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Tetrodotoxin-Insensitive Na^+ Channel Activator Palytoxin Inhibits Tyrosine Uptake into Cultured Bovine Adrenal Chromaffin Cells

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SUMMARY

The effects of the tetrodotoxin-insensitive Na^+ channel activator palytoxin on both the secretion of endogenous catecholamines and the formation of ^{14}C-catecholamines from [^{14}C]tyrosine were examined using cultured bovine adrenal chromaffin cells. Palytoxin was shown to cause the stimulation of catecholamine secretion in a concentration-dependent manner. However, this toxin caused the reduction rather than the stimulation of ^{14}C-catecholamine formation at the same concentrations. Palytoxin failed to cause any alteration in the activity of tyrosine hydroxylase prepared from bovine adrenal medulla. Furthermore, the uptake of [^{14}C]tyrosine into the cells was shown to be inhibited by this toxin under the conditions in which the suppression of ^{14}C-catecholamine formation was observed, and this inhibitory action on tyrosine uptake was closely correlated with that on catecholamine formation. The inhibitory action of palytoxin on tyrosine uptake into the cells was observed to be noncompetitive, and this effect was not altered by the removal of Na^+ from the incubation mixture. These results suggest that palytoxin may be able to inhibit the uptake of [^{14}C]tyrosine into the cells, resulting in the suppression of ^{14}C-catecholamine formation, probably through its direct action on the plasma membranes of bovine adrenal chromaffin cells.

Cultured adrenal chromaffin cells have been widely employed to investigate a possible role of ion channels in stimulus-secretion coupling, which is a series of events that is initiated by stimulation of the plasma membranes and results in the exocytotic secretion of catecholamines and other materials stored in chromaffin granules. The stimulation of chromaffin cells by different secretagogues is well known to stimulate the influx of extracellular Ca^{2+} into the cells, leading to a rise in the intracellular Ca^{2+} concentration, thus stimulating the subsequent process of exocytosis. The influx of extracellular Ca^{2+} into the cells has previously been shown to be mediated by two different types of Ca^{2+} channels, i.e., voltage-dependent and receptor-operated Ca^{2+} channels (1, 2). On the other hand, the stimulation of chromaffin cells by cholinergic agonists has been shown to increase the influx of Na^+ as well as Ca^{2+} into the cells. Furthermore, various neurotoxins, such as VTD, aconitine, batrachotoxin, and scorpion venom, have previously been shown to cause catecholamine secretion as a result of the activation of tetrodotoxin-sensitive Na^+ channels, which was thought to be accompanied by the stimulation of Ca^{2+} influx into the cells through voltage-dependent Ca^{2+} channels (3, 4). It thus seems quite reasonable to conclude that Na^+ channels are closely linked to Ca^{2+} channels and, therefore, play an important role in stimulus-secretion coupling in the adrenal chromaffin cell.

In the earlier studies, various marine toxins have been shown to cause an alteration in the ion permeability of cell membranes (5–8). A potent marine toxin isolated from the zoanthid *Palythoa* species, palytoxin, has, furthermore, been found to depolarize the plasma membranes in various excitable tissues in a Na^+-dependent but tetrodotoxin-insensitive manner (9–13). Palytoxin has previously been shown to stimulate the release of norepinephrine from adrenergic nerve endings (14). The stimulatory action of palytoxin on catecholamine secretion has been observed in clonal rat pheochromocytoma PC12 cells (15) and cultured bovine adrenal chromaffin cells (16), and this effect has, furthermore, been suggested to be mediated by an increase in tetrodotoxin-insensitive Na^+ influx into the cells. In view of these findings, this toxin is thought to depolarize the plasma membranes through activation of tetrodotoxin-insensitive Na^+ channels, leading to the activation of voltage-dependent Ca^{2+} channels and, thus, resulting in the stimulation of catecholamine release from the adrenergic tissue.

