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Mechanism of the Cardiotoxic Action of Palytoxin

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SUMMARY

Palytoxin (PTX) is a non-12-*O*-tetradecanoylphorbol-13-acetate-type tumor promoter that has potent cardiotoxic properties. In embryonic chick ventricular cells, PTX increased $[Ca^{2+}]_i$ ($K_{0.5}$ = 5 nM) in a manner that was dependent on the presence of extracellular Ca^{2+}. The action of PTX was not consequent to its depolarizing action, to the opening of voltage-dependent Ca^{2+} channels, to an intracellular Na^+ load, or to intracellular acidification. Flow cytometric analysis of the $[Ca^{2+}]_i$ distribution in PTX-treated cells showed that only the largest ventricular cells responded to the toxin. All ventricular cells responded to PTX by intracellar acidification. PTX also increased $^{22}Na^+$ uptake by cardiac cells ($K_{0.5}$ = 100 nM) via a pathway that was sensitive to 3,4-dichlorobenzamil ($K_{0.5}$ = 8 μM), suggesting a possible involvement of the Na^+/Ca^{2+} antiporter. We conclude that the action of PTX in chick cardiac cells is distinct from that in erythrocytes or in fibroblasts and that it likely involves several distinct mechanisms. A primary action of PTX could be to open a Ca^{2+} uptake pathway in the plasma membrane, which would then trigger $^{22}Na^+$ uptake by the Na^+/Ca^{2+} antiporter.

PTX is one of the most potent marine toxins known. It has been isolated from marine coelanterate and it consists of a long aliphatic, partially unsaturated chain with interspersed cyclic ether, hydroxyl, and carboxyl groups (1). PTX impairs the function of excitable cells via a complex but still undefined mechanism that includes Na^+ influx, Ca^{2+} influx, and K^+ efflux (2). PTX inhibits the (Na^+,K^+)ATPase in erythrocytes (3) and it has been proposed that all actions of PTX are secondary to its action on (Na^+,K^+)ATPase (2). In addition to these effects, PTX has tumor-promoting properties and it interferes with growth control pathways in fibroblasts, for instance by down-regulating epidermal growth factor action in a manner similar to that of phorbol esters (4, 5). However unlike phorbol esters, PTX does not bind to or activate protein kinase C (6).

In cardiac cells, PTX causes a sustained depolarization, arrhythmia, and contracture (7, 8). In this paper, we analyze the action of PTX on embryonic chick cardiac cells. These cells were chosen because several membrane structures involved in the control of ion movements across the sarcolemmal membrane have been studied in detail. These are the (Na^+,K^+)ATPase (9), the Na^+/H^+ antiporter (10), the Na^+/Ca^{2+} antiporter (9), the $Na^+/K^+/Cl^-$ cotransport system (11), and voltage-dependent Na^+ channels (12). The coupled operation of some of these systems, for instance following treatment of the cells with veratridine or sea anemone toxin (12) or digitalis (9), following intracellular acidification (10), or during the "oxygen paradox" (13), has also been dissected. We document here the changes in $[Ca^{2+}]_i$ and $[Na^+]_i$ induced by PTX and present evidence for a possible coupling mechanism between Na^+ and Ca^{2+} movements across the plasma membrane.

Experimental Procedures

PTX from *Palythoa caribaeorum* was kindly provided by Dr. E. Habermann, Liebig University, Giessen Germany. Dilutions of PTX were prepared in 1% bovine serum albumin solutions and stored at −20° until use. 3,4-DCB and EIPA were synthesized as previously described (14).

Chick ventricular cells were dissociated from 12-day-old chick embryos as previously described (10). The culture medium was Eagle's minimal essential medium (GIBCO) supplemented with 5% charcoal-treated fetal bovine serum (GIBCO), 50 units/ml penicillin, and 200 μg/ml streptomycin.

