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EFFECT OF TRIFLUOPERAZINE AND OTHER DRUGS ON MATRIX VESICLE FORMATION BY CHICKEN GROWTH PLATE CHONDROCYTES IN PRIMARY CELL CULTURE

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Abstract—Growth plate chondrocytes in primary culture release alkaline phosphatase (AP)-rich matrix vesicles (MV) into the culture medium. While these are thought to derive from the plasma membrane by a membrane fusion-dependent process, the mechanism is not fully understood. Recently, a cytosolic protein, synexin, has been shown to promote membrane fusion in a number of systems, and thus may be involved in MV formation. Since the action of synexin is selectively inhibited by trifluoperazine (TFP) and other phenothiazines, we examined the effects of these drugs, and imipramine, on cellular AP production and formation of AP-containing MV by cultured chondrocytes. In addition, we studied the effect on cell division, protein biosynthesis, and incorporation of palmitate into cellular and MV lipids. All of the drugs reduced cellular AP in a concentration-dependent manner; however, at the higher levels, chlorpromazine (CPZ) and TFP caused a transient sharp rise in MV AP activity. At IC_{50} levels, the drugs were more inhibitory to *cellular* AP than to *MV* AP, appearing to enhance significantly the transfer of available cellular AP into MV. In contrast, the drugs stimulated incorporation of [^{3}H]palmitate into cellular lipids, but either had no effect on, or actually inhibited, incorporation of the fatty acid into MV. At these levels, the drugs had little effect on cell division and protein biosynthesis. The inhibitory effect of IC_{50} levels of CPZ on palmitate incorporation into MV appears to have resulted from impairment of vesicle formation *per se*, since at these levels the drug stimulated incorporation of the fatty acid into the cells. The transient stimulatory effect of higher levels of CPZ on MV AP levels, and the enhanced transfer of AP into MV by the drugs generally, may result from the effect of the drugs on membrane structure. Since TFP was not inhibitory to MV formation, it is doubtful that synexin was directly involved.

Matrix vesicles (MV†) are membrane-bound *extracellular* microbodies that are known to be associated with initiation of endochondral and other types of biological calcification [1, 2]. These structures are rich in enzymes (e.g. alkaline phosphatase (AP) [3–6]) and lipids (e.g. phosphatidylserine [7, 8]) that are associated with the plasma membrane. While the exact mechanism by which these structures form is not well understood, their chemical composition [7–10] and morphology [2, 11–14] suggest that they derive from the chondrocyte plasma membrane by budding from the ends of microvilli. During this vesiculation process, fusion of apposing membranes within the microvillus must occur. While a number of factors are known to stimulate membrane fusion, Creutz *et al.* [15] have described recently a 47 kilodalton Ca^{2+}-binding cytosolic protein, synexin, which promotes binding and fusion of intracellular membrane-enclosed secretory vesicles with the inner surface of the plasma membrane of secretory cells. Fusion of the two membranes leads to release of the contents of the granule [15]. Although the cellular system differs, synexin, or a related protein, may be involved in the membrane fusion process that occurs during MV formation. Trifluoperazine (TFP) and another phenothiazine drug, promethazine (PMZ), have been shown to inhibit the activity of synexin [16, 17]. TFP has also been shown to profoundly disturb growth plate development and lipid metabolism when administered *in vivo* [18].

In this report, we examine the effects of TFP, PMZ and chlorpromazine (CPZ), and imipramine (IMP), a dibenzazepine antipsychotic, on cultured chondrocytes using a variety of variables to assess MV formation. These drugs were chosen because previous studies by Pollard *et al.* [17] had shown that they selectively inhibit synexin, the previously mentioned membrane fusogenic protein.

While our findings reveal marked inhibition of cellular AP levels by TFP, PMZ and CPZ, effects on MV levels were significantly less. The data indicate that transfer of cellular AP to MV was actually enhanced by these drugs. All three drugs enhanced [^{3}H]palmitate incorporation into cells, but only CPZ inhibited labeling of the fatty acid in MV. Since neither TFP nor PMZ specifically inhibited transfer of labeled cellular lipid to the vesicles, the data suggest that synexin is probably not involved in the mechanism of MV formation.

