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CHARACTERIZATION OF PALYTOXIN-INDUCED CATECHOLAMINE SECRETION FROM CULTURED BOVINE ADRENAL CHROMAFFIN CELLS

EFFECTS OF Na^+- AND Ca^{2+}-CHANNEL BLOCKERS

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Abstract—The effect of palytoxin (PTX), a potent marine toxin, on catecholamine (CA) secretion from cultured bovine adrenal chromaffin cells was examined. PTX at concentrations of over 10^{-10} M induced CA secretion concentration-dependently. About 40–50% of the total cellular CA was secreted during 20-min incubation with 3×10^{-8} M PTX. PTX-induced CA secretion was dependent on both extracellular Na^+ and Ca^{2+}. PTX caused increases in $[^{22}Na]^+$- and $[^{45}Ca]^{2+}$-influxes into the cells. Increase in $[^{22}Na]^+$-influx was observed at concentrations of over 10^{-11} M PTX and was maximal at 10^{-10} M PTX and then gradually decreased at higher concentrations that induced $[^{45}Ca]^{2+}$-influx and CA secretion. On the other hand, increase in $[^{45}Ca]^{2+}$-influx was observed at concentrations of over 10^{-10} M PTX and increased with increase in concentration of PTX. This concentration–response curve for PTX-induced $[^{45}Ca]^{2+}$-influx was similar to that for PTX-induced CA secretion. The CA secretion and $[^{22}Na]^+$- and $[^{45}Ca]^{2+}$-influxes induced by PTX were not affected by tetrodotoxin (TTX), but were significantly inhibited by quinidine and aprindine(mexiletine), antiarrythmic drugs known to block Na^+-channels. Ca^{2+}-channel blockers such as nifedipine, verapamil, Co^{2+}, Cd^{2+}, inhibited both CA secretion and $[^{45}Ca]^{2+}$-influx induced by PTX. These results indicate that PTX-induced CA secretion is mediated by activation of Na^+-dependent, TTX-insensitive voltage-dependent Ca^{2+}-channels, and is inhibited by quinidine and aprindine through their inhibitory effects on the Na^+- and Ca^{2+}-influxes into the cells induced by PTX.

Palytoxin (PTX) is a potent marine toxin isolated from zoanthid *Palythoa* species [1]. This toxin induces Na^+-dependent, tetrodotoxin (TTX)-insensitive depolarization of various excitable cells [2, 3], such as vascular smooth muscle [4, 5], cardiac muscle [6–8], skeletal muscle [9], spinal cord [10] and squid axons [11]. Electrophysiological studies by the patch-clamp technique have shown that PTX-induced depolarization is attributable to the opening of ion channels, which permits Na^+ influx [7, 8]. This PTX-induced depolarization causes an increase in permeability of the cell membrane to Ca^{2+}, leading to contraction of muscle and release of transmitter [12, 13]. PTX has also been shown to stimulate the efflux of K^+ from erythrocytes [14] and HeLa cells [15] and the release of [^{3}H]norepinephrine from pheochromocytoma cells (PC-12 cells) preloaded with [^{3}H]norepinephrine [16].

In this study, we examined the effect of PTX on catecholamine (CA) secretion from cultured bovine adrenal chromaffin cells to determine whether it also affects the secretory response of these cells in a Na^+-dependent, but TTX-insensitive manner. Its effect was compared with that of veratridine, which is known to induce CA secretion in a Na^+-dependent and TTX-sensitive manner [17]. The effects of antiarrythmic drugs, known to be Na^+-channel blockers, and Ca^{2+}-channel blockers on the responses to PTX were also examined.

Results showed that PTX at relatively low concentrations induced marked CA secretion which was dependent on both extracellular Na^+ and Ca^{2+}. PTX caused increases in $[^{22}Na]^+$- and $[^{45}Ca]^{2+}$-influxes into the cells. These responses to PTX were not affected by TTX, but were significantly inhibited by the antiarrythmic drugs quinidine and aprindine. Ca^{2+}-channel blockers such as nifedipine, verapamil, Co^{2+} and Cd^{2+} inhibited both PTX-induced CA secretion and $[^{45}Ca]^{2+}$-influx, indicating that PTX-induced CA secretion was mediated by activation of Na^+-dependent, TTX-insensitive voltage-dependent Ca^{2+}-channels. A preliminary report of this work has been published [18].