Incubation solutions used in biochemical experiments were derived from Earle's salt solution (140 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5 mM glucose, buffered at pH 7.4 with 25 mM HEPES-Tris). Na^+-free solutions were obtained by isoosmolar substitution with *N*-methyl-D-glucamine-Cl or KCl. When the external pH was changed, mixtures of Tris, HEPES and (2-[*N*-morpholino]ethane)sulfonic acid were used. Low Ca^{2+} solutions were prepared by using appropriate mixtures of $CaCl_2$ and EGTA.

For intracellular Ca^{2+} measurements, freshly dissociated ventricular cells were loaded for 1 hr with 5 μM indo-1/AM (Boehringer) at 37°, centrifuged, and resuspended in Earle's salt solution. The indo-1 fluo-

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ABBREVIATIONS: PTX, palytoxin; BCECF, 2′,7′-biscarboxyethyl-5(6) carboxyfluorescein; EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EIPA, ethylisopropylamiloride; 3,4-DCB, 3,4-dichlorobenzamil; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; pH_i, intracellular pH; pH_o, extracellular pH; AM, acetoxy methyl ester.

rescence ratio was measured by flow cytometry as previously described (14), using an ATC 3000 cell sorter (Odam Brücker). For intracellular pH measurements, freshly dissociated cells were loaded with 10 μM BCECF/AM (Calbiochem) for 1 hr at 37°, centrifuged, and resuspended in Earle's salt solution. Flow cytometric analysis of the BCECF fluorescence was performed as previously described (15). Cells analyzed by flow cytometry were quiescent.

For $^{22}Na^+$ uptake experiments, ventricular cells were seeded into 24-well tissue culture clusters and used after 2 days of culture. The culture medium was aspirated off and cells were incubated in K^+-free Earle's salt solution supplemented with 1 μCi/ml $^{22}Na^+$ (0.5 Ci/mg; Amersham), 100 nM PTX, and inhibitors. After a 3-min incubation, cells were washed three times with 100 mM $MgCl_2$ and harvested into 0.1 N NaOH, and the cell-associated radioactivity was counted.

Results

PTX promotes Ca^{2+} entry in a variety of excitable cells (7, 16–18), but not in fibroblasts (5) or erythrocytes (19). Fig. 1A shows that the addition of PTX to indo-1-loaded chick cardiac cells induced a large and rapid rise in $[Ca^{2+}]_i$. The $K_{0.5}$ value for PTX action on $[Ca^{2+}]_i$ was 5 nM (Fig. 1B). Fig. 1A further shows that, when the incubation solution contained 50 nM free Ca^{2+}, PTX had no effect on $[Ca^{2+}]_i$. Conversely when PTX-treated cells were exposed to EGTA to reduce free $[Ca^{2+}]_o$ to 50 nM, $[Ca^{2+}]_i$ levels returned to the basal control level within 2 min. These results suggested that PTX opened a Ca^{2+}-uptake pathway in the plasma membrane of chick cardiac cells. It could be a direct or an indirect action. One possibility for an indirect action of PTX is that, as previously described for toxins that stabilize an open form of the voltage-dependent Na^+ channels (12), PTX increased the membrane permeability to Na^+, loaded cells with Na^+, and triggered Ca^{2+} uptake by the Na^+/Ca^{2+} exchange system. This was checked by assaying the action of PTX under Na^+-free conditions. Fig. 2 shows that dilution of chick cardiac cells in a Na^+-free medium (using *N*-methyl-D-glucamine as a substitute) increased $[Ca^{2+}]_i$, probably because internal Na^+ was exchanged for external Ca^{2+} by the Na^+/Ca^{2+} exchange system. As expected, this action was dependent on the presence of external Ca^{2+} (Fig. 2). The addition of PTX 6 min after the shift to the Na^+-free solution still increased $[Ca^{2+}]_i$ to a large extent. Again, this action of PTX was not observed when $[Ca^{2+}]_o$ was 50 nM (Fig. 2). This experiment clearly indicated that the action of PTX on $[Ca^{2+}]_i$ could not be a consequence of a Na^+ load and was not mediated by Ca^{2+} influx via the Na^+/Ca^{2+} exchanger.