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† Abbreviations: MV, matrix vesicles; DMEM, Dulbecco's modified Eagle's medium; TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid; TFP, trifluoperazine; PMZ, promethazine; CPZ, chlorpromazine; IMP, imipramine; AP, alkaline phosphatase; PMSF, phenylmethylsulfonylfluoride; and TMS, 50 mM TES: 1.5 mM $MgCl_2$: 10% (w/w) sucrose buffer.

EXPERIMENTAL PROCEDURES

Cell culture. Chondrocytes from the hypertrophic zone of growth plate epiphyseal cartilage of leg bones from 8 to 10-week-old broiler strain chickens were released and cultured according to the method of Chin *et al.* [19]. The cells were seeded in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic, at a density of 2.7×10^4 cells/cm^2. Incubation was at 37° in a 5% CO_2–95% air atmosphere.

Alkaline phosphatase assay. Matrix vesicles were harvested from the spent media of chondrocyte cultures every 3 days as described previously [19]. In brief, cells were grown in 75 cm^2 flasks containing 12 ml of DMEM; the spent medium, collected every third day from day 3 to the end of the study, was incubated for 5 min at 37° with highly purified hyaluronidase (1 NF units/ml DMEM) to digest any proteoglycan present; matrix vesicles were then sedimented by differential centrifugation [19]. Alkaline phosphatase activity in the matrix vesicles was assayed according to the procedure of Cyboron and Wuthier [20].

Cell harvest. Chondrocytes were harvested from either 75 cm^2 culture flasks, 60 mm culture dishes, or 16 mm wells after removal of the DMEM. The cell layer was gently scraped from the culture vessel and sedimented at 1000 *g* for 10 min. The cell pellet was suspended in 50 mM TES buffer (pH 7.4) containing 1.5 mM $MgCl_2$ and 10% (w/w) sucrose (TMS), frozen, and later thawed and dispersed using a ground-glass homogenizer and sonication for 30 sec. Alternatively, with cells from 16 mm wells, after sedimentation they were resuspended in 0.1% Triton X-100 buffer (pH 7.4) containing 10 mM Tris, 0.5 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonylfluoride (PMSF), frozen, and later dispersed using sonication for 45 sec. Cellular AP activity and total cellular protein [21] were determined on these suspensions.

Incorporation of [^{3}H]*thymidine into chondrocyte DNA.* Cells, grown in 35 mm dishes with 2 ml of DMEM, were labeled for 3 days prior to assay by the addition of 0.1 to 1.25 μCi [^{3}H]thymidine per ml of freshly changed DMEM medium. After the labeling period, the used medium was removed, and the cell layer was washed with TMS buffer, fixed twice with methanol (5 min each), and then washed four times with water and finally solubilized by addition of 1 ml of 0.3 N NaOH. An aliquot was taken for liquid scintillation counting to determine [^{3}H]thymidine incorporation per culture dish [15]. The scintillation fluid was composed of Omnifluor in toluene (4 g/l) to which was added Triton X-100 (1:4, v/v).

Incorporation of [^{3}H]*palmitate into chondrocyte and matrix vesicle lipids.* Cells, grown in 16 mm multiwell dishes with 1 ml of DMEM, were labeled from the beginning of the culture at each successive feeding until 3 days prior to assay by addition of 0.5 to 1.0 μCi [^{3}H]palmitate (30 Ci/mmole) per ml of DMEM. Following the labeling period, the radiolabeled medium was removed, and the cells were washed once with unlabeled DMEM and then refed with unlabeled DMEM plus 10% fetal bovine serum. On the day of harvest, the DMEM was removed and assayed for incorporation of label into MV by differential centrifugation [19], washing of the vesicle pellet twice with TMS buffer, resuspending in deionized water, and counting an aliquot by scintillation counting as described above. For determination of cellular incorporation, the cell layer was washed once with TMS buffer, scraped from the culture surface, transferred to a centrifuge tube, sedimented, rewashed with the buffer, resedimented, and resuspended in 0.5 ml of Triton X-100 for counting as just described.