MATERIALS AND METHODS

Cell preparation and primary culture. Fresh bovine adrenal glands were obtained from a local slaughter house and chromaffin cells were separated enzymatically as described previously [19]. Briefly, the medulla was sliced with a hand slicer, and the slices were digested in medium containing 0.1% collagenase, 0.01% soybean trypsin inhibitor, and 0.5% bovine serum albumin in balanced salt solution [BSS: 135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgSO_4$, 2.2 mM $CaCl_2$, 10 mM glucose, and 20 mM *N*-2-hydroxyethylpiperazine - *N'* - 2 - ethanesulfonic acid (HEPES)/NaOH, pH 7.4]. The cells were plated on 24-well cluster plates at a density of 5×10^5 cells/well, and maintained for 3 days as monolayer

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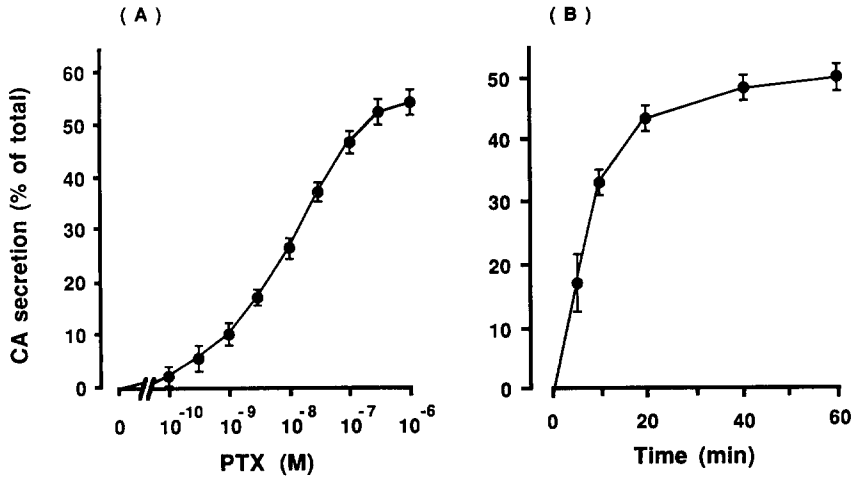

Fig. 1. (A) Concentration–response curve for PTX-induced catecholamine (CA) secretion from cultured bovine adrenal chromaffin cells. Cells were incubated with various concentrations of PTX at 37° for 10 min. CA secretion is shown as a percentage of the total cellular CA content. Spontaneous CA secretion was approx. 2–3%. PTX-induced secretion was calculated by subtracting the spontaneous values from observed values. Points and bars are means ± SEM for 3–4 experiments. (B) Time course of the PTX-induced catecholamine (CA) secretion from the cells. Cells were incubated with 3×10^{-8} M PTX for the indicated times. CA secretion is shown as a percentage of the total cellular CA content. Points and bars are means ± SEM for 3–4 experiments.

cultures in 1.5 mL of Eagle's minimum essential medium containing 5% heat-inactivated fetal calf serum, 2 mM glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL), gentamicin (400 μg/mL), fungizone (2.5 μg/mL), and cytosine arabinoside (10 μM) [20, 21].

Incubation media. BSS, used as normal assay medium, had the composition described above. Ca^{2+}-free solution was prepared by omitting $CaCl_2$ and adding 1 mM ethyleneglycolbis(aminoethyl-ether)tetra-acetate (EGTA) to BSS. Na^+-free solution was prepared by replacing NaCl in BSS by choline chloride keeping the solution isotonic.