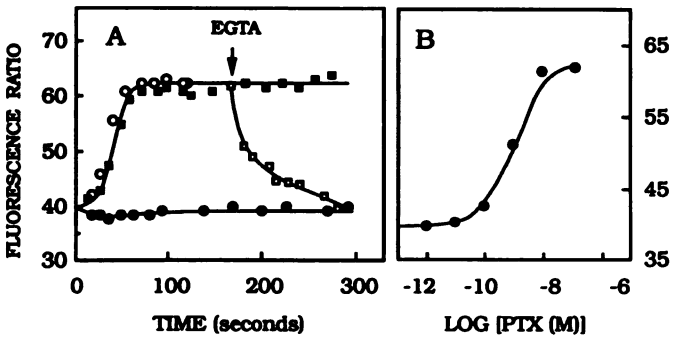

Fig. 1. Increase in $[Ca^{2+}]_i$ in chick cardiac cells by PTX. A, Cells were equilibrated in 140 mM Na^+ solutions in the absence (■, ●) or the presence (○) of 1 μM (±)-verapamil and in the presence of 1.8 mM external Ca^{2+} (■, ○) or of 50 nM Ca^{2+} (●). PTX (100 nM) was added at time 0. At the time indicated by the *arrow*, cells incubated in the 1.8 mM Ca^{2+} solution were treated with EGTA to reduce $[Ca^{2+}]_o$ to 50 nM (□). B, Dose-response curve for PTX action on $[Ca^{2+}]_i$. Indo-1 fluorescence was measured 3 min after the addition of different concentrations of PTX to cells equilibrated in Earle's salt solution.

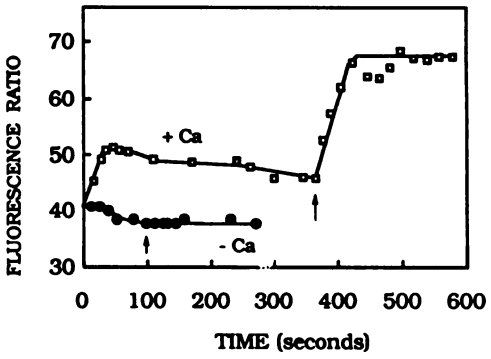

Fig. 2. Lack of prevention of PTX action on $[Ca^{2+}]_i$ by Na^+-free conditions. Cells were diluted into a 140 mM *N*-methyl-D-glucamine Earle's salt solution containing 1.8 mM Ca^{2+} (□) or 50 nM Ca^{2+} (●). At the times indicated by the *arrows*, 100 nM PTX was added.

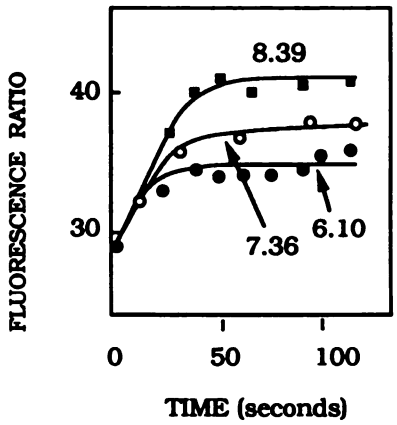

Fig. 3. Action of PTX on $[Ca^{2+}]_i$ at different external pH values. Na^+-depleted cells were incubated in a 145 mM K^+ modified Earle's salt solution at pH 6.10 (●), 7.36 (○), or 8.39 (■) and were treated with 100 nM PTX. All experiments were performed in the presence of 1 μM (±)-verapamil to block the activity of L-type Ca^{2+} channels.

