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Effects of Methylmercury and Trimethyltin on Cardiac, Platelet, and Aorta Eicosanoid Biosynthesis and Platelet Serotonin Release

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ALLY, A., R. BUIST, P. MILLS AND K. REUHL. *Effects of methylmercury and trimethyltin on cardiac, platelet, and aorta eicosanoid biosynthesis and platelet serotonin release.* PHARMACOL BIOCHEM BEHAV 44(3) 555–563, 1993.– The effects of two organometals on heart, platelet, and aorta prostaglandin biosynthesis were examined in vitro. Methylmercuric chloride (MMC, 39–796 nM) increased the biosynthesis of thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) in the heart and stimulated the biosynthesis of PGI_2 in incubates of aorta rings. The aorta biosynthesis of PGI_2 was monitored by its inhibition of platelet aggregation and serotonin [5-hydroxytryptamine (5-HT)] secretion, while the metabolites 6-keto-$PGF1_\alpha$ and TxB_2 were quantified by radioimmunoassay. In platelet experiments, low concentrations of MMC (5 μM) enhanced aggregation to adenosine diphosphate (ADP) and at high concentrations MMC (50–100 μM) directly stimulated aggregation and 5-HT secretion. These effects of MMC were inhibited by nonsteroidal antiinflammatory drug, thromboxane synthetase, and phospholipase A_2 inhibitors. Trimethyltin (TMT), another highly toxic organometal, had no affect on prostaglandin biosynthesis in either heart or aorta incubates. TMT did not increase platelet aggregation responses to ADP, nor did it directly trigger aggregation. At moderate concentrations, TMT (20–45 μM) slightly depressed ADP aggregation; however, this was paradoxically associated with increased 5-HT secretion. In platelets pretreated with either NDGA or ASA, TMT in the absence of aggregation enhanced 5-HT secretion in response to ADP. TMT, unlike MMC, did not stimulate platelet TxB_2 biosynthesis from exogenous [^{3}H]arachidonic acid, whereas MMC stimulates heart, vascular, and platelet eicosanoid biosynthesis. TMT, unlike MMC, does not directly activate the arachidonic acid cascade.

Methylmercury Trimethyltin PGs 5-HT

BECAUSE of their widespread industrial use, organometallic compounds pose environmental risks that impact on human health. Methylmercury (MMC), in particular, is a persistent poison originating from the methylation of inorganic mercury and bioaccumulation in the food chain (14). The ingestion of foodstuffs containing this material has resulted in a variety of neurological, visual, and neuromuscular symptoms (8,10,18) and a high death rate in humans (8,35).

The effects of occupational exposure to organic mercurial compounds have been documented over 35 years ago (19). While much is known of the clinical signs following the outbreaks of MMC poisoning in Japan (26), Iraq (8), and Canada (18), the precise mechanism(s) of toxicity is still unknown. MMC has been shown to alter blood physiology (31) and change brain lipid profile (34) and is associated with an elevated incidence of human hypertension (55.4% at age 40 years) in MMC-polluted areas (15). The suggestion that lipid peroxidation may be responsible for MMC toxicity has been made (17), and indeed vitamin E, which inhibits lipid peroxidation, has been shown to reduce methylmercury toxicity (37,38).

Organotin compounds have a wide range of biologic effects and are therefore used in a variety of applications. As a result, the potential health impact of these classes of compounds are of concern. It has been reported that organotins cause hemolysis, affect platelet aggregation (28) and 5-hydroxytryptamine (5-HT) uptake and release (21), and lower ATP concentrations in hepatocytes (1,2). MMC and trimethyltin (TMT) are similar in being lipid-soluble alkylmethyl cations that could influence heart, vascular, and platelet function through changes in eico-

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sanoid biosynthesis. Platelets exhibit excitation-contraction (secretion) responses and are a partial model for vascular smooth muscle cells [for review, see (32)]. In addition, a remarkably good correlation between platelets and brain synaptosomes has been confirmed for the uptake of 5-HT. As a result platelets are used as a peripheral model for central neurons in transmitter uptake studies (9). The present study was undertaken to determine the effects of MMC and TMT on cardiac, platelet and vascular eicosanoid biosynthesis.

METHOD

Isolated perfused heart

Male Sprague-Dawley specific pathogen-free rats (200–250 g) (NRC, Ontario, Canada) were anesthetized with pentobarbital (40 mg/kg), a midline thoracotomy made, and the heart rapidly removed to a beaker containing ice-cold Krebs-Henseleit (K-H) buffer (to arrest contractility). The aorta was cannulated for retrograde perfusion at a constant flow rate of 8 ml/min with K-H buffer containing glucose as described in (23). The K-H buffer was continuously gassed with 95% O_2/5% CO_2, maintaining a pH of 7.4 at 37°C. Ventricular mechanical activity was monitored with a strain gauge (Grass FT03, Grass Instruments, Quincy, MA) at a resting tension of 3 g and recorded on a Grass Model 7 polygraph. The coronary effluent was collected in chilled tubes and stored at −70°C until assayed by radioimmunoassay (RIA) for 6-keto-PGF1$_a$ and TxB_2 using RIA kits obtained from New England Nuclear (Mississauga, Ontario).

Platelet Preparation

Platelets were prepared from rat blood obtained under anesthesia in 0.1 vol acid-citrate-dextrose solution (2.2 g sodium citrate, 0.8 g citric acid, 2.2 g D-glucose in 100 ml H_2O). Platelet-rich plasma (PRP) was separated by centrifugation at 200× *g* for 10 min. After adjusting the volume to give 10^8 platelets/ml, the PRP was equilibrated for 30 min, mixed gently, and divided into aliquots.