The stimulation of catecholamine secretion is generally known to be accompanied by an increase in the ability for catecholamine biosynthesis in adrenergic tissues (17). It, therefore, seems possible that palytoxin can stimulate the biosynthesis as well as the release of catecholamines in the adrenal medulla. In the present study, the effect of palytoxin on catecholamine biosynthesis was investigated in cultured bovine adrenal chromaffin cells, and ^{14}C-catecholamine formation from [^{14}C]tyrosine was found to be unexpectedly inhibited,

ABBREVIATIONS: VTD, veratridine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N*^{1},*N*′-tetraacetic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; $DMPH_4$, 6,7-dimethyl-5,6,7,8-tetrahydropterin; DOPA, 3,4-dihydroxyphenylalanine.

rather than stimulated, by the toxin under the conditions in which the stimulation of catecholamine secretion was observed. This suppression of ^{14}C-catecholamine formation was, furthermore, shown to be due to the inhibition of [^{14}C]tyrosine uptake into the cells. Thus, the first evidence for the inhibitory action of palytoxin on the tyrosine transport system in adrenergic cells is provided here.

Experimental Procedures

Cell preparation and culture. Chromaffin cells were enzymatically prepared from fresh bovine adrenal medulla according to the method reported previously (18). Isolated cells were plated on 24-well plastic cluster plates, at a density of 5×10^5 cells/well, and maintained for 3 or 4 days as monolayer cultures at 37°, in a humidified atmosphere containing 5% CO_2, in 1.5 ml of Eagle's minimum essential medium containing 5% heat-inactivated newborn calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, 2 μg/ml fungizone, and 10 μM cytosine arabinoside.

Determination of ^{14}C-catecholamine formation. Cells were washed with 1 ml of balanced salt solution (135 mM NaCl, 5.6 mM KCl, 1.2 mM $MgCl_2$, 2.2 mM $CaCl_2$, 10 mM glucose, 20 mM HEPES, pH 7.35) and then incubated with palytoxin at 37° for 30 min, in 500 μl of balanced salt solution containing 20 μM L-[^{14}C]tyrosine (0.25 μCi/well). At the end of incubation period, the medium was removed by aspiration, and the cells were washed twice with 1 ml of ice-cold balanced salt solution and then lysed by addition of 500 μl of 0.4 M perchloric acid and subjection to a freeze-thaw cycle. The cell lysates were centrifuged at 9000 × *g* for 5 min, and the amounts of ^{14}C-catecholamines in the acid extracts were then determined as reported previously (19).

Determination of [^{14}C]tyrosine uptake. Cells were incubated at 0° and 37° for 30 min in the mixture containing L-[^{14}C]tyrosine, with or without palytoxin, washed with ice-cold balanced salt solution as described above, and lysed by addition of 250 μl of 0.4 M perchloric acid, followed by a freeze-thaw cycle. Radioactivities in the acid extracts were determined, and the amount of tyrosine taken up into the cells was calculated on the basis of the specific activity of [^{14}C]tyrosine in the incubation mixture. Tyrosine uptake (expressed as the temperature-dependent fraction) was calculated by subtraction of the values obtained at 0° from those obtained at 37°.

Determination of catecholamine release. Cells were incubated at 37° for 30 min in 250 μl of balanced salt solution containing palytoxin. The medium was removed at the end of incubation period, and the cells were then lysed by addition of 250 μl of 10% acetic acid, followed by a freeze-thaw cycle. Both the medium and the cell lysates were centrifuged at 9000 × *g* for 2 min, and the amounts of catecholamines in the supernatant fractions were determined as described previously (20, 21). Catecholamine secretion was expressed as the percentage of total cellular catecholamine content released during the incubation period.

Determination of tyrosine hydroxylase activity. Tyrosine hydroxylase was prepared from bovine adrenal medulla according to the method reported previously (22), and the enzyme activity was determined by measurement of the amount of L-DOPA formed from L-tyrosine during the incubation. The mixture, containing 200 mM Tris-acetate (pH 6.0), 0.1 mg (1700 units) of catalase, 100 mM 2-mercaptoethanol, 0.2 mM L-tyrosine, 1 mM $DMPH_4$, and the enzyme (approximately 100 μg of protein) in a final volume of 500 μl, was incubated at 37° for 10 min, and the reaction was then stopped by addition of 500 μl of 1 M perchloric acid. The mixture was centrifuged at 9000 × *g* for 5 min, and the amount of DOPA contained in the supernatant fraction was measured by a high performance liquid chromatography method, as reported previously (23).

To keep the solution isotonic, NaCl in the Na^+-free medium was substituted by equimolar choline chloride, and the Ca^{2+}-free medium contained 1 mM EGTA to remove trace amounts of Ca^{2+}.

Standard deviation (SD) of the difference between two groups was calculated as $(SD_1^2 + SD_2^2)^{1/2}$, and Student's *t* test was used to determine statistical significance.