Another indirect mechanism by which PTX could increase $[Ca^{2+}]_i$ is by depolarizing cells and opening voltage-dependent Ca^{2+} channels (2, 20). This hypothesis was unlikely for the following reasons. First, the previous experiments already showed that a large effect of PTX on $[Ca^{2+}]_i$ could be observed when cells were incubated under Na^+-free solutions. These conditions prevent the depolarization induced by PTX (8). Second, an involvement of L-type or T-type Ca^{2+} channels was ruled out by the observations that neither (±)-verapamil (Fig. 1A) nor divalent cations (Zn^{2+}, Cd^{2+}, Mn^{2+}, and Co^{2+}, tested at 1 mM) prevented the action of PTX on $[Ca^{2+}]_i$. Third, depolarizing conditions, obtained by incubation of the cells in a 145 mM K^+ solution, did not promote an increase in $[Ca^{2+}]_i$ comparable to that produced by PTX. Fourth, PTX still increased $[Ca^{2+}]_i$ in cells that had been depolarized by incubation in a 145 mM K^+ solution (Fig. 3). These results indicated (i) that depolarizing membrane conditions did not mimic the action of PTX on $[Ca^{2+}]_i$ and (ii) that changes in membrane potential did not alter the action of PTX. These were good indications that the action of PTX on $[Ca^{2+}]_i$ could not be consequent to its depolarizing action.

Another action of PTX in chick cardiac cells is to increase

the membrane permeability to H^+ (15). This leads to intracellular acidification and to a secondary activation of the Na^+/H^+ antiporter. As a consequence, the acidifying action of PTX is enhanced under Na^+-free conditions and by derivatives of amiloride that prevent Na^+/H^+ exchange activity (15). It could be that the action of PTX on $[Ca^{2+}]_i$ was consequent to its acidifying action. It is well known that a close relationship exists between $[Ca^{2+}]_i$ and pH_i, possibly because Ca^{2+} and H^+ share common intracellular buffering sites (21). One way to test the hypothesis that PTX-induced changes in $[Ca^{2+}]_i$ were consequent to changes in pH_i is to analyze the action of PTX on $[Ca^{2+}]_i$ in K^+-depolarized cells that were incubated in solutions of different pH values. It has already been reported that, in K^+-depolarized chick cardiac cells, PTX produced opposite effects on pH_i depending on the value of pH_o (15). When pH_o was <7.4, a cellular acidification was observed. When it was >7.4, a cellular alkalinization was observed. Finally, when pH_o was 7.4, no change in pH_i was observed. This is simply a consequence of the fact that, under depolarizing membrane conditions, the action of PTX is to equilibrate pH_i with pH_o (15). Fig. 3 shows that, in K^+-depolarized cells, the action of PTX on $[Ca^{2+}]_i$ was larger when the pH of the incubation solution was alkaline. The important point is, however, that PTX increased $[Ca^{2+}]_i$ irrespective of the value of pH_o and that this action did not correlate with the action of PTX on pH_i. The obvious conclusion is, therefore, that the PTX-induced change in $[Ca^{2+}]_i$ could not be a consequence of the acidifying action of PTX.

One advantage of the flow cytometry technique is that it provides information about the distribution of $[Ca^{2+}]_i$ values in the cell population analyzed. In all conditions in which PTX increased $[Ca^{2+}]_i$, we observed that the distribution of $[Ca^{2+}]_i$ values was heterogeneous. For instance, Fig. 4C shows a unimodal distribution of $[Ca^{2+}]_i$ values in the control cardiac cell population. After treatment with PTX, the distribution became bimodal (Fig. 4D). Only one third of the population increased its Ca^{2+} content in response to PTX. This is good evidence that PTX did not cause holes to form in the membranes of cells and did not increase the membrane permeability to ions in a nonspecific manner. The results confirm that PTX had no ionophore properties by itself (2, 18). Fig. 4, A and B, compares the pH_i distributions in control and PTX-treated cells. PTX shifted the whole distribution of pH_i values to more acidic values. Thus, the cellular heterogeneity in the $[Ca^{2+}]_i$ response was not accompanied by a cellular heterogeneity in the pH_i response. Fig. 5, *left*, shows the size distribution of the ventricular cell population. Four classes of cells were defined (Fig. 5, *left*). Class 1 merely represents cell debris that arose during the dissociation procedure. We checked that cells of the size classes 2–4 had identical mean indo-1 fluorescence ratios before the addition of PTX. Fig. 5, *right*, shows the distributions of the indo-1 fluorescence ratio, 3 min after the addition of 100 nM PTX. The $[Ca^{2+}]_i$ distribution in the small class 2 cells was nearly identical to that of control untreated cells, indicating that few of the class 2 cells responded to PTX. Conversely, in the larger class 4 cells, PTX induced a large shift in the $[Ca^{2+}]_i$ distribution. Further analysis of the data showed that 9% of class 2 cells, 32% of class 3 cells, and 63% of class 4 cells responded to PTX with an increase in $[Ca^{2+}]_i$. Thus, PTX increased $[Ca^{2+}]_i$ in the largest cells of the ventricular cell population. Knowing that a ventricular cell population is composed of both muscle and nonmuscle cells (often referred to as fibroblasts) and that muscle cells are much larger than fibroblasts (22), the results suggest that PTX acted specifically to raise $[Ca^{2+}]_i$ in cardiomyocytes. This conclusion agrees with the observation that, in 3T3 fibroblasts, PTX had no action on $[Ca^{2+}]_i$ (5).