Aorta Rings

The abdominal aorta was dissected free of adventitia, washed, and transferred to cold K-H buffer. Aorta rings were prepared and stored in cold buffer until required by methods similar to those previously described (12). For in vitro PGI_2 biosynthesis, rings were preincubated with K-H buffer alone or buffer plus MMC or TMT in an ice bath for 20 min, the incubation fluid replaced with new buffer, and then transferred to a 37°C water bath and incubated for an additional 5 min. A 10-μl aliquot of the tissue incubation buffer was immediately added to a cuvette containing PRP, and adenosine diphosphate (ADP) aggregation determined after equilibrating for 1 min. In this bioassay, 1×10^{-8} M PGI_2 inhibits platelet aggregation by 61.2 ± 15%.

Platelet Experiments

Aggregation studies were performed using a single-channel agrometer (Chrono-Log Corp., Havertown, PA). Four-hundred and 50 microliters of control or pretreated PRB were preincubated at 37°C. After 1 min, the activating agent was added and the change in optical density recorded on a strip chart recorder.

In experiments in which TxB2 biosynthesis was deter-

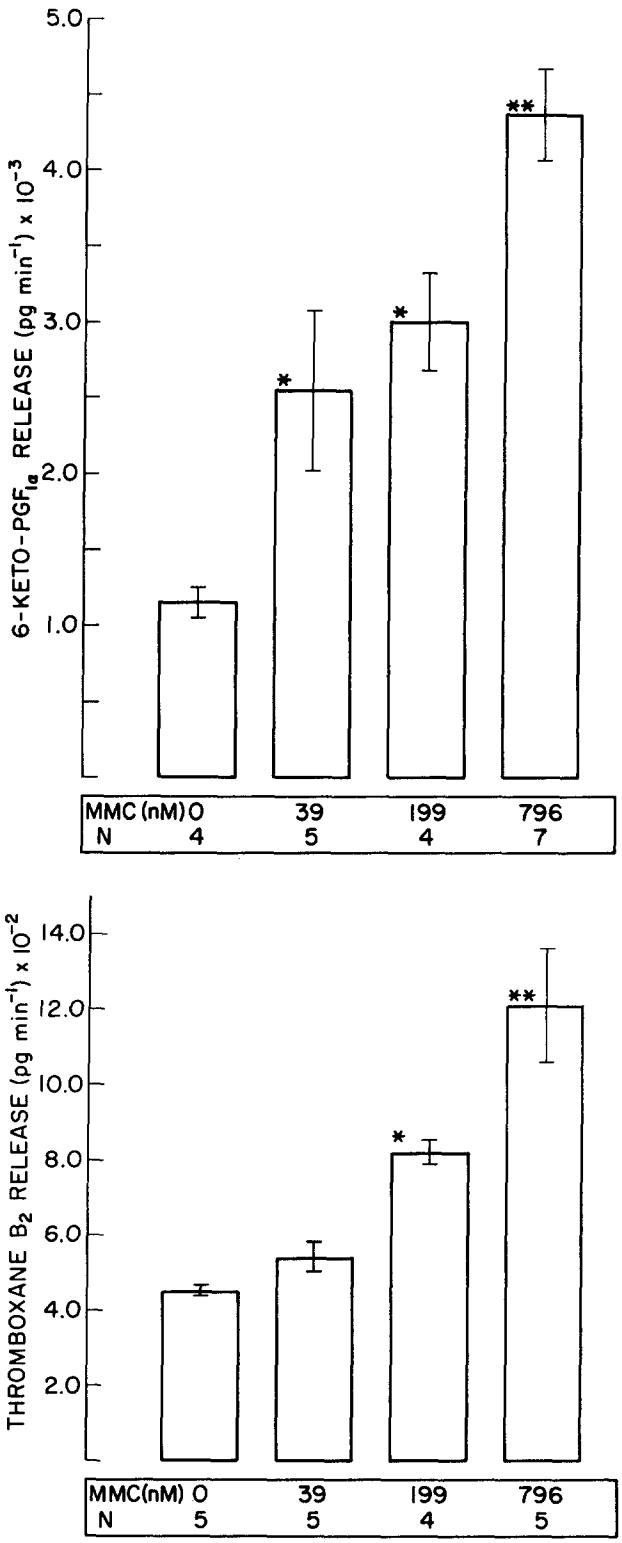

FIG. 1. Changes in coronary fluid PGI_2 (6-keto-PGF1$_a$) and TxA_2 after 25-min perfusion with buffer or methylmercury chloride (MMC) solution. Coronary effluent collected from 25–30 min. The number of preparations and MMC concentrations are shown. All values are mean ± SD for four to seven preparations.

mined, 1 μCi [^{3}H]arachidonic acid diluted with nonlabeled compound in a final concentration of 50 μM was incubated with PRP during the aggregation. Incubates were centrifuged at 4,000× g for 10 min and the supernatants acidified to pH 4 with 1 M citric acid and extracted with 3 × 3-ml ethylacetate. The organic phases were pooled, dehydrated with anhydrous $MgSO_4$, and evaporated under a stream of dry nitrogen. The residue was resolubilized in 100 μl ethylacetate and stored at −70°C until analyzed.