Chemicals. L-[U-^{14}C]Tyrosine was obtained from New England Nuclear. $DMPH_4$ was obtained from Aldrich Chemical Co. VTD and monensin were from Sigma Chemical Co. Other chemicals were of commercially available reagent grade. Palytoxin was a kind gift from Dr. I. Muramatsu of Fukui Medical School (Fukui, Japan).

Results

The effect of palytoxin on secretory function was examined using cultured bovine adrenal chromaffin cells. As shown in Fig. 1, catecholamine secretion was markedly stimulated by palytoxin, and this effect was observed in a manner dependent on its concentration. Approximately 35% of cellular catecholamine content was released by 10^{-9} M palytoxin, and the maximum effect (approximately 60% of endogenous catecholamines released) was obtained at about 10^{-6} M. Under these conditions, the formation of ^{14}C-catecholamines from [^{14}C]tyrosine was suppressed rather than stimulated by palytoxin, and this formation was almost completely suppressed at 10^{-6} M. In contrast, both catecholamine release and ^{14}C-catecholamine formation were stimulated by VTD, an activator of voltage-dependent Na^+ channels (Fig. 2). Thus, this toxin was shown to cause suppression of ^{14}C-catecholamine formation from [^{14}C]tyrosine in cultured bovine adrenal chromaffin cells under the conditions in which the stimulation of catecholamine release was observed.

To elucidate a possible mechanism of the inhibitory action on ^{14}C-catecholamine formation observed here, the direct effect of palytoxin on tyrosine hydroxylase activity was then studied, using enzyme prepared from bovine adrenal medulla. As shown in Fig. 3, the addition of this toxin to the incubation mixture failed to cause any notable change in enzyme activity at the concentrations producing inhibition of ^{14}C-catecholamine formation in intact cells. Palytoxin was clearly shown to have no direct effect on tyrosine hydroxylase activity *in vitro*, thus suggesting the possibility that the suppression of ^{14}C-catecholamine formation observed here might not be related to the inhibition of this enzyme within the cells.

To test the possibility that the suppression of ^{14}C-catecholamine formation by palytoxin was due to the reduction of precursor supply to the catecholamine biosynthetic pathway in chromaffin cells, the effect of this toxin on [^{14}C]tyrosine uptake, as well as ^{14}C-catecholamine formation, was then investigated. As shown in Fig. 4, palytoxin caused a marked decrease in the uptake of [^{14}C]tyrosine into the cells under the conditions in which the suppression of ^{14}C-catecholamine formation was observed, and the suppression of ^{14}C-catecholamine formation by palytoxin was also shown to correlate with that of [^{14}C]tyrosine uptake. As shown in Fig. 5, the values for [^{14}C]tyrosine uptake and ^{14}C-catecholamine formation obtained in the presence of different concentrations of this toxin could be fit to a straight line, with a correlation coefficient of 0.942. The reduction of ^{14}C-catecholamine formation caused by palytoxin was, therefore, considered to be due to inhibition of [^{14}C]tyrosine uptake into the cells. In contrast, VTD was found to cause a significant increase in ^{14}C-catecholamine formation but failed to have any effect on [^{14}C]tyrosine uptake into the cells. In this case, a correlation between these parameters was, therefore, not observed (data not shown).