PTX activated a $^{22}Na^+$ flux component in chick cardiac cells that was as important as the $^{22}Na^+$ uptake component activated by a mixture of veratridine and *Anemonia sulcata* toxin II (15). The PTX-activated $^{22}Na^+$ flux component was insensitive to tetrodotoxin. It was suppressed by lowering of $[Ca^{2+}]_o$ to 50 nM. Fig. 6A shows that, as previously described (15), the PTX-activated $^{22}Na^+$ flux component could be partly suppressed by EIPA, an inhibitor of the Na^+/H^+ antiporter. Fig. 6A further shows that, in the presence of EIPA to block Na^+/H^+ exchange

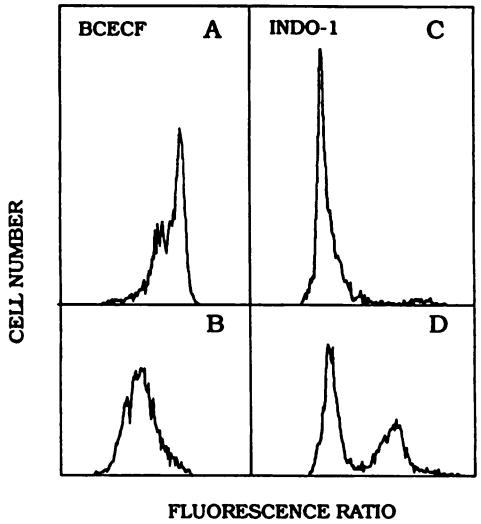

Fig. 4. Distribution of indo-1 and BCECF fluorescence ratios in control and PTX-treated cells. A and B, Distribution of the BCECF fluorescence ratio in chick ventricular cells before (A) and 3 min after (B) the addition of 100 nM PTX. The incubation solution was a Na^+-free Earle's solution. Acidic pH_i values correspond to low values of the BCECF fluorescence ratio. C and D, Distribution of the indo-1 fluorescence ratio in chick ventricular cells before (C) and 3 min after (D) the addition of 100 nM PTX. The incubation solution was 140 mM Na^+ Earle's solution. Histograms displayed in each panel are based on the analysis of 5000 cells.

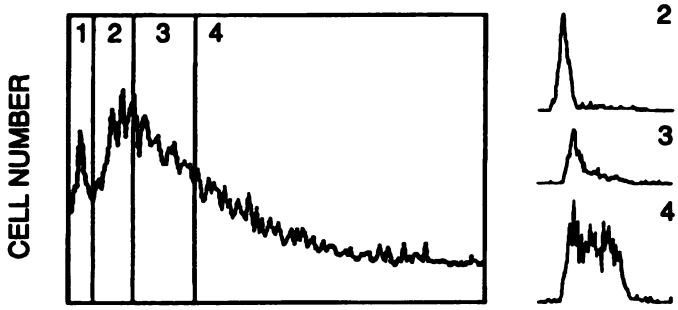

Fig. 5. Preferential response of large ventricular cells to PTX by an increase in $[Ca^{2+}]_i$. *Left*, distribution of the cell volumes in a population of freshly dissociated chick ventricular cells. The *vertical lines* define four classes of cell size, numbered *1* to *4*. Class 1 merelyl represents cell debris that arose during the dissociation procedure. *Right*, distribution of the indo-1 fluorescence ratio in PTX (100 nM, 3 min)-treated ventricular cells of the size classes defined in the *left panel*. All data in Figs. 4 and 5 are from the same batch of cells.