Determination of catecholamine secretion from the cells. Plated cells were washed with 1 mL of BSS and then incubated at 37° for 10 min in 250 μL of BSS containing PTX and/or different agents. At the end of the incubation period, the medium was removed and the cells were lysed by adding 250 μL of 10% acetic acid and then freeze–thawing. Both the medium and the cell lysate were centrifuged in an Eppendorf centrifuge operated at maximum speed (approx. 8800 *g*) for 2 min, and the supernatant fractions were then used for catecholamine (CA) assay. CA was measured fluorometrically as described previously [22]. CA secretion was expressed as the percentage of the total cellular content secreted during the incubation period.

Measurements of $[^{22}Na]^+$- and $[^{45}Ca]^{2+}$-influxes into the cells. Plated cells were incubated with PTX and/or different drugs at 37° for 10 min in BSS containing $[^{22}Na]Cl$ (3 μCi/mL) or $[^{45}Ca]Cl_2$ (3 μCi/mL). Then, the medium covering the cells was discarded and the cells were washed four times with 1 mL of ice-cold Na^+-free or Ca^{2+}-free BSS. The cells were then solubilized by 1% Triton X-100, and the radioactivity in the lysate was measured in a liquid scintillation counter. The amounts of $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ taken up into the cells were calculated on the basis of the specific activities of radioactive $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ in the reaction mixture and expressed in nanomoles per well (5×10^5 cells). In experiments on Na^+-uptake, ouabain, a Na^+–K^+ activated ATPase inhibitor, which is usually used to prevent the efflux of $[^{22}Na]^+$ taken up by the cells, was not used, because ouabain (10^{-6}–10^{-4} M) was found to cause marked inhibition of PTX-induced CA secretion.

Chemicals. PTX was kindly donated by Dr Muramatsu (Fukui Medical School, Fukui, Japan). $[^{45}Ca]Cl_2$ and $[^{22}Na]Cl$ were purchased from New England Nuclear Co. (Boston, MA). Tetrodotoxin was obtained from Sankyo Co. (Tokyo, Japan). Nifedipine, verapamil, $CoCl_2$ and $CdCl_2$ were obtained from Wako Pure Chemical Co. (Osaka, Japan). Veratridine, quinidine and disopyramide were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Aprindine (Mexiletine) was a gift from Mitsui Pharmaceutical Co. (Tokyo, Japan). Other chemicals used were of commercial reagent grade. The final concentration of dimethylsulfoxide, which was used to dissolve the hydrophobic drugs, in the incubation medium was less than 1%, which did not affect CA secretion or cation uptake by the cells.

Statistics. All values are expressed as means ± standard error of means (SEM) and Student's *t*-test was used to determine statistical significance.

RESULTS

Effect of PTX on CA secretion

PTX caused marked CA secretion from cultured bovine adrenal chromaffin cells. As shown in Fig.

Table 1. Effects of removal of Ca^{2+} and Na^+ from the medium on catecholamine secretion induced by PTX and veratridine

	PTX-induced CA secretion (% of total)	Veratridine-induced CA secretion (% of total)
Complete medium	32.35 ± 0.32	20.85 ± 0.20
Ca^{2+}-free medium	1.40 ± 0.17	0.86 ± 0.04
Na^+-free medium	9.65 ± 0.40	6.17 ± 0.37

Cultured bovine adrenal chromaffin cells were incubated with PTX (3×10^{-8} M) or veratridine (10^{-4} M) in normal BSS medium, Ca^{2+}-free medium (BSS without $CaCl_2$ and with 1 mM EGTA), and Na^+-free medium (BSS with choline chloride instead of NaCl) at 37° for 10 min. Catecholamine (CA) secretion is shown as a percentage of the total cellular CA content.

Values are means ± SEM for 3–4 experiments.

1A, PTX induced CA secretion concentration-dependently at concentrations of 10^{-10}–10^{-6} M during 10 min incubation. This CA secretion was rapid, continued for at least 20 min and then leveled off (Fig. 1B). About 40–50% of the cellular CA was secreted during 20 min incubation with 3×10^{-8} M PTX.