Materials. The following materials were obtained from the Sigma Chemical Co. (St. Louis, MO): trifluoperazine·HCl (TFP), promethazine·HCl (PMZ), chlorpromazine·HCl (CPZ), imipramine·HCl (IMP), alkaline phosphatase substrate (*p*-nitrophenylphosphate), *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES), hyaluronidase (Type VI-S), and trypsin (Type III). Dulbecco's modified Eagle's medium (DMEM) with L-glutamine, sodium pyruvate and glucose (1 g/l), fetal bovine serum, and antibiotic-antimycotic were purchased from Gibco (Grand Island, NY). [9,10-^{3}H]Palmitate (30 Ci/mmole), [1,2-*Me*-^{3}H]thymidine (102 Ci/mmole) and Omnifluor were obtained from New England Nuclear (Boston, MA). Collagenase (CLS-II) was purchased from the Worthington Biochemical Corp. (Freehold, NJ). Sterile culture flasks (75 cm^2) and culture dishes (60 mm and 35 mm) were obtained from Corning Glass Works (Corning, NY). All other chemicals were of reagent grade and were supplied by Fisher Scientific (Pittsburgh, PA).

RESULTS

Trifluoperazine (TFP), when added as the cells approached confluency, inhibited formation of AP-rich MV by chondrocytes in culture in a time- and concentration-dependent manner (Fig. 1). Half-maximum inhibition of MV AP levels occurred at approximately 10 μM TFP (Fig. 1, inset). Morphologically, TFP-treated cells appeared similar to the control cells, except for a small, but detectable increase in vacuolization of the drug-treated cells. At 20 μM TFP, formation of AP-rich MV was transiently stimulated (Fig. 1), but after 12 days of treatment, the cells became rounded and died. Addition of 10 μM TFP early in the culture (from day 3) produced an equivalent toxic effect (data not shown). Cellular AP levels were also inhibited by TFP in a concentration-dependent fashion (Fig. 2). Cellular protein synthesis, on the other hand, remained relatively unaffected up to 15 μM TFP. Further, 10 μM TFP caused a minimal effect on cellular division, as indicated by [^{3}H]thymidine incorporation into chondrocyte DNA (Fig. 3). The TFP-treated cells followed the same pattern of labeling, levels of [^{3}H]thymidine incorporation being about 90% of that of untreated control cells. Note the progressive incorporation of [^{3}H]thymidine (indicative of cell division) throughout the 23-day test period. Also note that 10 μM TFP caused only minor (*ca.* 11%) inhibition of the rate of cellular division, whereas formation of AP-rich MV and production of cellular

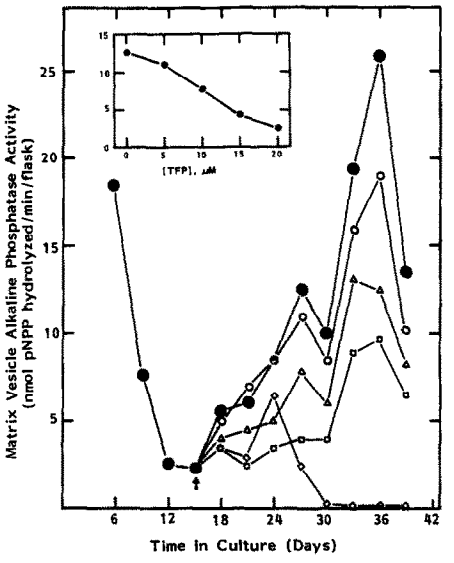

Fig. 1. Time effect of graded levels of trifluoperazine (TFP) on formation of AP-rich MV by primary cultures of chicken epiphyseal growth plate chondrocytes. Arrow denotes the beginning of drug treatment. MV AP is that activity in the spent culture medium which is sedimentable at 100,000 *g* for 1 hr (see Experimental Procedures). *Inset:* Concentration–response curve of inhibition of AP-rich MV formation by TFP (day 27 of culture). In this and subsequent comparable figures, values for the Y-axis of the inset are the same as those for Y-axis of the main figure. Note that inhibition of MV AP by TFP became gradually greater with time. The drug concentrations tested were 0 μM (●), 5 μM (○), 10 μM (△), 15 μM (□), and 20 μM (◇).

AP (Figs. 1 and 2 respectively) were inhibited much more severely.