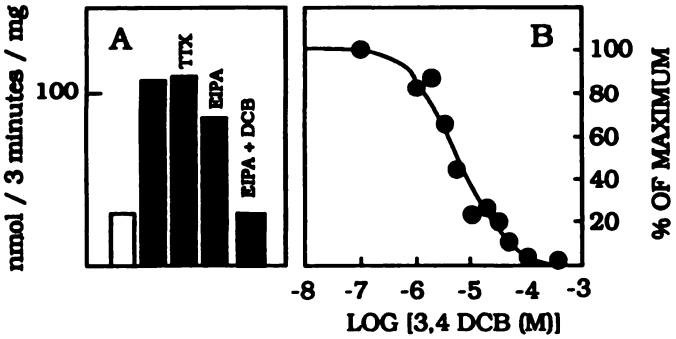

Fig. 6. Reversal by 3,4-DCB of PTX action on $^{22}Na^+$ uptake. A, Influences of 1 μM tetrodotoxin (*TTX*), 10 μM EIPA, and 0.1 mM 3,4-DCB on PTX (100 nM)-activated $^{22}Na^+$ uptake. Time of uptake was 3 min. B, Dose-response curve for 3,4-DCB inhibition of the PTX-activated $^{22}Na^+$ uptake. Experiments were performed in the presence of 10 μM EIPA.

activity, the PTX-activated $^{22}Na^+$ flux component could be completely suppressed by 3,4-DCB, an inhibitor of the Na^+/Ca^{2+} antiporter. The $K_{0.5}$ value for 3,4-DCB inhibition of the PTX-activated $^{22}Na^+$ flux component was 8 μM (Fig. 6B).

Discussion

PTX has a diversity of actions in cardiac cells. It increases the membrane permeability to Na^+, produces a depolarization, and increases $[Ca^{2+}]_i$. An important point is to determine whether these actions are independent of each other or whether they are consequences of a primary action that has still to be identified. Habermann (2), on the basis of detailed investigations in erythrocytes, proposed that the primary action of PTX is to bind to the (Na^+,K^+)ATPase. It would then convert the enzyme into an open channel permeable to Na^+ and K^+. The resulting depolarization was proposed to open voltage-dependent Ca^{2+} channels in excitable cells and to increase $[Ca^{2+}]_i$ (2, 20). Partial evidence for such a mechanism is that PTX opens a nonselective channel that is permeable to Na^+ and K^+, but not to Ca^{2+}, in cardiomyocytes from guinea pig and rat (20, 23). This channel was not shown, however, to be an altered form of the (Na^+,K^+)ATPase. The data reported here indicate that, in chick cardiac cells, PTX increased $[Ca^{2+}]_i$ in a manner that was dependent on external Ca^{2+} but that did not involve L-type or T-type Ca^{2+} channels and that was not consequent to its depolarizing action. This is because (i) depolarizing membrane conditions did not mimic PTX action on $[Ca^{2+}]_i$ and (ii) PTX action on $[Ca^{2+}]_i$ was observed both under polarized membrane conditions (when the incubation solutions contained 140 mM *N*-methyl-D-glucamine) and under depolarized membrane conditions (when the incubation solutions contained 140 mM Na^+ or 145 mM K^+). The observations (i) that PTX increased $[Ca^{2+}]_i$ when cells were incubated in Na^+-free solutions and (ii) that the $K_{0.5}$ value for PTX action on $[Ca^{2+}]_i$ (5 nM) (Fig. 1B) was much lower than the $K_{0.5}$ value for PTX action on $^{22}Na^+$ uptake (100 nM) (15) further indicated that PTX did not increase $[Ca^{2+}]_i$ by loading cells with Na^+ and by triggering Ca^{2+} uptake by the Na^+/Ca^{2+} exchange system. Thus, PTX increased $[Ca^{2+}]_i$ by a mechanism that was distinct from the mechanism proposed by Habermann (2) and from the mechanism of action of toxins of the voltage-dependent Na^+ channels (12). Sauviat (24) recently showed that in voltage-clamped frog atrial fibers incubated in a Na^+-free solution, PTX shifted the Ca^{2+} current-voltage curve towards more negative potentials. This action is consistent with an $[Na^+]_o$-independent intracellular accumulation of Ca^{2+}. Taken together, these observations, thus, suggest that PTX opened a membrane channel that was permeable to Ca^{2+}, that was normally quiescent, and that was probably not voltage dependent. It could belong to the still poorly defined class of "receptor-operated channels," such as the ones that are activated by vasoconstricting hormones in aortic myocytes (25, 26) and by ADP in platelets (27).