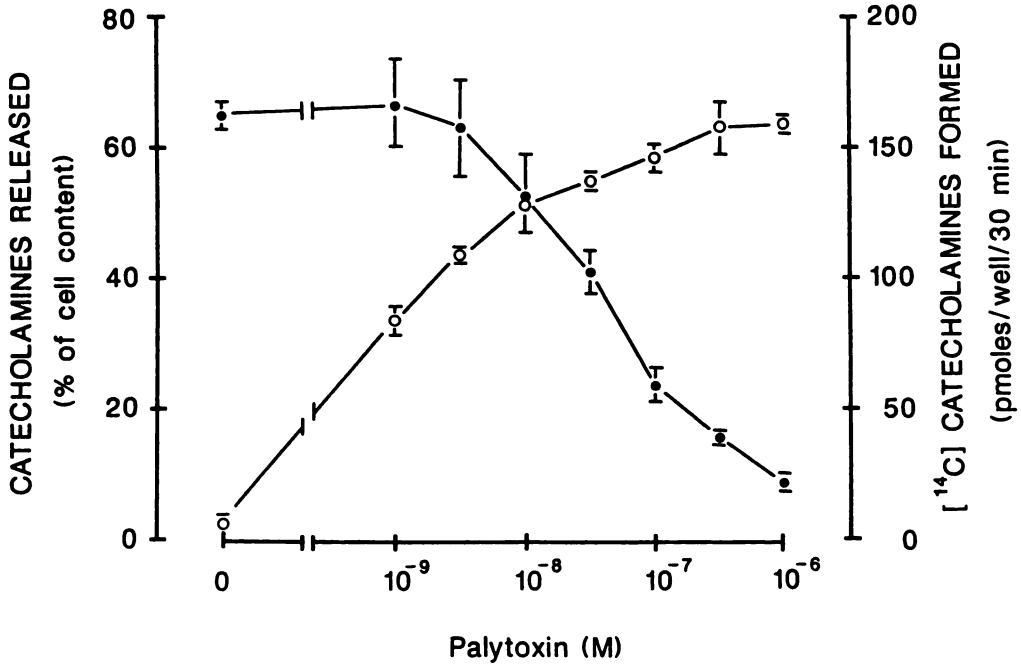

Fig. 1. Effects of palytoxin on both catecholamine secretion and ^{14}C-catecholamine formation in cultured bovine adrenal chromaffin cells. Cells were incubated with different concentrations of palytoxin, and catecholamine secretion (○) and ^{14}C-catecholamine formation from [^{14}C] tyrosine (●) were independently determined, as described in the text. Values are the mean ± standard deviation of three experiments.

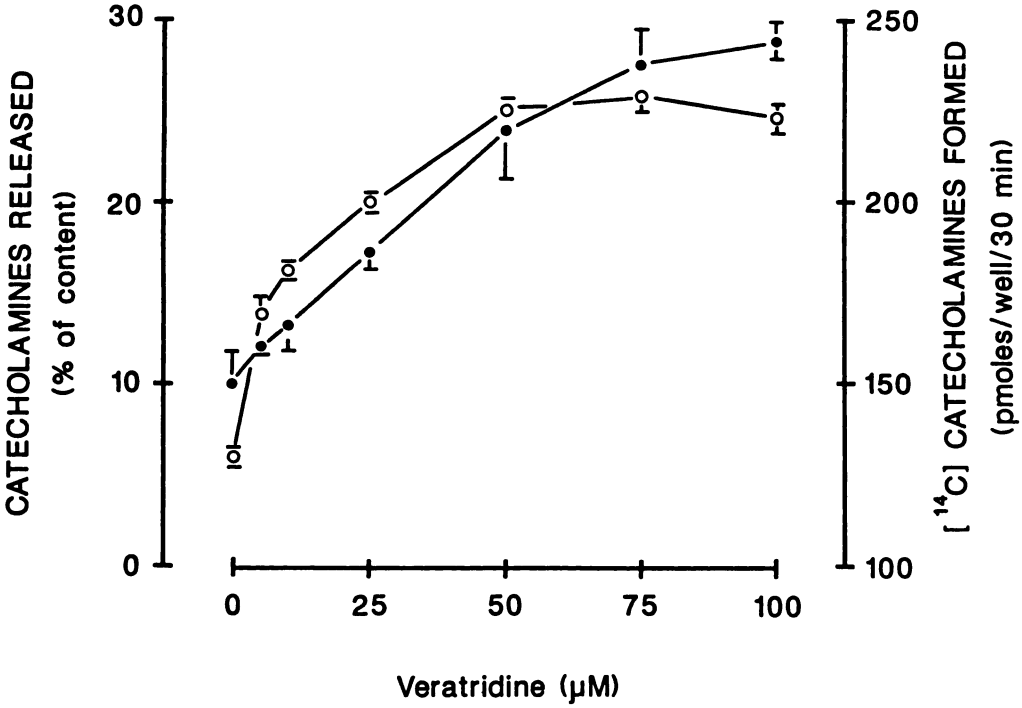

Fig. 2. Effects of VTD on catecholamine secretion and ^{14}C-catecholamine formation in cultured bovine adrenal chromaffin cells. Cells were stimulated by various concentrations of VTD, and catecholamine secretion (○) and ^{14}C-catecholamine formation (●) were independently determined, as described in the text. Values are the mean ± standard deviation of three experiments.