Prostaglandin Separation

High-performance liquid chromotography (HPLC) separation of the arachidonic acid metabolites was by reverse-phase

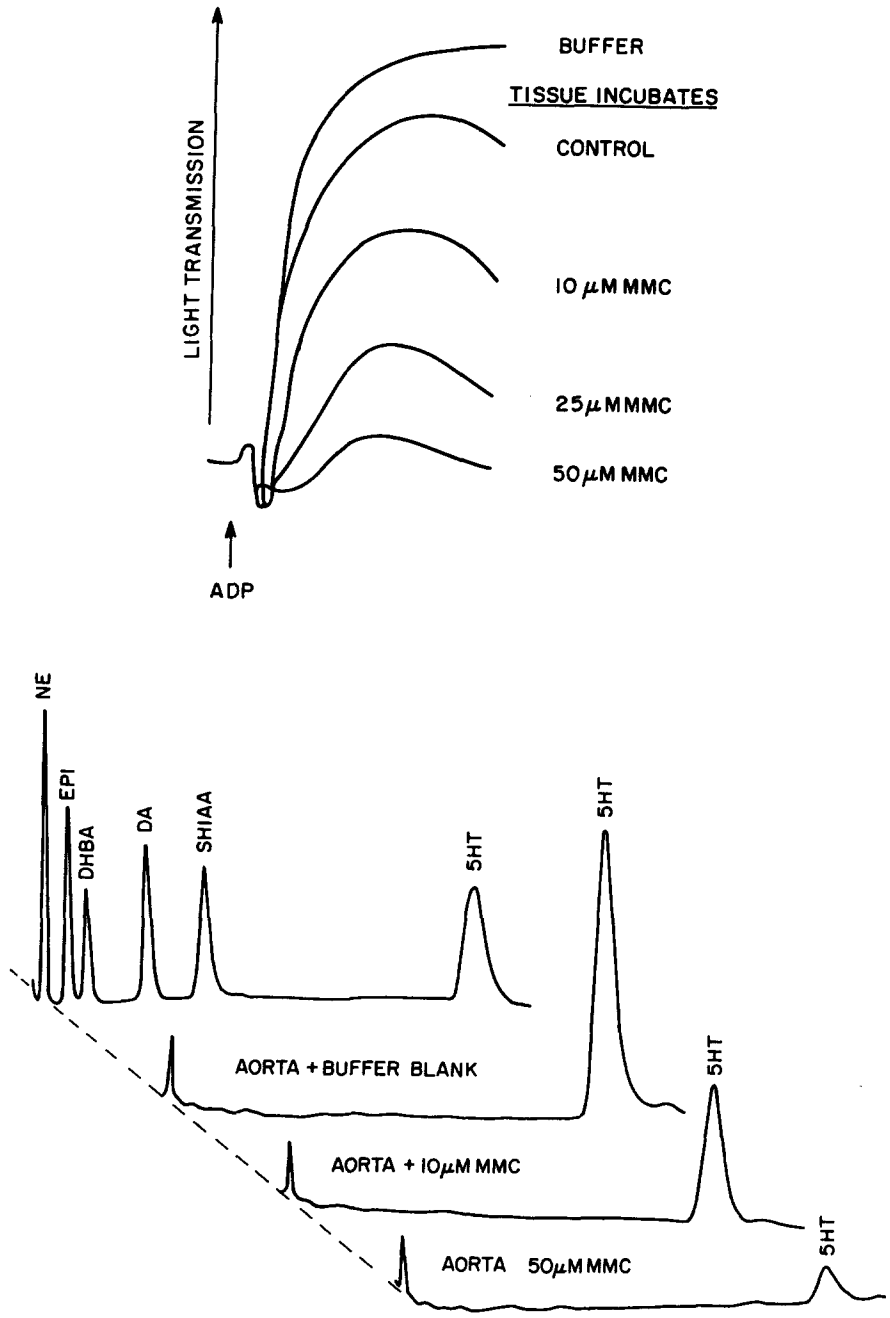

FIG. 2. (A). Bioassay of PGI_2 production by aorta rings preincubated with methylmercury chloride (MMC). Inhibition of adenosine diphosphate (ADP)-induced platelet aggregation indicating increased biosynthesis of this inhibitory eicosanoid. (B). Determination of MMC-induced PGI_2 inhibition of platelet aggregation and 5-hydroxytryptamine (5-HT) secretion (total 5-HT content 92.2 ng/ml incubate).

chromatography as previously described (4) using a modular chromatograph (Waters Chromatography Division, Toronto, Ontario, Canada).

5-HT Analysis

5-HT was analyzed by direct injection of the filtered PRP supernatant (8,000× *g*, 1 min) upon completion of the aggregation test. 5-HT analysis was by isocratic ion-pair HPLC with electrochemical detection as previously described (6).

Malondialdehyde Assay

To PRP at $T = 0$ min MMC or TMT was added and mixed. Samples were centrifuged 4 min after addition of either MMC or TMT and 1 ml PRP supernatant was mixed with 0.4 ml 40% TCA in 1 N HCL and the proteins precipitated. One milliliter of this supernatant (0.714 ml original volume) was mixed with 0.2 ml 0.1 M sodium-2-thiobarbituric acid and incubated at 80°C for 20 min. The optical density (OD) was determined in an HP 8451 spectrometer (Hewlett-Packard Instruments, Toronto, Ontario, Canada) at 507, 532, and 557 nm. The mean value of OD 507 and 557 was subtracted from OD 532 to give the absorbance of the chromophore (27). A standard curve was prepared by hydrolyzing 1 mmol tetrahydroxypropane (Aldrich, Milwaukee, WI) in 0.5 l 0.05 N HCL at 50°C for 60 min. This solution was brought up to 1 l volume and stored in amber bottles at 4°C in the dark for no more than 1 month.

Electron Microscopy

Platelets were fixed for transmission electron microscopy (EM) after agrometry using a modification of the procedure in (24). In brief, an equal volume of 0.1 M phosphate buffer, pH 7.4, containing 0.05% glutaraldehyde and PRP were mixed and the platelets allowed to settle overnight (1× *g*, 8–12 h). The bulk of the plasma was aspirated and the platelets resuspended gently in 0.1 M phosphate buffer. The mixture was centrifuged (1,000× *g*, 10 min), the supernatant removed, and replaced with 4% glutaraldehyde in 0.1 M phosphate buffer. Platelets were incubated for an additional 1 h in a refrigerator and the pellets processed as described in (6) for EM. Ultrathin sections (65–70 nm) cut with an LKB ultramicrotome II, were stained with uranyl acetate and lead citrate and examined with a Zeiss 10 C electron microscope (33).