Effects of removal of Ca^{2+} *and* Na^+ *from the medium*

CA secretion from chromaffin cells is known to be initiated by increase in intracellular Ca^{2+}. Moreover, PTX is known to cause membrane depolarization by increasing Na^+-influx into the cells. Therefore, we examined the dependencies of PTX-induced CA secretion on extracellular Ca^{2+} and Na^+. As shown in Table 1, CA secretion induced by PTX was almost abolished by removal of Ca^{2+} from the medium and was significantly decreased by removal of Na^+ from the medium. CA secretion induced by veratridine, a voltage-dependent Na^+ channel activator, was also dependent on the presence of Na^+ and Ca^{2+} in the medium.

Effects of PTX on $[^{22}Na]^+$*- and* $[^{45}Ca]^{2+}$*-influxes into the cells*

Since PTX-induced CA secretion from the cells required the presence of external Na^+ and Ca^{2+}, we examined whether PTX increased the influxes of Na^+ and Ca^{2+} into the cells. PTX was found to induce influxes of both $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into the cells, the time courses of both influxes being similar to that of CA secretion induced by PTX (data not shown). However, the concentration–response curves for $[^{22}Na]^+$-influx and $[^{45}Ca]^{2+}$-influx induced by PTX were different. As shown in Fig. 2, $[^{22}Na]^+$-influx was observable at PTX concentrations of over 10^{-11} M and maximal at 10^{-10} M and decreased at higher concentrations. On the other hand, $[^{45}Ca]^{2+}$-influx induced by PTX was observed at concentrations of over 10^{-10} M and increased with increase in concentration of PTX from 10^{-10}–10^{-6} M. This concentration–response curve for PTX-induced $[^{45}Ca]^{2+}$-influx was quite similar to that for PTX-induced CA secretion.

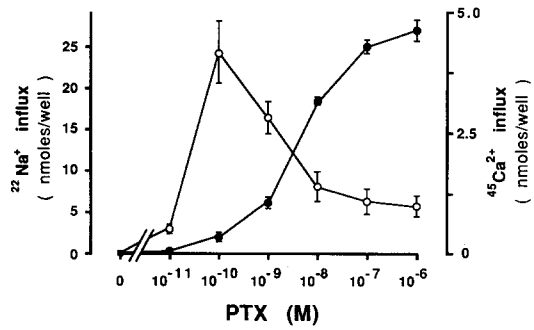

Fig. 2. Concentration–response curve for PTX-induced influxes of $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into cultured bovine adrenal chromaffin cells. Cells were incubated at 37° for 10 min with $[^{22}Na]Cl$ or $[^{45}Ca]Cl_2$ in the presence of various concentrations of PTX, as described in the Materials and Methods. The influxes of $[^{22}Na]^+$ (○) and $[^{45}Ca]^{2+}$ (●) into the cells are shown in nmol/well. Points and bars are means ± SEM for three experiments.

Effect of TTX on PTX-induced CA secretion

To determine the characteristics of PTX-induced CA secretion, we examined the effect of TTX, an inhibitor of voltage-dependent Na^+-channels, on CA secretion induced by PTX. As shown in Fig. 3, even at a high concentration of 10^{-6} M, TTX did not inhibit PTX-induced CA secretion, although it inhibited veratridine-induced CA secretion concentration-dependently. TTX also did not affect increase in $[^{22}Na]^+$-influx into the cells induced by PTX (Table 2). These results indicated that PTX induced Na^+-influx into the cells by activation of TTX-insensitive Na^+-channels or pathways, resulting in an increase in cell membrane permeability to Ca^{2+} and initiation of CA secretion.

