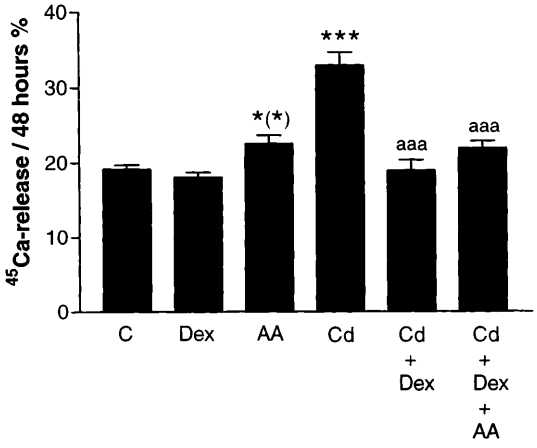

Fig. 2 Effects of dexamethasone (Dex, 10 μM), arachidonic acid (AA, 10 μM), Cd (0.8 μM) and combinations thereof on Ca-release from calvarial bones during 48 h incubation. Values represent means ±SEM, $n = 12$. Statistical significance compared to control: $^{*(*)}P < 0.02$, $^{***}P < 0.001$. Statistical significance compared to Cd-induced Ca release: $^{aaa}P < 0.001$

three inhibitors potently inhibited Cd-induced Ca release. The 50% inhibitory concentration (IC_{50}) was 1×10^{-9} M for NS-398, 4.1×10^{-8} M for DFU and 7×10^{-9} M for meloxicam.

Effects of dexamethasone and cox-2 inhibitors on Cd-induced PGE_2 production by calvarial bones

Basal PGE_2 production in unstimulated calvaria was very low at around the detection limit for the assay method (> 20 pg/ml incubation medium). The presence of Cd during the incubation period stimulated PGE_2 production (mean 132 pg/ml of incubation medium). Addition of dexamethasone (10 μM), meloxicam (50 nM), DFU (10 μM) or NS-398 (10 nM) significantly depressed this Cd-stimulated PGE_2 production, again to values below the detection limit for the assay.

Involvement of PKC in Cd-induced Ca release from calvarial bones

The presence of 20 nM PMA during the 48-h incubation significantly stimulated Ca release from the calvaria (control, 14.4 ± 0.4; PMA, 21.0 ± 1.2%). The PKC inhibitor calphostin C (0.5 μM) inhibited PMA-induced Ca release (to 12.7 ± 0.5%; Fig. 4). Cd-induced Ca release was also inhibited by 0.5 μM calphostin C (Cd, 24.3 Cd + calphostin C, 13.2 ± 0.7%). The effects of PMA and Cd on Ca release were not additive (PMA, 21 ± 1.2; Cd, 24.3 ± 1.0; PMA + Cd, 23.4 ± 1.3%). PMA-induced Ca release was inhibited by NS-398 (PMA, 21 ± 1.2; PMA + NS-398, 16.2 ± 1.0%). PTH-induced (10 nM) Ca release was totally inhibited by calphostin C (PTH, 33.4 ± 2.0; PTH + calphostin C, 15 ± 1.1% ; $P < 0.001$).