Data Analysis

Values are reported as mean ± SD. Nonpaired two-sided Students *t*-test was used for comparison of mean values. Differences were considered significant for $p < 0.05$.

TABLE 1

RELEASE OF PROSTAGLANDINS BY INCUBATES OF AORTA RINGS IN THE PRESENCE OF MMC AND TMT

	pg/min/mg/Aorta	
Treatment	6-keto-PGF1$_1$	TxB$_2$
Spontaneous release	1.78 ± 0.46	0.22 ± 0.17
MMC (50 μM)	14.22 ± 2.22*	1.38 ± 0.19*
TMT (50 μM)	2.16 ± 0.47	0.22 ± 0.12

Mean ± SD, n = 4–5 experiments with duplicate determination.
*$p < 0.001$ (*t*-test).

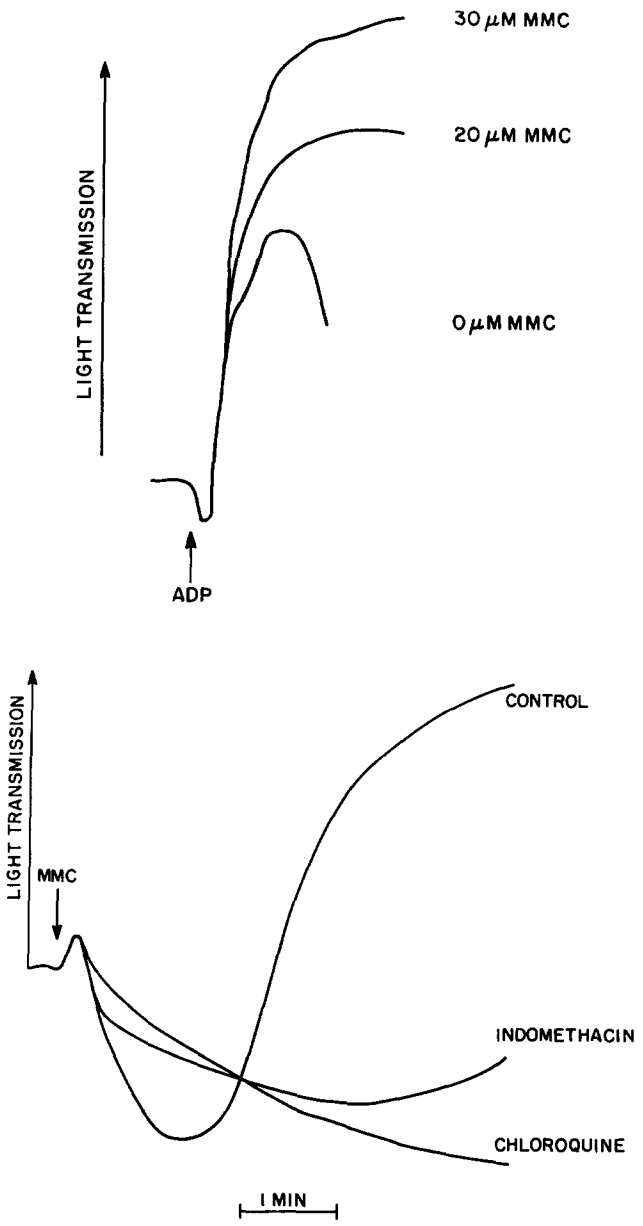

FIG. 3. (A). Enhancement of adenosine diphosphate (ADP) platelet aggregation by low concentrations of methylmercury chloride (MMC). Note the conversion of the reversible platelet aggregation (0 μM MMC) to irreversible aggregation by 20–30 μM MMC. Higher concentrations of MMC can directly trigger platelet aggregation. This is shown in (B) in response to 50 μM MMC (25 nM/0.5 ml platelet-rich plasma). The aggregation is blocked by the phospholipase A_2 inhibitor chloroquine and the arachidonate cyclooxygenase inhibitor indomethacin.

RESULTS

MMC perfused through the isolated heart increased the biosynthesis of PGI_2 (300%) and TxA_2 (200%) measured as immunoreactive 6-keto-PGF1$_a$ and TxB_2 (Figs. 1a and 1b). Note that the effect of MMC is concentration dependent and does not appear to be an all-or-none response. In contrast,

TMT perfused through the heart had no effect on either 6-keto-$PGF1_a$ or TxB_2 biosynthesis.

The effect of MMC on blood vessel prostaglandin biosynthesis was evaluated using a platelet bioassay to detect the generation of biologically active PGI_2. MMC stimulated PGI_2 release from aortic rings in a concentration-dependent manner, inhibiting ADP-triggered platelet aggregation (Fig. 2). Preincubation of aortic rings with indomethacin or chloroquine blocked the biosynthesis of this inhibitory prostaglandin by 96 ± 1.4%. Similarly, infusion of chloroquine through the heart reduced the release of immunoreactive prostaglandins by more than 90%.

MMC 50 μM stimulated the release of sufficient PGI_2 to inhibit both platelet aggregation and 5-HT release by 80–90% (Fig. 2). The biosynthesis of PGI_2 and TxB_2 by aorta rings is shown in Table 1. MMC but not TMT increased the biosynthesis of both prostaglandins above the basal rate. In two determinations using higher concentrations of TMT (200 μM), no stimulation of prostaglandin synthesis was detected.