Effects of antiarrythmic drugs (Na^+*-channel blockers) on CA secretion induced by PTX*

As PTX-induced CA secretion was Na^+-dependent, but TTX-insensitive, we examined the effects of antiarrythmic drugs, known to be Na^+-channel blockers, on CA secretion and $[^{22}Na]^+$- and $[^{45}Ca]^{2+}$-influxes induced by PTX. As shown in Fig. 4, quinidine inhibited not only veratridine-induced CA secretion, but also PTX-induced CA secretion

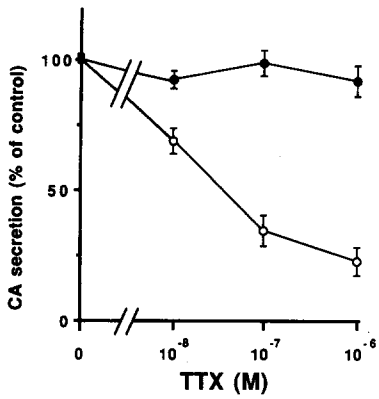

Fig. 3. Effect of tetrodotoxin (TTX) on catecholamine secretion induced by PTX or veratridine. Cells were incubated with PTX (3×10^{-8} M) (●) or veratridine (10^{-4} M) (○) at 37° for 10 min in the presence of various concentrations of TTX. Results were expressed as percentage of the control values, which were calculated to be 32.4 ± 0.3% and 20.9 ± 0.2% of the total cellular content for PTX-, and veratridine-induced secretion, respectively. Points and bars are means ± SEM for three experiments.

concentration-dependently. Aprindine (mexiletine) also inhibited veratridine-induced CA secretion concentration-dependently, and significantly inhibited PTX-induced CA secretion at concentrations of over 10^{-4} M. Disopyramide, another antiarrythmic drug, inhibited veratridine-induced CA secretion, but even at 10^{-4} M, it did not inhibit PTX-induced CA secretion. It is interesting that among the antiarrythmic drugs tested, quinidine and aprindine were found to inhibit not only TTX-sensitive CA secretion induced by veratridine, but also TTX-insensitive CA secretion induced by PTX. As shown in Table 2, quinidine and aprindine were also found to inhibit the influxes of $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into the cells induced by PTX.

Effects of Ca^{2+}-channel blockers on CA secretion induced by PTX

To determine whether PTX-induced CA secretion is mediated by activation of voltage-dependent Ca^{2+}-channels, we examined the effects of so-called Ca^{2+}-channel blockers on CA secretion induced by PTX. The organic Ca^{2+}-channel blockers nifedipine and verapamil were found to inhibit PTX-induced CA secretion (Fig. 5). These Ca^{2+}-channel blockers also inhibited CA secretion induced by veratridine. The inorganic Ca^{2+}-channel blockers Co^{2+} and Cd^{2+} also inhibited both PTX-induced and veratridine-induced CA secretion, their inhibitory effects being especially marked (Fig. 6). As shown in Table 2, these Ca^{2+}-channel blockers inhibited $[^{45}Ca]^{2+}$-influx induced by PTX, but did not significantly affect $[^{22}Na]^+$-influx induced by PTX. These results indicated that like veratridine-induced CA secretion, PTX-induced CA secretion was also induced by increase in Ca^{2+}-influx through voltage-dependent Ca^{2+}-channels.

Table 2 summarizes the effects of removal of Na^+ from the medium and the additions of various agents on the influxes of $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into the cells induced by PTX.

DISCUSSION

In this study we found that PTX at relatively low concentrations (10^{-10}–10^{-6} M) induced CA secretion from cultured bovine adrenal chromaffin cells concentration-dependently. About 40–50% of cellular CA was secreted during 20 min incubation with 3×10^{-8} M PTX. The stimulatory effect of PTX on

Table 2. Effects of removal of Na^+ from the medium and the presence of various agents on influxes of $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into the cells induced by PTX

	PTX-induced	
	$[^{22}Na]^+$-influx (nmol/well)	$[^{45}Ca]^{2+}$-influx (nmol/well)
Control	24.7 ± 0.9	3.8 ± 0.2
Na^+-free	—	1.2 ± 0.1*
TTX (10^{-6} M)	24.2 ± 0.8	3.7 ± 0.2
Quinidine (10^{-4} M)	12.3 ± 0.3*	2.4 ± 0.1*
Aprindine (10^{-4} M)	11.2 ± 0.1*	2.1 ± 0.2*
Disopyramide (10^{-4} M)	21.7 ± 0.5	3.3 ± 0.2
Nifedipine (10^{-4} M)	20.8 ± 0.4	0.7 ± 0.1*
Verapamil (10^{-4} M)	22.5 ± 0.6	1.9 ± 0.2*
Co^{2+} (10^{-3} M)	21.7 ± 0.8	0.7 ± 0.1*
Cd^{2+} (10^{-5} M)	22.6 ± 0.7	0.6 ± 0.1*