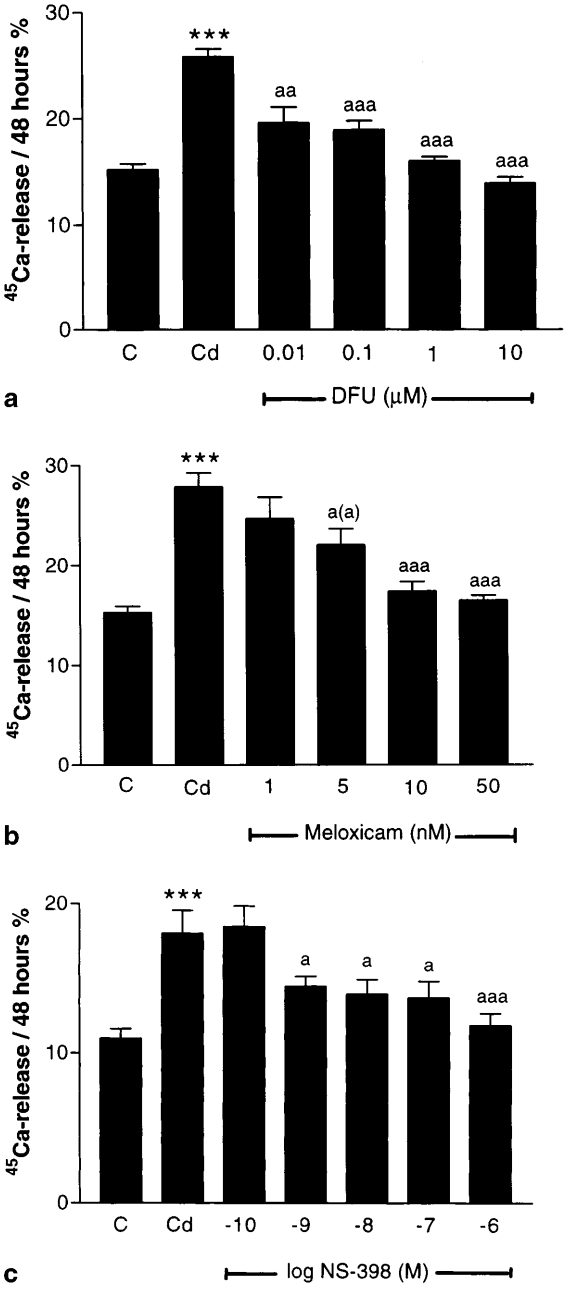

Fig. 3A–C Dose-response effects of: **A** DFU, **B** meloxicam, and **C** NS-398 on Cd-induced (0.8 μM) Ca release from calvarial bones during 48 h incubation. Basal Ca-release was not affected by the cox-2 inhibitors. Values represent means ±SEM, $n = 12–18$. Statistical significance compared to control: $^{***}P < 0.001$. Statistical significance compared to Cd-induced Ca release: $^{a}P < 0.05$, $^{a(a)}P < 0.02$, $^{aa}P < 0.01$, $^{aaa}P < 0.001$

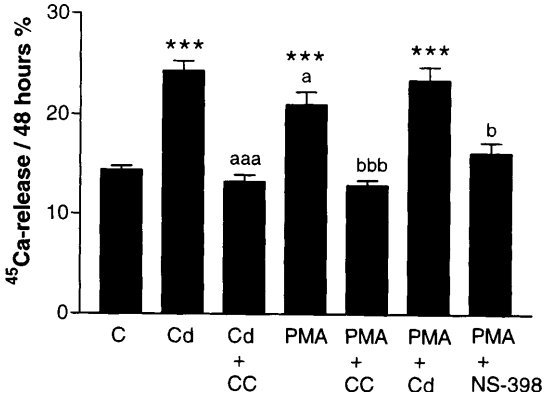

Fig. 4 Effects of cadmium (Cd, 0.8 μM) and phorbol 12-myristate 13-acetate (PMA, 20 nM), used separately, together and in combination with calphostin C (CC, 0.5 μM) or NS-398 (1 μM) on Ca release from calvarial bones during 48 h incubation. Values are means ±SEM, $n = 12–18$. Statistical significance compared to control: $^{***}P < 0.001$. Statistical significance compared to Cd-induced Ca release: $^{a}P < 0.05$, $^{aaa}P < 0.001$. Statistical significance compared to PMA-induced Ca-release: $^{b}P < 0.05$, $^{bbb}P < 0.001$

Discussion

Over the past few years, there has been a growing awareness that the current acceptable limits for cadmium exposure might be too high and that deleterious effects on the skeleton and the kidneys may occur at concentrations previously not recognized as harmful (Järup et al. 1998). Several mechanisms have been suggested to explain the effects of Cd on the skeleton (Kjellström 1992). Some investigators have concluded that the main effect of Cd is kidney damage (Itokawa et al. 1974, 1978; Kajikawa et al. 1981), and that effects on the skeleton are secondary and possibly due to increased leakage of calcium or decreased hydroxylation of 25-hydroxy vitamin D_3. Other researchers have pointed out that the major effect of Cd is impairment of bone formation, which may be due to destabilisation of collagen via inhibition of lysyl oxidase (Iguchi and Sano 1982) or collagen synthesis (Long 1997b). A third possibility is that Cd stimulates bone resorption, which is supported by data obtained in vitro and in vivo by Bhattacharyya et al. (1988), Wang and Bhattacharya (1993), and Wang et al. (1994).