Promethazine (PMZ) produced a similar concentration-dependent inhibition of MV AP levels in the cultured chondrocytes (Fig. 4). Half-maximum

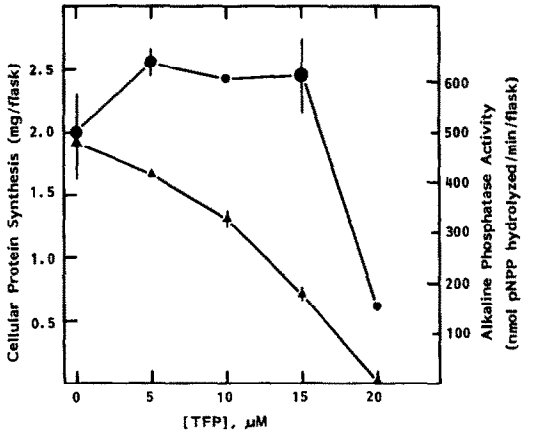

Fig. 2. Concentration-dependent effect of TFP on cellular synthesis of protein and AP by cultured chondrocytes. Note that at concentrations of TFP up to 15 μM cellular protein synthesis (●) was either unaffected, or was slightly stimulated, whereas cellular AP production (▲) was progressively depressed. Assays were run on day 39 at the termination of the culture (see Fig. 1). Values are means ± S.E.M., N = 2.

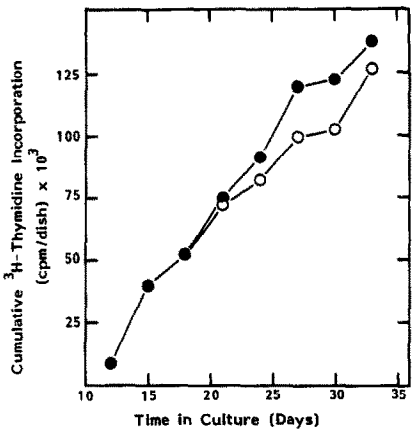

Fig. 3. Time effect of TFP on DNA synthesis by cultured chondrocytes as measured by cumulative incorporation of [^{3}H]thymidine. Successive culture dishes of chondrocytes were given [^{3}H]thymidine for a period of 3 days at which time the cells were harvested and the trichloroacetic acid-precipitable counts were measured (see Experimental Procedures). The graph illustrates [^{3}H]thymidine incorporation by untreated (●) and 10 μM TFP-treated (○) cells.

inhibition occurred at approximately 29 μM (Fig. 4, inset). PMZ also caused a minor increase in vacuolization, similar to that seen with TFP. Note that in contrast to TFP, however, inhibition of PMZ was most marked during the initial stages of drug administration and decreased with time. Cellular AP levels were also strongly inhibited by PMZ in a concentration-dependent manner (IC_{50} = 27 μM) and, in contrast to TFP, PMZ caused a con-

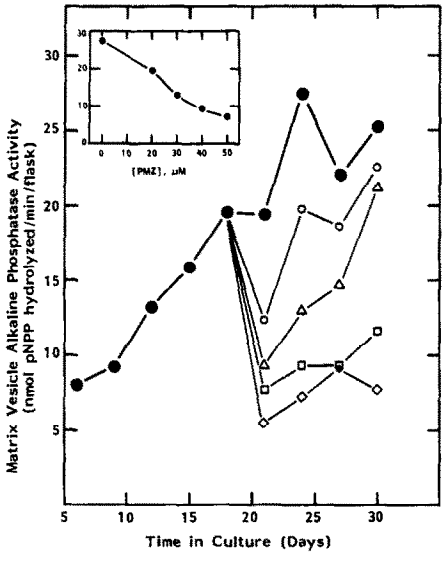

Fig. 4. Time effect of graded levels of promethazine (PMZ) on formation of AP-rich MV by cultured chondrocytes. *Inset:* Concentration–response curve of inhibition of formation of AP-rich MV by PMZ. As in Fig. 1 and subsequent insets, values on Y-axis of the inset are the same as those for the Y-axis of the main figure. The concentrations of PMZ tested were 0 μM (●), 20 μM (○), 30 μM (△), 40 μM (□), and 50 μM (◇).