Cultured bovine adrenal chromaffin cells were incubated at 37° for 10 min with PTX and $[^{22}Na]Cl$ or $[^{45}Ca]Cl_2$ in normal BSS medium, Na^+-free medium (BSS with choline chloride instead of NaCl) or normal medium in the presence of various agents, as described in Materials and Methods. $[^{22}Na]^+$-influx into the cells induced by PTX (10^{-10} M) and $[^{45}Ca]^{2+}$-influx into the cells induced by PTX (3×10^{-8} M) are shown in nmol/well. Values are means ± SEM for 3–4 experiments.

* $P < 0.005$ vs control value.

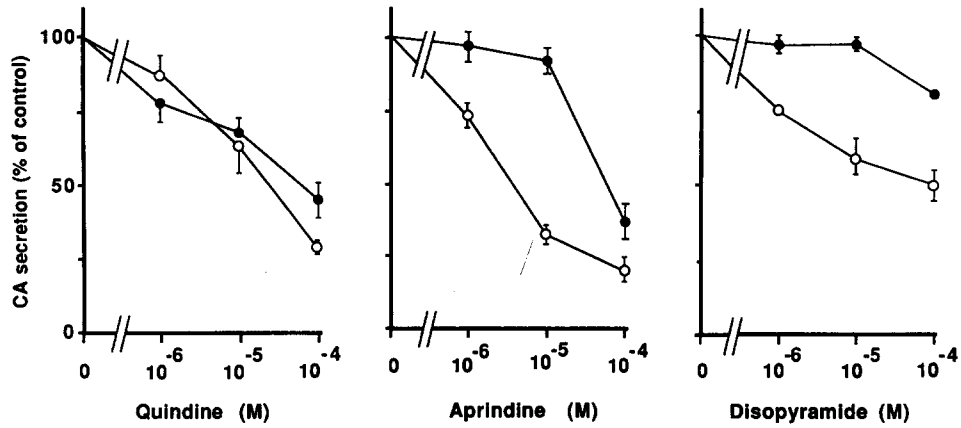

Fig. 4. Effects of quinidine, aprindine and disopyramide on catecholamine secretion induced by PTX and veratridine. Cells were incubated with PTX (3×10^{-8} M) (●) or veratridine (10^{-4} M) (○) at 37° for 10 min in the presence of various concentrations of quinidine, aprindine or disopyramide. Results were expressed as percentages of the control values, which were calculated to be 32.4 ± 0.3%, and 20.9 ± 0.2% of the total cellular content for PTX-, and veratridine-induced secretion, respectively. Points and bars are means ± SEM for three experiments.