Initially, we could not reproduce the potent Cd-induced Ca release from calvaria as previously observed (Carlsson and Lundholm 1996). We only observed a significant Cd-induced Ca release at the two highest Cd concentrations (control, 13.3 ± 0.6; 0.8 μM Cd, 18 ± 1; 1.6 μM Cd, 18.9 ± 1%; $P < 0.001$ for both). We found no Ca release at Cd concentrations between 0.1 and 0.4 μM. At that time, our breeding stock consisted mainly of mice purchased from a Swedish breeder (BK Universal) and were of the strain Balb/c BKl. When the whole breeding stock was replaced with Balb/c Abom animals from a Danish breeder (Bomholt), the potent Cd-induced Ca release presented in Figs. 1–4 and in Carlsson and Lundholm (1996) was again observed. We have not made a systematic comparision of the animals from the two breeders, hence we cannot explain the indicated difference. Ca release from cultured calvaria of the Swedish-bred mice could be induced by other stimuli, i.e. parathyroid hormone, interleukin-1α and PGE_2 to the same extent as in the Danish-bred mice. This finding raises several questions about genetically determined differences in the sensitivity to Cd-induced

skeletal effects. It is likely that sensitivity to the skeletal effects of Cd, also among humans, may vary for as yet unidentified genetic reasons. This means that some individuals may be more sensitive to Cd-induced skeletal damage than others and that exposure limits generally claimed to be safe may in fact be too high for these individuals.

In the present study, we confirmed the previous findings (Carlsson and Lundholm 1996) that submicromolar concentrations of Cd present in the culture medium during 48 h incubation significantly stimulated Ca release from the calvaria. We found that the maximal stimulatory effect occurred at Cd concentrations between 0.4 and 0.8 μM; higher Cd concentrations had an inhibitory effect. This indicates that Cd has multiple effects on bone metabolism and that higher concentrations of Cd may affect other cellular reactions, resulting in inhibition of Ca release. In the earlier study (Carlsson and Lundholm 1996), it was found that higher Cd concentrations suppressed tartrate-resistant acid phosphatase. This enzyme is assumed to be of importance for bone resorption by the osteoclasts (Minkin 1982).

Dexamethasone significantly inhibited Cd-induced Ca release in our experiments. It is known that dexamethasone inhibits the expression of cox-2 (Kawaguchi et al. 1994) and also decreases the activity of phospholipase A_2, which releases arachidonic acid from the cell membrane. We found that addition of exogenous arachidonic acid increased basal Ca release slightly but could not overcome the inhibitory effect of dexamethasone on Cd-induced Ca release. This indicates that dexamethasone suppressed Cd-induced Ca release by inhibiting cox-2 expression, and not by decreasing the availability of arachidonic acid through inhibition of phospholipase A_2. In other words, the Cd-induced Ca release was dependent on expression of cox-2. To verify the involvement of cox-2 in Cd-induced Ca release, we also determined the dose-response relationship for three different cox-2 inhibitors: meloxicam, NS-398 and DFU. All three compounds significantly inhibited Cd-induced Ca release when present in nanomolar concentrations in the culture medium. The IC_{50} values for inhibition of Cd-induced Ca release are in good accordance with data in the literature (Vane and Botting 1996; Pairet and Engelhardt 1996) and suggest that this inhibition was due to inhibition of cox-2.

Cd is also a potent activator of PKC in several cell types (Bagchi et al. 1997), including osteoblasts. Long (1997b) noted that a Cd concentration as low as 7.5×10^{-11} M induced half-maximal activation of PKC in rat osteosarcoma cells (ROS 17/2.8), whereas such activation was caused by Ca at a concentration of 3.6×10^{-7} M. Sato and co-workers (1996) observed that interleukin-1 stimulated osteoclast formation in mouse bone marrow cells via PGE_2 production and that the effect was abolished by cycloheximide, indicating that de novo protein synthesis is required for PGE_2 production. The same authors noted that IL-1-induced osteoclast formation was inhibited by both NS-398 and calphostin C, and the latter drug also suppressed IL-1-induced expression of cox-2 mRNA (Sato et al. 1996).

It has been suggested that the nuclear targeting of PKC induces phosphorylation of transcription factors, which are thus activated and can induce the expression of cox-2 (Vezza et al. 1996). Cox-2 catalyses the formation of PGE_2, which stimulates osteoclast formation; Ono et al. (1998) reported that in mouse bone marrow cells such formation is mediated through the EP4 subtype of PGE_2 receptors. The effects of PGE_2 on bone are concentration-dependent: low levels stimulate bone formation (Baylink et al. 1996) whereas high levels stimulate osteoclast formation and bone resorption (Raisz and Martin 1984).