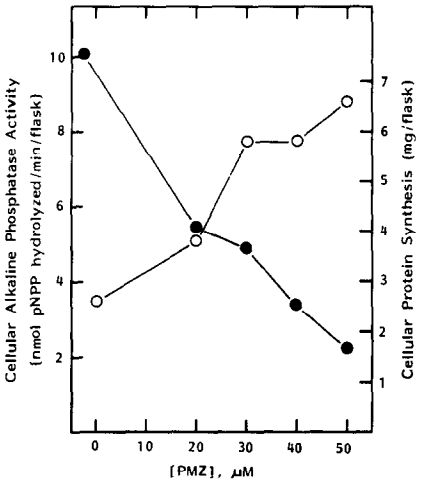

Fig. 5. Concentration effect of graded levels of PMZ on *cellular* AP activity (●) and protein synthesis (○) by cultured chondrocytes. Cellular assays were conducted on day 30 at the termination of the culture (see Fig. 4).

centration-dependent *increase* in cellular protein synthesis (Fig. 5). At the higher levels, PMZ inhibited cellular division, as indicated by decreased [^{3}H]thymidine incorporation (Fig. 6).

The two other drugs tested on the cultured chondrocytes, chlorpromazine (CPZ) and imipramine (IMP), gave similar results. Cultures treated with 40 or 50 μM CPZ produced a transient sharp increase in MV alkaline phosphatase levels shortly after addition of the drug, but a rapid decline in activity then followed (Fig. 7). Half-maximum inhibition occurred at approximately 14 μM. Morphologically, 20 and 30 μM CPZ caused increased cellular vacuolization, whereas 40 and 50 μM drug produced many rounded and unattached cells. Cellular AP activity was strongly suppressed by CPZ in a concentration-dependent manner (IC_{50} = 14 μM); total cellular protein was unaffected by the lower CPZ concentrations (20–30 μM) that suppressed vesicle AP levels, but

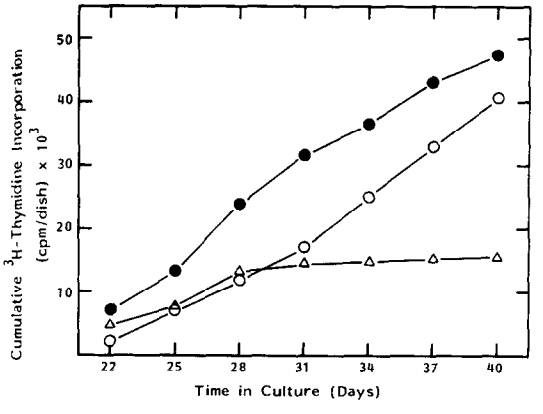

Fig. 6. Time effect of PMZ on DNA synthesis by cultured chondrocytes as measured by [^{3}H]thymidine incorporation. Graph illustrates [^{3}H]thymidine incorporation by untreated (●), 30 μM PMZ-treated (○), and 40 μM PMZ-treated (△) cells.

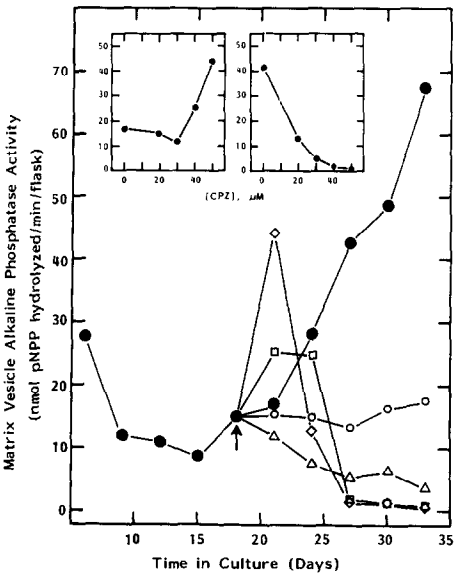

Fig. 7. Time effect of graded levels of chlorpromazine (CPZ) on formation of AP-rich MV by cultured chondrocytes. *Left inset:* Early concentration–response effect on MV formation (day 21). *Right inset:* Effect after 9 days of drug treatment (day 27). Note the transient stimulation of MV release at the higher levels of CPZ (40 and 50 μM). This was followed by rapid decline and cell death. The concentrations of CPZ tested were 0 μM (●), 20 μM (○), 30 μM (△), 40 μM (□), and 50 μM (◇).

was inhibited at the higher CPZ levels (Fig. 8). Note that at concentrations of CPZ up to 30 μM, cellular protein synthesis was little affected, whereas cellular AP production was severely suppressed. IMP also inhibited MV (Fig. 9) and cellular AP levels (Fig. 10) in a concentration-dependent manner. Again, cellular protein synthesis was elevated in the highest concentration in IMP-treated cultures (Fig. 10).