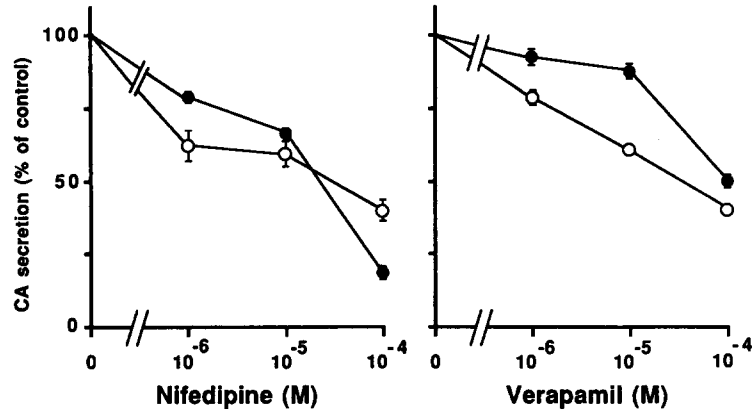

Fig. 5. Effects of nifedipine and verapamil on catecholamine secretion induced by PTX and veratridine. Cells were incubated with PTX (3×10^{-8} M) (●) or veratridine (10^{-4} M) (○) at 37° for 10 min in the presence of various concentrations of nifedipine or verapamil. Results are shown as percentages of the control values, which were calculated to be 32.4 ± 0.3% and 20.9 ± 0.2% of the total cellular content for PTX-, and veratridine-induced secretion, respectively. Points and bars are means ± SEM for three experiments.

CA secretion seemed to be greater than those of other secretagogues such as ACh, nicotine, high K^+ and veratridine [17, 23]. In a recent experiment on perfused bovine adrenal medulla we found that on stimulation with PTX, dopamine-β-hydroxylase in chromaffin granules was secreted together with CA, whereas DOPA-decarboxylase localized in the cytoplasmic fraction was not, indicating that PTX-induced CA secretion occurred in an exocytotic manner (unpublished).

PTX-induced CA secretion required the presence of both Na^+ and Ca^{2+} in the incubation medium, like that induced by veratridine, a voltage-dependent Na^+ channel activator. However, PTX-induced CA secretion was not affected by TTX, which inhibits veratridine-induced CA secretion. This Na^+-dependent, but TTX-insensitive effect of PTX was consistent with reports of the depolarizing effect of PTX on many excitable cells [2, 3] and the stimulatory effect of PTX on norepinephrine release from sympathetic nerve endings [12, 13] or PC-12 cells [16], none of which effects are affected appreciably by TTX.

PTX induced influxes of both $[^{22}Na]^+$ and $[^{45}Ca]^{2+}$ into the cells, and these influxes were also not affected by the presence of TTX. The concentration–response curves for $[^{22}Na]^+$-influx and $[^{45}Ca]^{2+}$-influx induced by PTX were quite different. Increase in $[^{22}Na]^+$-influx was observable at a concentration of 10^{-11} M PTX and about maximal at 10^{-10} M PTX

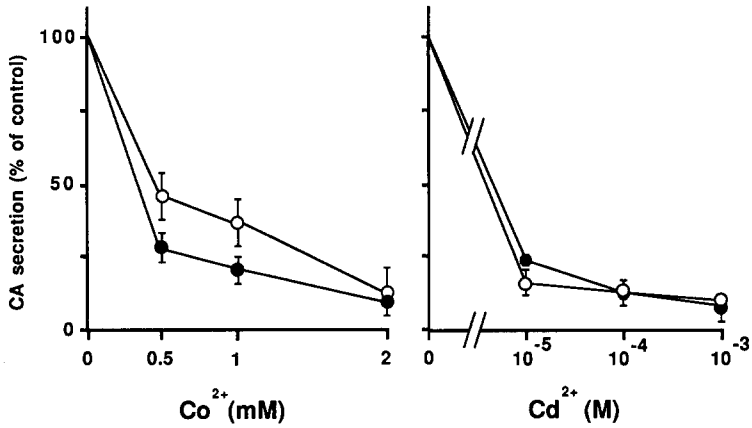

Fig. 6. Effects of Co^{2+} and Cd^{2+} on catecholamine secretion induced by PTX and veratridine. Cells were incubated with PTX (3×10^{-8} M) (●) or veratridine (10^{-4} M) (○) at 37° for 10 min in the presence of various concentrations of Co^{2+} or Cd^{2+}. Results are shown as percentages of the control values, which were calculated to be 32.4 ± 0.3%, and 20.9 ± 0.2% of the total cellular content for PTX-, and veratridine-induced secretion, respectively. Points and bars are means ± SEM for three experiments.