The addition of low concentrations of MMC to platelet incubates exposed to submaximal amounts of ADP resulted in the transformation of the reversible aggregation into an irreversible aggregation (Fig. 3A). TMT in similar experiments did not enhance platelet aggregation.

Higher concentrations of MMC, in the absence of ADP, did themselves trigger platelet aggregation. This can be seen

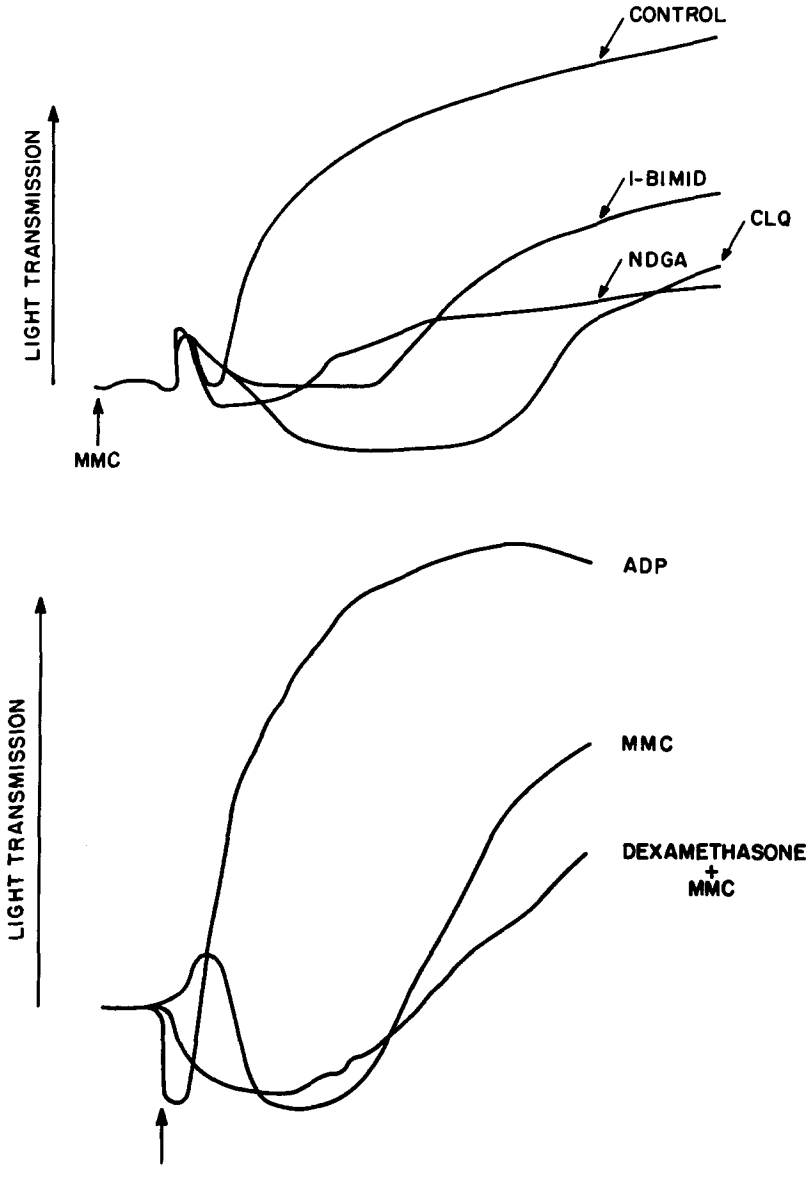

FIG. 4. Inhibition of methylmercury chloride (MMC)-induced platelet aggregation by compounds that block various steps in prostaglandin biosynthesis. (A). Thromboxane A_2 synthetase inhibition (1-benzylimidazole); inhibition of the cyclooxygenase and lipoxygenase pathways [nordihydroguaiaretic acid (NDGA)]; inhibition of phospholipase A_2 (chloroquine).