Another action of PTX in chick cardiac cells is to increase the membrane permeability to H^+ (15). Knowing that PTX-induced changes in $[Ca^{2+}]_i$ were not consequences of PTX-induced changes in pH_i (Fig. 3), we asked whether changes in pH_i were consequences of the changes in $[Ca^{2+}]_i$. The flow cytometric data reported in Fig. 4 show that the action of PTX on $[Ca^{2+}]_i$ was restricted to one third of the cell population analyzed, whereas changes in pH_i were observed in all cells. These results clearly indicate that the acidifying action of PTX cannot be a consequence of its action on $[Ca^{2+}]_i$ and suggest that PTX had two independent targets in chick cardiac cells, a H^+-conducting pathway that was present in all cells and a Ca^{2+}-conductive pathway that was mainly located in the largest cells. $K_{0.5}$ values for PTX actions on pH_i and on $[Ca^{2+}]_i$ were identical (5 nM), suggesting a similar sensitivity of the two ion-transporting systems to PTX.

A third action of PTX in cardiac cells is to increase the membrane permeability to Na^+. This leads to its well known depolarizing action (8). The uptake pathway involved may be the channel characterized by patch-clamp experiments in guinea pig and rat cardiomyocytes (20, 23). It is a voltage-independent, nonselective channel that is permeable to Na^+ and K^+ but not to Ca^{2+}. Opening of this channel by PTX is observed in the 10–30 pM range of concentrations (20, 23). We show here that PTX increased the rate of $^{22}N^+$ uptake by chick cardiac cells. The $K_{0.5}$ value for PTX action was, however, 100 nM (15). The difference between the two values suggests either that the channels characterized in patch-clamp experiments were not present in chick cardiac cells or that they did not contribute to the measured flux. It is well known that most of the toxins that affect the opening of voltage-dependent Na^+ channels in excitable cells, as measured by electrophysiology (peptide neurotoxins from sea anemone or scoprion venoms, alkaloid toxins, and pyrethroids), have almost no action on $^{22}Na^+$ uptake by themselves. Their action is only observed when mixtures of synergistic toxins are used to stabilize an open form of the channel (28).

The PTX-activated $^{22}Na^+$ flux component had characteristic pharmacological properties. It was insensitive to tetrodotoxin and was partially sensitive to EIPA and 3,4-DCB, two derivatives of amiloride that are the best inhibitors known so far of the Na^+/H^+ antiporter and the Na^+/Ca^{2+} antiporter, respectively (29). The inhibitory action of EIPA is due to the fact that PTX, via its acidifying action, activates Na^+/H^+ exchange activity (15). Similarly, it could be that the PTX-activated and 3,4-DCB-inhibitable $^{22}Na^+$ flux component was a consequence of the Ca^{2+} load imposed by PTX and was due to Na^+/Ca^{2+} exchange activity. The observations (i) that the PTX-activated $^{22}Na^+$ uptake component was dependent on external Ca^{2+} and (ii) that the $K_{0.5}$ value for 3,4-DCB inhibition of the rate of $^{22}Na^+$ uptake (8 μM) (Fig. 6) was similar to the value reported for 3,4-DCB inhibition of Na^+/Ca^{2+} exchange in cardiac vesicles