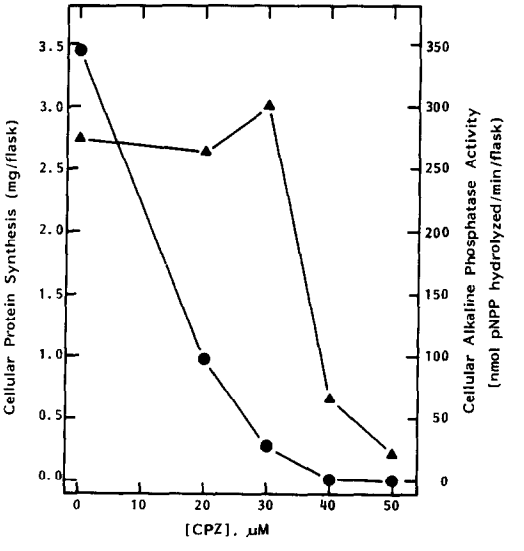

Fig. 8. Concentration-dependent effect of CPZ on *cellular* synthesis of proteins (▲) and AP (●) by cultured chondrocytes. Cellular assays were run on day 33 at the termination of cell culture (see Fig. 7).

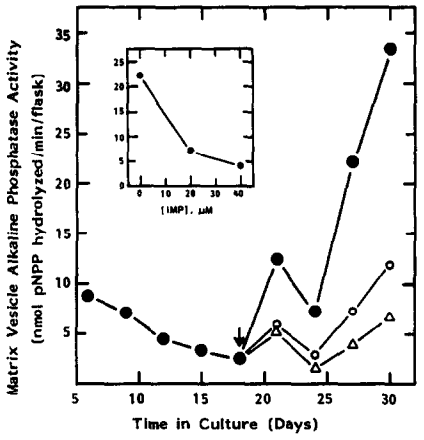

Fig. 9. Time effect of graded levels of imipramine (IMP) on formation of AP-rich MV by cultured chondrocytes. *Inset*: Concentration–response curve of inhibition of MV formation (day 27) by IMP. The concentrations of IMP tested were 0 μM (●), 20 μM (○), and 40 μM (△).

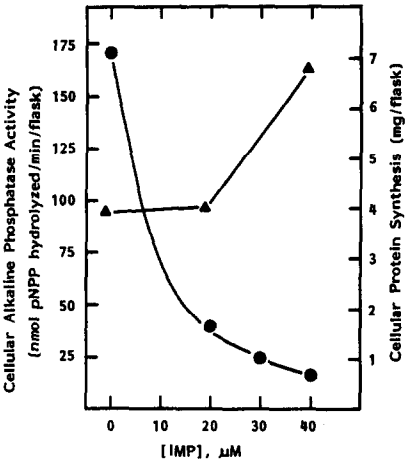

Fig. 10. Concentration-dependent effect of IMP on *cellular* synthesis of protein (▲) and AP (●) by cultured chondrocytes. Note that at the highest level (40 μM) IMP stimulated general protein synthesis while markedly reducing cellular AP activity.

To determine whether the inhibitory effects of the drugs on MV AP levels were caused by suppression of vesicle formation, as well as by inhibition of cellular AP levels, a series of experiments was conducted on the effects of IC_{50} levels of TFP, PMZ and CPZ on incorporation of [^{3}H]palmitate into the cultured cells and MV, as well as on cellular and MV AP and cellular protein levels. Table 1 shows that all three drugs markedly inhibited *cellular* AP levels to values about 20–30% of the control, but while drugs inhibited *MV* AP levels, the effects were much smaller, ranging from 65 to 85% of the control. Relating the effects on the cells to those on MV, the