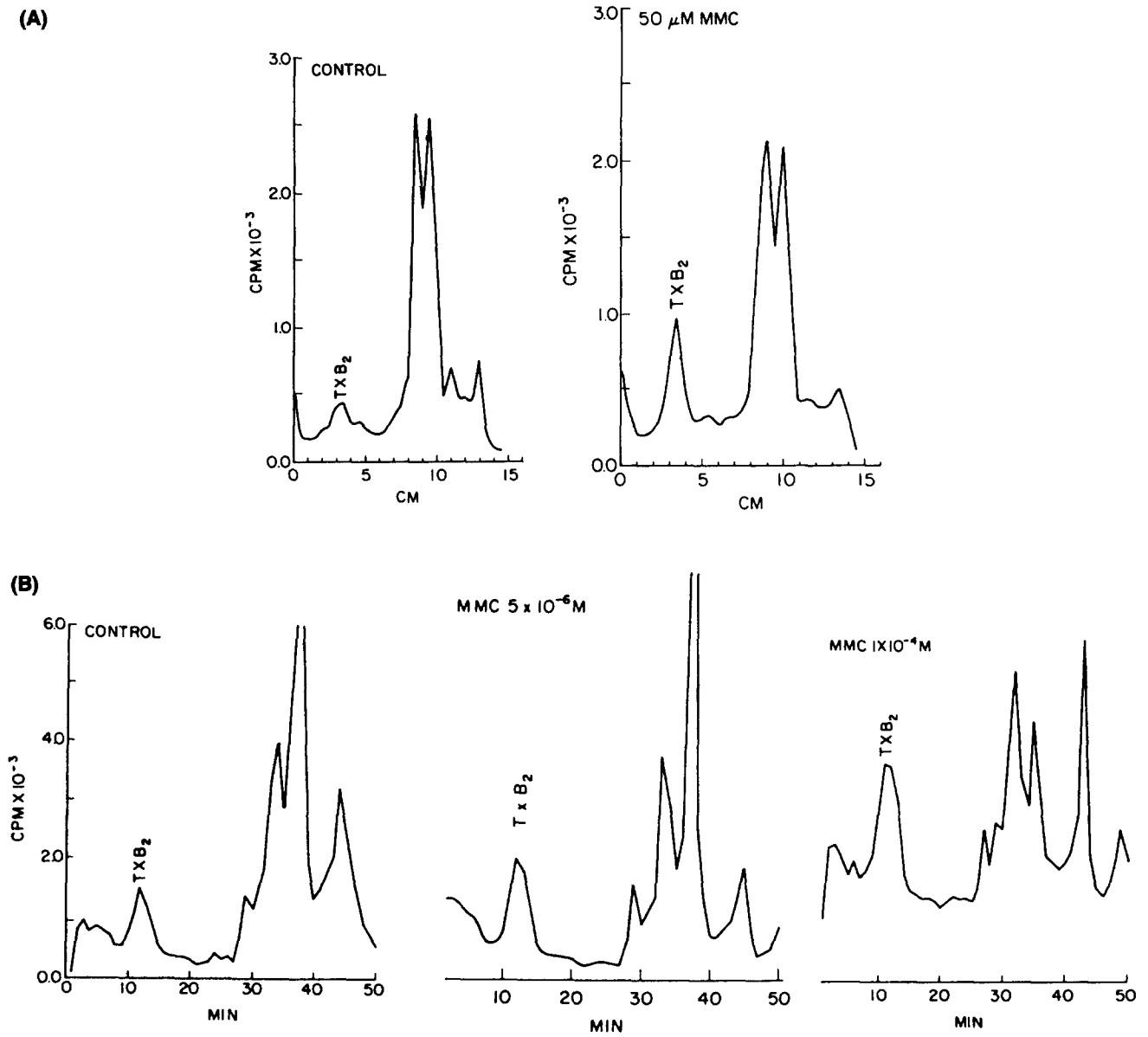

FIG. 5. Effects of methylmercury chloride (MMC) on platelet thromboxane biosynthesis in response to adenosine diphosphate (ADP) stimulation. Platelets were preincubated at room temperature with MMC and transferred to the aggrometer (37δC). Radiolabeled arachidonic acid was added and after 1 min aggregation triggered with ADP. Separation of the metabolites by TLC with liquid scintillation counting of 0.5-cm zones of the TLC plate (A) and the high-performance liquid chromotagraphy radio chromatographs of samples from similar experiments (B).

in Fig. 3B after a 10- to 20-s delay following the addition of MMC platelets irreversibly aggregated. This effect was prevented by pretreatment with either a phospholipase A_2 inhibitor or a cyclooxygenase inhibitor (Fig. 3B); these platelets displayed a decrease in light transmission (trace goes below baseline). Similar results were obtained using a number of structurally unrelated compounds that inhibited different steps in the biosynthesis of platelet TxB2 (Fig. 4). NDGA, a dual lipoxygenase and cyclooxygenase inhibitor, prevented MMC-triggered platelet aggregation, as did 1-benzylimidazole, a thromboxane synthetase inhibitor. Phospholipase inhibition with either chloroquine or dexamethasone was also effective in blocking MMC aggregation (Fig 4).

MMC at concentrations up to 1×10^{-4} M stimulated platelet TxB_2 biosynthesis by some 170% above that produced with ADP-triggered aggregation (Fig. 5). The effect of MMC and TMT on platelet polyunsaturated fatty acid metabolism is shown in Table 2. MMC stimulated malondialdehyde (MDA) biosynthesis, an effect blocked by indomethacin. In contrast, TMT did not stimulate MDA generation (Table 2). MMC did not increase either MDA synthesis (Table 2) or aggregation (Fig. 3B) in platelets pretreated with indomethacin. Similar results were obtained using platelets pretreated with either ASA or NDGA (cyclooxygenase inhibitors).

TMT by itself did not trigger platelet aggregation. It did, however, partially inhibit ADP-stimulated platelet aggrega-

TABLE 2

PRODUCTION OF MALONDIALDEHYDE (μM) BY PLATELETS EXPOSED TO MMC AND TMT

Treatment	MDA (μM)	
	Control	+5 μM INDO
Spontaneous release	0.142 ± 0.06	0.142 ± 0.08
MMC (50 μM)	1.415 ± 0.55*	0.133 ± 0.05
TMT (50 μM)	0.132 ± 0.08	0.156 ± 0.05

Mean ± SD, n = 5–6 experiments with duplicate determination.
*$p < 0.001$ (t-test).

tion (Fig. 6A) while paradoxically stimulating 5-HT secretion (Fig. 6A). In a dose-response experiment in which TMT was preincubated with platelets for 30 min prior to ADP challenge, it was found that TMT could increase 5-HT secretion in response to ADP stimulation by up to 700% of control (above that secreted in its absence) without influencing platelet aggregation or 5-HT secretion in response to either thrombin or collagen. In the absence of added ADP, MMC but not TMT stimulates platelet 5-HT secretion (Table 3). Cyclooxygenase inhibition with indomethacin prevented MMC (100 μM) -stimulated platelet 5-HT secretion. In contrast, TMT (up to 100 μM) had no effect on 5-HT secretion under these conditions.

EM examination of platelet aggregates showed that TMT-pretreated platelets had fewer dense granules after aggregation when compared to those stimulated with ADP alone (Fig. 6). These platelets maintain intact membranes showing the absence of nonspecific lysis.