and then decreased at higher concentrations. In contrast, PTX at 10^{-9}–10^{-6} M induced concentration-dependent increase in $[^{45}Ca]^{2+}$-influx into the cells. The extent of increase in $[^{22}Na]^{+}$-influx into the cells induced by 10^{-10} M PTX was similar to that induced by 10^{-4} M veratridine [24–26]. On the other hand, the concentration-dependent increase in $[^{45}Ca]^{2+}$-influx induced by PTX at concentrations of over 10^{-10} M PTX was similar to the concentration–response curve for CA secretion induced by PTX. In PC-12 cells also, PTX induced increase in $[^{22}Na]^{+}$-influx at lower concentrations than those required for increase in $[^{45}Ca]^{2+}$-influx and [^{3}H]norepinephrine secretion [16]. These observations suggest that PTX at low concentrations (10^{-10} M) selectively increased the cell membrane permeability to Na^{+} ion [7] and possibly also increased K^{+} efflux [14, 15], but did not increase permeability to Ca^{2+}, whereas at concentrations of over 10^{-10} M, PTX caused membrane depolarization, resulting from increased Na^{+} permeability, and so increased Ca^{2+} influx into the cells, resulting in initiation of CA secretion from the cells. However, there is a possibility that PTX has two separate effects, one on Na^{+}-influx and the other on Ca^{2+}-influx and that both could be important for the stimulatory effect on secretion depending on the concentration of PTX used. The observation that the accumulation of $[^{22}Na]^{+}$ in the cells decreased with increase in Ca^{2+} influx into the cells may be explained as due to acceleration of the Na^{+}–Ca^{2+} exchange reaction, although acceleration of Na^{+}–K^{+} exchange or Na^{+}–H^{+} exchange could be involved. The exact mechanism of this phenomenon is unknown.

Previous studies showed that the effect of PTX on cell membrane permeability was not ionophoretic, like the effects of the Na^{+}-ionophore monesin and the Ca^{2+}-ionophore A23187 [16]. In this study, to obtain further information on the characteristics of Na^{+}-dependent, TTX-insensitive CA secretion, we examined the effects of antiarrythmic drugs, which are known to block Na^{+}-channels, and Ca^{2+}-channel blockers on the CA secretion induced by PTX. We found that the antiarrythmic drug, quinidine inhibited not only veratridine-induced CA secretion, but also PTX-induced CA secretion in a concentration-dependent manner (10^{-6}–10^{-4} M). Aprindine (mexiletine), another antiarrythmic drug, that also inhibited veratridine-induced CA secretion concentration dependently, inhibited PTX-induced CA secretion at a high concentration of 10^{-4} M. However, disopyramide, another antiarrythmic drug, did not inhibit PTX-induced CA secretion although it inhibited veratridine-induced CA secretion. Quinidine and aprindine also inhibited the influxes of both $[^{22}Na]^{+}$ and $[^{45}Ca]^{2+}$ into the cells induced by PTX. An interesting finding was that quinidine and aprindine inhibited PTX-induced Na^{+}-dependent, but TTX-insensitive CA secretion through their inhibitory effects on Na^{+}- and Ca^{2+}-influxes induced by PTX. The organic Ca^{2+}-channel blockers nifedipine and verapamil and the inorganic Ca^{2+}-channel blockers Co^{2+} and Cd^{2+} all inhibited CA secretion and $[^{45}Ca]^{2+}$ influx induced by PTX, without significantly affecting PTX-induced $[^{22}Na]^{+}$-influx into the cells. The inhibitory effects of the inorganic Ca^{2+}-channel blockers were especially marked. These results suggested that PTX-induced CA secretion was due to increase in Ca^{2+}-influx through activation of Na^{+}-dependent, TTX-insensitive voltage-dependent Ca^{2+}-channels.

It is unknown whether TTX-insensitive Na^{+}-channels are normally present in the membrane of adrenal chromaffin cells or whether these channels are newly formed in the presence of PTX. Although exact mechanism of this Na^{+} entry pathway induced by PTX is not known, PTX should be useful in studies on the membrane cation channel or pathway that regulates CA secretion from adrenal chromaffin cells. Quinidine and aprindine, which inhibit Na^{+}-dependent and TTX-insensitive CA secretion

induced by PTX, should also be useful in these studies.

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