Table 1. Effects of phenothiazines on chondrocytes and matrix vesicles

A. Cellular variables

Treatment	Alkaline phosphatase* (act./well)	(% of control)	[^{3}H]Palmitate incorporation† (cpm/well)	(% of control)	Protein (μg/well)	(% of control)
Control	11.0 ± 1.3	100 ± 0	11.1 ± 0.9	100 ± 0	59.6 ± 6.7	100 ± 0
TFP	2.3 ± 0.4‡	19 ± 4‡	17.9 ± 2.3§	160 ± 17‖	60.2 ± 8.9	105 ± 7
CPZ	3.1 ± 0.3‡	31 ± 5‡	17.0 ± 2.2§	160 ± 13‖	52.3 ± 9.7	96 ± 8
PMZ	2.6 ± 0.4‡	23 ± 4‡	14.4 ± 1.4	137 ± 7‖	54.6 ± 10.8	92 ± 9

B. Matrix vesicle variables

Treatment	Alkaline phosphatase* (act./flask)	(% of control)	(MV/cell)	(% of control)
Control	7.3 ± 1.3	100 ± 0	0.70 ± 0.10	100 ± 0
TFP	4.4 ± 0.7	64 ± 5‡	2.40 ± 0.49‖	284 ± 67‖
CPZ	5.9 ± 1.0	84 ± 6§	2.11 ± 0.33‖	280 ± 39‡
PMZ	4.7 ± 0.7	68 ± 6‡	2.11 ± 0.25‖	287 ± 38‡

Treatment	[^{3}H]Palmitate incorporation† (cpm/well)	(% of control)	(MV/cell)	(% of control)
Control	6.2 ± 1.1	100 ± 0	5.3 ± 0.7	100 ± 0
TFP	7.7 ± 1.0	122 ± 22	5.3 ± 1.3	96 ± 22
CPZ	4.8 ± 1.0	68 ± 5	3.2 ± 0.8	57 ± 11‖
PMZ	7.2 ± 1.2	123 ± 15	5.2 ± 0.9	102 ± 16

Values are means ± S.E.M. of 7–9 samples each.

* Alkaline phosphatase activity is expressed in nmoles *p*-nitrophenylphosphate hydrolyzed/min at pH 10.3 (see Experimental Procedures). MV/cell relates activity for MV and cells derived from the same batch of cells and cultured at the same time in the same medium (±drug). MV were obtained from 75 cm^2 flasks; cells were grown in 16 mm wells.

† [^{3}H]Palmitate incorporation into cells = total cpm × 10^{-5}; incorporation into MV = total cpm × 10^{-3}. Palmitate values for MV/cell = values × 10^3 and are as described above for AP except that here the cells and MV were obtained from the same culture well.

‡–‖ Difference from control significant at: ‡ $P \leqq 0.001$; § $P \leqq 0.05$; and ‖ $P \leqq 0.01$.

drugs actually enhanced significantly the amount of the cellular AP appearing in the MV. Values for MV/cellular AP were nearly three times higher in the drug-treated cultures than in the control.

DISCUSSION

Antipsychotics are known to be inhibitors of many enzyme systems modulated by calmodulin, phenothiazines being some of the most potent [22]. These drugs bind to calmodulin in a Ca^{2+}-dependent manner and prevent activation of the target enzyme [23]. However, several calmodulin-independent systems also are inhibited by these drugs [24–26]. Creutz *et al.* [15] have shown that TFP inhibits synexin-induced chromaffin granule attachment to the plasma membrane, and, subsequently, membrane fusion. This is a calmodulin-independent process. Synexin has been found in several types of tissues [27]. Table 2 lists published IC_{50} values for drug-related inhibition of calmodulin-dependent phosphodiesterase [22, 23], synexin-dependent membrane fusion [27], and two other enzyme systems [25, 26], as well as our current data on drug-related inhibition of cellular and MV AP synthesis by growth plate chondrocytes. The IC_{50} levels for inhibition of cellular and MV AP activity by all drugs except TFP are significantly lower than those needed for inhibition of phosphodiesterase activity. This indicates that a calmodulin-*independent* process is involved in MV formation. This is further supported by the fact that the potency of TFP inhibition of MV AP is only about three times that of PMZ, whereas in calmodulin-dependent systems, TFP is twenty times more potent than PMZ. In addition, the IC_{50} levels for IMP and CPZ inhibition of cellular and matrix vesicle AP production are much lower than the corresponding values for phosphodiesterase inhibition. Finally, other studies have clearly shown that calmodulin *does not* enhance membrane fusion [27].