DISCUSSION

Enhanced 6-keto-PGF1$_a$ and TxB_2 release from perfused rat hearts exposed to MMC was determined by RIA (Fig. 1). This effect on prostaglandin biosynthesis was not specific to PGI_2 because TxB_2 was similarly enhanced, arguing against selective stimulation of prostacyclin synthetase (Fig. 1). MMC also increased the biosynthesis of prostaglandins in aorta rings (Fig. 2, Table 1) and platelets (Fig. 5). Therefore, MMC stimulates biosynthesis in three major components of the cardiovascular system—heart, blood vessels, and platelets. In contrast, TMT had no similar effect on prostaglandin biosynthesis in either heart, blood vessels, or platelets.

This effect of MMC was dose related (Figs. 1 and 2). The selective inhibition of platelet aggregation following the addition of MMC plus aorta incubation buffer to platelet incubates indicated that the bioactive product was most likely PGI_2 (12). Further evidence in support of this was that both indomethacin (10 μM) and tranylcypromine (100 μM, a prostacyclin synthetase inhibitor) blocked the production of this material. Incubation buffer from aortic rings pretreated with indomethacin or tranylcypromine had only a minor inhibitory effect on ADP-triggered platelet aggregation.

We have previously shown that MMC inhibits platelet 12-lipoxygenase (5). Whether MMC inhibits heart or vascular lipoxygenase(s) has not been determined. Such an inhibition if it occurs will in principle divert free arachidonic acid to the cyclooxygenase enzyme complex, further enhancing prostaglandin biosynthesis.

The interpretation of MMC effects on endothelial PGI_2

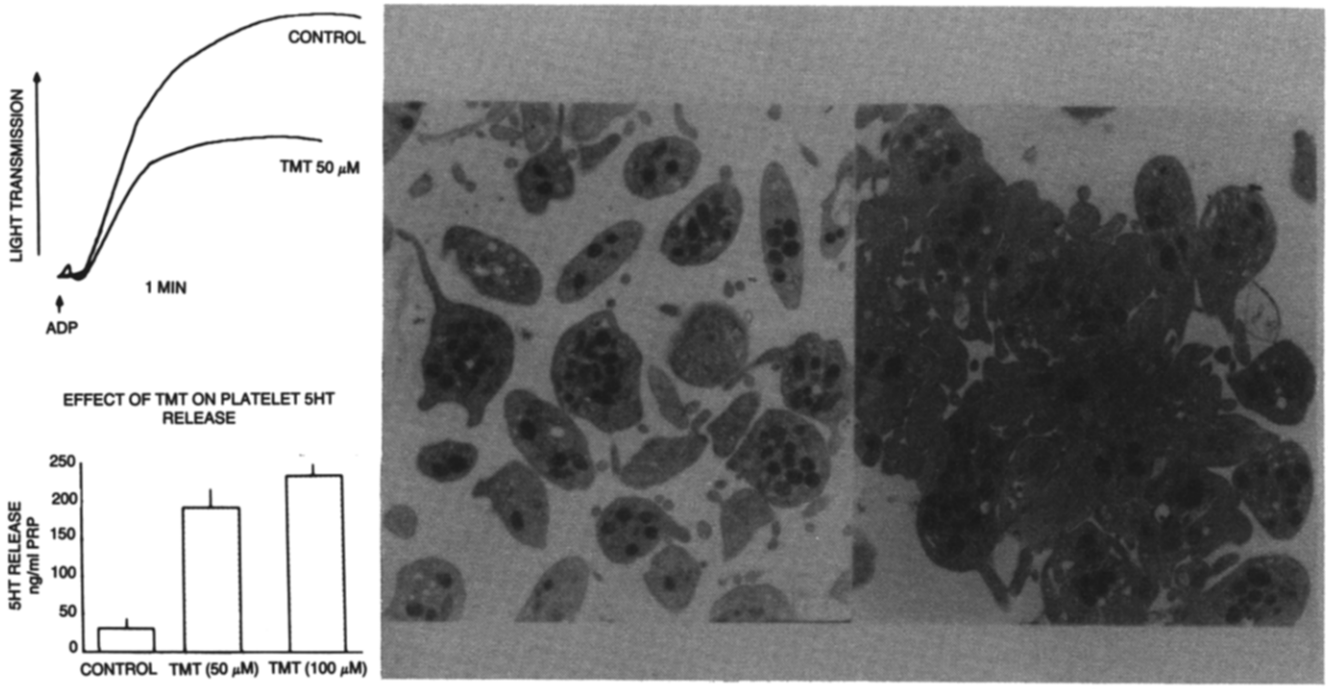

FIG. 6. Effect of trimethyltin (TMT) on platelet aggregation. TMT produces a reduction of adenosine disphosphate (ADP) aggregation (A), which was associated with increased 5-hydroxytryptamine (5-HT) secretion (B). Electron micrographs show that control untreated platelets have denser aggregates (more platelets clumped together) yet retain many dense granules; in contrast, TMT-treated platelets have more diffuse aggregates, retainining fewer dense granules.

TABLE 3

RELEASE OF ENDOGENOUS 5-HT BY MMC AND TMT IN THE PRESENCE AND ABSENCE OF INDOMETHACIN (A CYCLOOXYGENASE INHIBITOR)

Treatment	% Platelet 5-HT	
	Control	+5 μM INDO
Buffer	1.70 ± 0.33	1.71 ± 0.61
MMC (5 μM)	2.01 ± 0.35	3.52 ± 1.12
MMC (100 μM)	78.92 ± 2.63*	6.71 ± 1.40*
TMT (5 μM)	1.92 ± 0.78	2.10 ± 0.39
TMT (100 μM)	2.41 ± 0.86	2.20 ± 0.62

Mean ± SD, n = 5–6 experiments with duplicate determination.
*$p < 0.001$ (t-test).