(30) are consistent with this hypothesis. The report by Sauviat (24) that, in voltage-clamped frog atrial fibers, PTX increased the magnitude and duration of the tail current is also consistent with this hypothesis. The Na^+/Ca^{2+} exchanger is a major membrane ion exchange system in cardiac cells. It operates in a bidirectional way and is regulated by transmembrane Na^+ and Ca^{2+} gradients and by the membrane potential (31). Its main function is to promote Ca^{2+} efflux during repolarization. A decrease in the transmembrane Na^+ gradient, either by loading of cells with Na^+ (for instance in response to digitalis or to toxins of the voltage-dependent Na^+ channel) or by reduction of $[Na^+]_o$ (9, 12, 13), is well known to promote Ca^{2+} influx by the exchanger. Conversely, a decrease in the transmembrane Ca^{2+} gradient, either by an increase in $[Ca^{2+}]_i$ or by a decrease in $[Ca^{2+}]_o$, raised $[Na^+]_i$ (32). Although all data presented here are consistent with the proposal that most of the $^{22}Na^+$ that entered cells in response to PTX was via the Na^+/Ca^{2+} exchange system, they do not prove it unambiguously. 3,4-DCB has multiple sites of action in cardiac cells that are not all related to the blockade of the Na^+/Ca^{2+} antiporter (33, 34). On the other hand, the hypothesis implies that Na^+ influx was in the same cells as the ones that responded to PTX by a rise in $[Ca^{2+}]_i$. This could not be demonstrated at the present time.

The mechanism of action of PTX is clearly different from that of toxins that open voltage-dependent Na^+ channels. Two key experiments distinguish the two mechanisms. First, Na^+-free conditions prevent Ca^{2+} accumulation in response to toxins of the voltage-dependent Na^+ channel (12); they do not prevent Ca^{2+} accumulation in response to PTX (Fig. 2). Second, Ca^{2+}-free conditions do not prevent veratridine activation of $^{22}Na^+$ uptake; they block PTX activation of $^{22}Na^+$ uptake. In PC12 cells, PTX was reported to increase both $^{22}Na^+$ and $^{45}Ca^{2+}$ influxes in a tetrodotoxin-insensitive manner (18). The observation that Na^+-free conditions did not suppress the PTX-activated $^{45}Ca^{2+}$ flux component in PC12 cells (18) suggests that PTX had very similar actions in chick cardiac cells and in PC12 cells. A different mechanism of PTX action was observed in 3T3 fibroblasts (5, 6). In these cells, low concentrations of PTX increased $^{22}Na^+$ uptake without modifying either $[Ca^{2+}]_i$ or pH_i. Taken together, these results, therefore, suggest that PTX has different mechanisms of action in different cell types. This proposal, which means that PTX probably recognizes different receptor sites that have different tissue distributions, should not be surprising. It is well known that tetrodotoxin and sea anemone toxins recognize different forms of voltage-dependent Na^+ channels that may even be present in the same cell type (35). It is also well known that a variety of hormones (epinephrine, histamine, serotonin, acetylcholine, etc.) act via several subtypes of membrane receptors that are coupled to different signal-transducing pathways and that may even be structurally unrelated. We cannot exclude, however, the possibility that the PTX preparations used in most studies were mixtures of closely related structures that have different targets in the plasma membrane. The actions of PTX reported here were observed in a range of concentrations at which PTX alters cardiac contractility (2). The actions of PTX on $[Ca^{2+}]_i$, on pH_i and the resulting actions on Na^+/H^+ exchange, and possibly on Na^+/Ca^{2+} exchange can all contribute to the cardiotoxicity of PTX.

Acknowledgments

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