To obtain further information on the inhibitory action on the uptake of tyrosine observed here, the effect of this toxin on uptake was examined again, in the presence of different concentrations of tyrosine, and palytoxin was found to cause a decrease in the V_{max} value, without any significant alteration in the apparent K_m value for tyrosine under the experimental conditions used here (Fig. 6). Thus, the uptake of tyrosine into the cells was clearly shown to be inhibited by palytoxin in a noncompetitive manner. The effects of VTD and monensin, a Na^+ ionophore, on tyrosine uptake into the cells were also examined and were compared with that of palytoxin. As shown in Table 1, the uptake of tyrosine into the cells was markedly inhibited by palytoxin, whereas neither VTD nor monensin had any significant effect on uptake under the same conditions. In addition, the inhibitory action of palytoxin on tyrosine uptake was studied in the absence of extracellular Na^+ or Ca^{2+}, and it was found that both tyrosine uptake itself and the inhibitory action of this toxin on the uptake were not significantly affected by the removal of Na^+ or Ca^{2+} from the incubation mixture (Table 2). Thus, palytoxin seemed to cause the inhibition of tyrosine uptake into the cells through its novel action on the cell membranes, which might be independent of intracellular as well as extracellular Na^+ and Ca^{2+}.

Discussion

Palytoxin has previously been reported to cause norepinephrine release from rat pheochromocytoma PC12 cells, probably

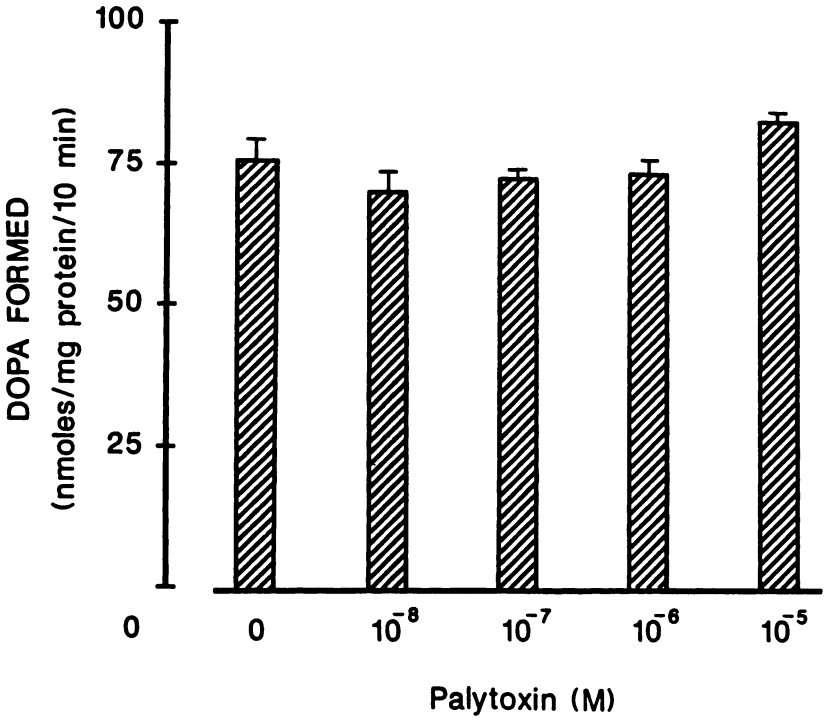

Fig. 3. Effect of palytoxin on tyrosine hydroxylase prepared from bovine adrenal medulla. Enzyme was incubated with various concentrations of palytoxin, and DOPA formation was then determined as described in the text. Values are the mean ± standard deviation of three experiments.