biosynthesis must be considered in the context of PGI_2 being one of three endothelial-derived factors—prostacyclin, endothelium-derived relaxing factor (EDRF, identified as nitric oxide), and endothelin. Both PGI_2 and EDRF relax vascular smooth muscle whereas EDRF and endothelin stimulate prostacyclin release (16,20). If EDRF and endothelin are released following MMC exposure, then endothelial PGI_2 synthesis may be indirectly stimulated. Because a) MMC also increased platelet TxB_2 biosynthesis and b) neither EDRF nor endothelin are synthesized by platelets, they are not necessary for MMC-triggered prostaglandin biosynthesis. The blockade by indomethacin, NDGA, and chloroquine of MMC effects (Figs. 3 and 4) supports the conclusion that MMC activates the release of membrane arachidonic acid. These data do not rule out effects of MMC on EDRF and endothelin synthesis or release. The data in Figs. 1 and 2 and Table 1 show enhanced prostaglandin biosynthesis upon exposure to MMC. Because prostaglandins are not stored but are synthesized de novo, this means membrane phospholipid arachidonic acid must have been released upon exposure to MMC (i.e., phospholipase A_2 activation).

Low concentrations MMC augmented ADP-triggered aggregation (Fig. 3A) while high concentrations directly triggered platelet aggregation by a prostaglandin-dependent pathway (Fig. 3B), as shown by its blockade by either indomethacin or chloroquine. Moderate amounts of MMC by stimulating a low level of TxA_2 biosynthesis enhances ADP aggregation (ADP is a weak aggregating agent). Higher concentrations of MMC initiate a surge of TxA_2, which acts as a calcium ionophore, initiating the fusion of α- and dense cored-platelet granules with the surface-connecting system of the membrane, releasing the stored contents, for example, 5-HT (Table 2), ADP, and ATP (3). In contrast, strong aggregating agents such as thrombin (a soluble activator), which are potent calcium mobilizers, induce secretion independently of TxA_2.

It has been suggested that one mechanism of mercurial toxicity may involve lipid peroxidation and the generation of free radicals (17). The ability of indomethacin, NDGA, and chloroquine to block MMC actions (Figs. 3 and 4) suggest that MMC activates arachidonic acid metabolism and this generates the endoperoxides PGH_2 and PGG_2. These are short lived and difficult to measure. Instead, platelet lipid peroxidation was assessed by measuring MDA production in the presence of MMC and TMT (Table 2). MMC enhanced MDA production over ninefold, an effect blocked by indomethacin, whereas TMT had no effect on platelet lipid peroxidation. This inhibition by indomethacin suggests that the source of the lipid peroxides was the cyclooxygenase complex. In support of an effect of MMC on general lipid peroxidation, MMC (10 mg/kg, 3–4 days) exposure increased in vivo lipid peroxidation, detected as MDA, and exhaled ethane and pentane (39), while membrane phosphatidylcholine was selectively decreased after in vivo MMC dosing (36). This effect of MMC on polyunsaturated fatty acid (PUFA) mobilization does not appear to be specific because 48 h after MMC dosing PUFAs, including arachidonic acid, are mobilized (7), resulting in lower tissue content.

In rat platelets, trisubstituted tins in the absence of any other stimuli inhibited [^{14}C]5-HT uptake and stimulated the release of preloaded [^{14}C]5-HT (21). This is contrary to the present data on release of endogenous 5-HT. TMT did not by itself stimulate platelet aggregation or the release of endogenous 5-HT. It has been previously noted (21) that there were differences in the release of radiolabeled 5-HT and that of endogenous 5-HT in response to organotins. This suggests that different storage compartments, perhaps cytosolic, are examined using [^{14}C]5-HT and the two sets of data cannot be directly compared. TMT in the concentrations used here did not trigger rat platelet aggregation (Fig. 6); however, both TMT and TET have been reported to induce human platelet aggregation (30). This may reflect specie differences because we have no explanation for this.

Rat platelets exposed to TMT actually exhibited diminished aggregation in response to ADP yet secreted more 5-HT (Fig. 6). The concentration of TMT used here did not by itself result in platelet lysis as evidenced by the lack of a significant release of 5-HT in the absence of an aggregating stimuli (Table 3).

The EM data obtained showed fewer dense granules in platelets coincubated with TMT and ADP in comparison to those incubated with ADP alone. The ability of TMT-pretreated platelets to undergo shape change and aggregation following ADP stimulation argues against nonspecific membrane lysis. This is in agreement with the conclusions of other workers that the effects of organotins on platelets are not the result of membrane lysis (21), because platelets harvested from animals dosed with organotins had lower levels of 5-HT or in some cases were 5-HT depleted. This may be explained by the data presented here, showing that TMT enhances 5-HT secretion even though it depresses platelet aggregation (Fig. 6). In vivo, this would mean that the platelets would remain in the circulation. We previously reported that TMT causes partial depletion of adrenal catecholamines, with no indication of ultrastructural damage (6), while TMT inhibits transmitter uptake by isolated synaptosomes (13). These data are consistent with TMT being an indirect-acting excitotoxin, eliciting the release of stored intracellular material by either platelets, adrenal or neurons, while inhibiting their reuptake by a mechanism dissimilar from that of MMC.

SUMMARY

We have shown that MMC increases cardiac, vascular, and platelet prostaglandin biosynthesis, whereas TMT had no effect. Low concentrations of MMC enhanced ADP aggregation and secretion of 5-HT. Higher concentrations of MMC actually directly triggered platelet aggregation and 5-HT secretion by a prostaglandin-dependent mechanism. In contrast, TMT partially depressed platelet aggregation while enhancing secretion of 5-HT by a non-prostaglandin-dependent mechanism; thus, TMT causes its effects by a mechanism(s) independent of this pathway.

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