In some respects, our findings are similar to the inhibitory effects of the phenothiazines observed by Pollard *et al.* [17] on synexin-dependent membrane aggregation and fusion in chromaffin granule secretion. However, data on the effects of TFP, PMZ and CPZ on MV versus cellular AP and [^{3}H]palmitate labeling (Table 1) show that the primary effect of the drugs was on cellular AP levels, and only secondarily on MV AP. Further, the fact that only CPZ (but neither TFP nor PMZ) inhibited palmitate incorporation into MV suggests that MV form by a synexin-*independent* mechanism. However, the fact that MV are enriched in phosphatidylserine [7–9], as opposed to phosphatidylinositol, suggests that a synexin-like protein may be involved. Studies have shown that synexin promotes Ca^{2+}-dependent fusion of membrane regions containing *phosphatidylserine* but inhibits those containing phosphatidylinositol [28, 29].

It is important to realize that exocytosis of granule secretion and emiocytosis of vesicle formation from cell microvilli may be related processes. Since the orientation of the lipid bilayer in secretory vesicles is the opposite of that of the plasma membrane, in both microvillar vesiculation and secretory vesicle exocytosis, apposition of the phosphatidylserine-rich side of the membranes would be the first step in the membrane fusional processes. Ca^{2+} and some

Table 2. Comparison of inhibition of cellular and matrix vesicle alkaline phosphatase production and other membrane-related systems by phenothiazine and related drugs

	Cellular AP activity*		Matrix vesicle AP activity*		Synexin-dependent membrane fusion†	
Drug	IC_{50} (μM)	Relative inhibition‡	IC_{50} (μM)	Relative inhibition‡	IC_{50} (μM)	Relative inhibition‡
TFP	13	1.0	10	1.0	4	1.0
PMZ	27	2.1	29	2.9	16	4.0
CPZ	14	1.1	14	1.4		
IMP	9	0.7	12	1.2	NI§	0
	Calmodulin-dependent PDE activity‖		Protein kinase C activity‖		Sarcoplasmic reticulum Ca^{2+}/Mg^{2+}-ATPase**	
Drug	IC_{50} (μM)	Relative inhibition‡	IC_{50} (μM)	Relative inhibition‡	IC_{50} (μM)	Relative inhibition‡
TFP	17	1.0	25	1.0	10	1.0
PMZ	340	20.0			40	3.7
CPZ	40	2.4	30	1.2	200	18.5
IMP	125	7.4	200	8.0		

* This paper.
† Synexin-dependent membrane aggregation and fusion, Pollard *et al.* [17].
‡ Inhibition relative to TFP.
§ NI = noninhibitory (Pollard, personal communication).
‖ Weiss and coworkers [22, 23].
¶ Mori *et al.* [26].
** Volpe *et al.* [25].

membrane fusion-stimulating protein could be critically involved. While the assembly of actin microfilaments and microtubules has been shown to affect MV formation [30], it is unlikely that the proteins themselves directly stimulate membrane fusion.

Verkleij *et al.* [31] have shown that phosphatidic acid-containing model membranes treated with CPZ favor the hexagonal H_{II} phase, as opposed to bilayer configuration. Cullis and DeKruijff [32] and Sundler *et al.* [33] have shown that the formation of H_{II} phase is favorable, but not essential, to fusion of model membranes. These observations may partially explain the markedly stimulatory effect of CPZ on vesicle formation at the two highest drug concentrations.

Finally, comment must be made of the fact that cellular AP was inhibited more severely by the antipsychotics than was the AP level in MV. This, and the lack of effect of TFP and PMZ on palmitate incorporation in MV, indicate that much of the effect of the drugs on MV AP levels resulted from their effects on cellular AP synthesis. The latter is closely dependent on the state of confluency of the cultured cells [19]. Hence, the depression of cellular AP suggests that these drugs also act prior to the actual membrane fusion event. Since phenothiazine drugs have been shown to interfere with mitochondrial oligomycin-sensitive ATPase [24], Ca^{2+}/Mg^{2+}-ATPase in endoplasmic (sarcoplasmic) reticulum [25] and Ca^{2+}/phosphatidylserine-dependent protein kinase C [26], it is possible that the observed effects on cellular AP production are related to interferences with related enzymes in the chondrocytes. Further study will be required to clarify this issue.

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