We found that PTH-induced Ca release was also inhibited by calphostin C, in agreement with the findings of Sprague et al. (1996) showing that the bone-resorbing effect of PTH in cultured neonatal rat calvaria depend primarily on PKC activation. Moreover, PTH has been shown to induce cox-2 mRNA expression, cox-2 protein production, and PGE_2 synthesis in cultured neonatal mouse calvaria (Kawaguchi et al. 1994). In summary, our results indicate that Cd-induced Ca release from mouse calvaria in culture depends on activation of PKC by Cd. This induces the expression of cox-2, which catalyses the formation of PGE_2.

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References

Akatsu T, Takahashi N, Udagawa N, Imamura K, Yamaguchi A, Sato K, Nagata N, Suda T (1991) Role of prostaglandins in interleukin-1-induced bone resorption in mice in vitro. J Bone Miner Res 6: 183

Bagchi D, Bagchi M, Tang L, Stohs SJ (1997) Comparative in vitro and in vivo protein kinase C activation by selected pesticides and transition metal salts. Toxicol Lett 91: 31–37

Baylink TM, Mohan S, Fitzsimmons RJ, Baylink DJ (1996) Evaluation of signal transduction mechanisms for the mitogenic effects of prostaglandin E2 in normal human bone cells in vitro. J Bone Miner Res 11: 1413–1418

Beyersmann D, Block C, Malviya AN (1994) Effects of cadmium on nuclear protein kinase C. Environ Health Perspect 102: 177–180

Bhattacharyya MH, Whelton BD, Stern PH, Peterson DB (1988) Cadmium accelerates bone loss in ovariectomized mice and fetal rat limb bones in culture. Proc Natl Acad Sci USA 85: 8761–8765

Buchet JP, Lauwerys R, Roels H, Bernard A, Bruaux P, Claeys F, Ducoffre G, dePlaen P, Staessen J, Amery A (1990) Renal effects of cadmium body burden of the general population. Lancet 336 (8717): 699–702

Carlsson L, Lundholm CE (1996) Characterisation of the effects of cadmium on the release of calcium and on the activity of some enzymes from neonatal mouse calvaria in culture. Comp Biochem Physiol 115 C: 251–256

deBrum-Fernandes AJ, Laporte S, Heroux M, et al. (1994) Expression of PGH2 synthase-1 and PGH2 synthase-2 in human osteoblasts in culture. Biochem Biophys Res Commun 198: 955–960

Funk CD, Funk LB, Kennedy ME, Pong AS, Fitzgerald GA (1991) Human platelet/erythro-leukemia cell prostaglandin G/H synthase: cDNA cloning, expression and gene chromosomal assignment. FASEB J 5: 2304–2312

Herschman HR (1996) Prostaglandin synthase 2. Biochim Biophys Acta 1299: 1 125–140

Iguchi H, Sano S (1982) Effect of cadmium on the bone collagen metabolism of rat Toxicol Appl Pharmacol 62: 126–136

Itokawa Y, Abe T, Tabei R, Tanaka S (1974) Renal and skeletal lesions in experimental cadmium poisoning. Arch Environ Health 28: 149–154

Itokawa Y, Nishino K, Takashima M, Nakata T, Kaito H, Okamoto E, Daijo K, Kawamura J (1978) Renal and skeletal lesions in experimental cadmium poisoning of rats: histology and renal function. Environ Res 15: 206–217

Järup L, Berglund M, Elinder CG, Nordberg G, Vahter M (1998) Health effects of cadmium exposure – a review of the literature and a risk estimate. Scand J Work Environ Health 24 (Supp 1): 1–52

Kajikawa K, Nakanishi I, Kuroda K (1981) Morphological changes of the kidney and bone of rats in chronic cadmium poisoning. Exp Mol Pathol 34: 9–24

Kawaguchi H, Raisz LG, Voznesensky OS, Alander CB, Hakeda Y, Pilbeam CC (1994) Regulation of the two prostaglandin G/H synthases by parathyroid hormone, interleukin-1, cortisol and prostaglandin E2 in cultured neonatal mouse calvariae. Endocrinology 135: 1157–1164

Kjellström T (1992) Mechanism and epidemiology of bone effects of cadmium. In: Norberg GF, Herber RFM, Alessio L (eds) Cadmium in the human environment: toxicity and carcinogenicity. International Agency for Research on Cancer, Lyon, pp 301–310

Lerner UH (1987) Modifications of the mouse calvarial technique improve the responsiveness to stimulators of bone resorption. J Bone Miner Res 2: 375–383

Ljunggren Ö, Ransjö M, Lerner UH (1991) In vitro studies on bone resorption in neonatal mouse calvaria using a modified dissection technique giving four samples of bone from each calvaria. J Bone Miner Res 6: 543–550

Long GJ (1997a) Cadmium perturbs calcium homeostasis in rat osteosarcoma (ROS 17/2.8) cells; a possible role for protein kinase C. Toxicol Lett 91: 91–97

Long GJ (1997b) The effect of cadmium on cytosolic free calcium, protein kinase and collagen synthesis in rat osteosarcoma (ROS 17/2.8) cells. Toxicol Appl Pharmacol 143: 189–195

Maciel FMB, Sarrazin P, Morisset S, Lora M, Patry C, Dumais R, deBrum-Fernades A (1997) Induction of cyclooxygenase-2 by parathyroid hormone in human osteoblasts in culture. J Rheumatol 24: 2429–2435

Minkin C (1982) Bone acid phosphatase: tartrate-resistant acid phosphatase as a marker of osteoclast function. Calcif Tissue Int 34: 285–290

Miyahara T, Takata M, Mort-Uchi S, Miyata M, Nagai M, Sugure A, Matsusita M, Kozuka H, Kuze S (1992) Stimulative effects of cadmium on bone resorption in neonatal parietal bone cultures. Toxicology 73: 93–99

Morita I, Suzuki Y, Toriyama K, Murota S (1990) Induction of cyclooxygenase in osteoblasts and bone metabolism. Adv Prostaglandin Thromb Leukotriene Res 21: 839–842

Nogawa K (1981) Itai-Itai disease and follow-up studies. In: Nriagu JO (ed) Cadmium in the environment. Wiley, NewYork, pp 1–37

Ono K, Akatsu T, Murakami T, Nishikawa M, Yamamoto M, Kugai N, Motoyoshi K, Nagata N (1998) Important role of EP4, a subtype of prostaglandin (PG) E receptor, in osteoclast-like cell formation from mouse bone marrow cells induced by PGE2. J Endocrinol 158: R1–R5

Pairet M, Engelhardt G (1996) Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. Fundam Clin Pharmacol 10: 1–15

Raisz LG, Martin TJ (1984) Prostaglandins in bone and mineral metabolism. In: Peck WA (ed) Bone and mineral research, vol 2 Elsevier Science Publishers, Amsterdam, pp 286–310

Sato T, Morita I, Sakaguchi K, Nakahama K-I, Smith WL, Dewitt DL, Murota S-I (1996) Involvement of prostaglandin endoperoxide H synthase-2 in osteoclast-like cell formation induced by interleukin-1-beta. J Bone Miner Res 11: 392–400

Smith WL, Meade EA, DeWitt DL (1994) Interactions of PGH synthase isozymes-1 and -2 with NSAIDs. Ann NY Acad Sci 744: 50–57

Sprague SM, Popovtzer MM, Dranitzki-Elhalel M, Wald H (1996) Parathyroid hormone-induced calcium efflux from cultured bone is mediated by protein kinase C translocation. Am J Physiol 271: F1139–F1146

Staessen J, Amery A, Bernard A, Bruaux P, Buchet JP, Claeys F, dePlaen P, Ducoffre G, Fagard R, Lauwerys RR (1991) Effects of exposure to cadmium on calcium metabolism: a population study. Br J Ind Med 48: 710–714

Suzuki Y, Morita I, Yamane Y, Murota S (1989) Cadmium stimulates prostaglandin E_2 production and bone resorption in cultured mouse calvaria. Biochem Biophys Res Commun 158: 508–513

Vane JR. Botting RM (1996) Mechanism of action of anti-inflammatory drugs. Scand J Rheumatol 25 (Suppl 102): 9–21

Vezza R, Habib A, Li H, Lawson JA, Fitzgerald GA (1996) Regulation of cyclooxygenases by protein kinase C. J Biol Chem 271: 30028–30033

Wang C, Bhattacharyya MH (1993) Effect of cadmium on bone calcium and ^{45}Ca in nonpregnant mice on a calcium-deficient diet: evidence of direct effect of cadmium on bone. Toxicol Appl Pharmacol 120: 228–239

Wang C, Brown S, Bhattacharyya MH (1994) Effect of cadmium on bone calcium and ^{45}Ca in mouse dams on a calcium-deficient diet: evidence of Itai-Itai syndrome. Toxicol Appl Pharmacol 127: 320–330