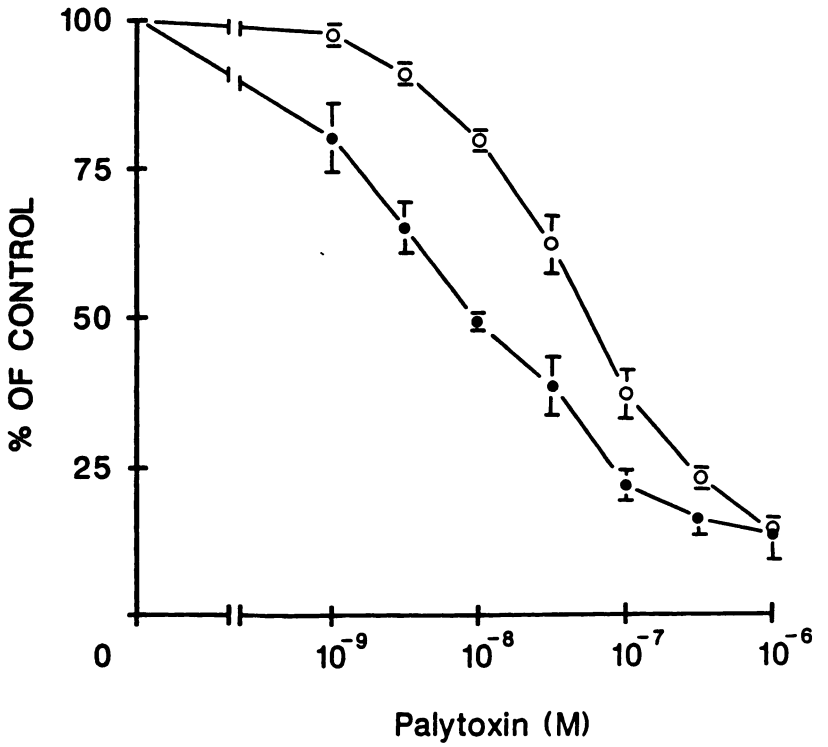

Fig. 4. Effects of palytoxin on ^{14}C-catecholamine formation and [^{14}C]tyrosine uptake in cultured bovine adrenal chromaffin cells. Cells were incubated with different concentrations of palytoxin, and [^{14}C]tyrosine uptake (●) and ^{14}C-catecholamine formation (○) were determined as described in the text. Results are expressed as percentage of the control values, which were estimated as 369.2 ± 18.7 and 109.8 ± 3.9 pmol/well/30 min for tyrosine uptake and catecholamine formation, respectively. Values are the mean ± standard deviation of three experiments.

through its stimulatory action on tetrodotoxin-insensitive Na^+ channels (15). The present study showed that palytoxin could stimulate catecholamine secretion from cultured bovine adrenal chromaffin cells. In our separate study, the secretory action of palytoxin has already been shown to be dependent on extracellular Na^+ and Ca^{2+} and was inhibited by various Ca^{2+} channel blockers but not by a voltage-dependent Na^+ channel inhibitor, tetrodotoxin (16). On the other hand, because the stimulation of catecholamine secretion by various secretagogues is commonly known to be accompanied by the stimulation of catecholamine biosynthesis in adrenergic tissue (17), we examined the effect of palytoxin on the formation of ^{14}C-catecholamines from [^{14}C]tyrosine and found that this toxin markedly inhibited, rather than stimulated, ^{14}C-catecholamine formation under the conditions in which the stimulation of catecholamine secretion by this toxin was observed (Fig. 1). In contrast, both the secretion of endogenous catecholamines and the formation of ^{14}C-catecholamines were shown to be significantly stimulated by VTD (Fig. 2). These results seem to suggest the possibility that palytoxin may directly inhibit the catecholamine biosynthetic enzymes within the cells or indirectly inhibit them, probably through stimulation of tetrodotoxin-insensitive Na^+

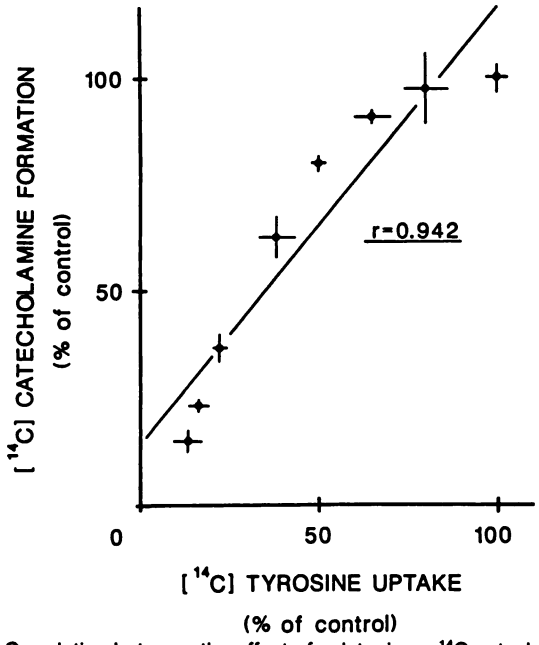

Fig. 5. Correlation between the effect of palytoxin on ^{14}C-catecholamine formation and that on [^{14}C]tyrosine uptake in cultured bovine adrenal chromaffin cells. For the linear regression analysis, the values of tyrosine uptake were plotted against those of catecholamine formation. Values were taken from Fig. 4.

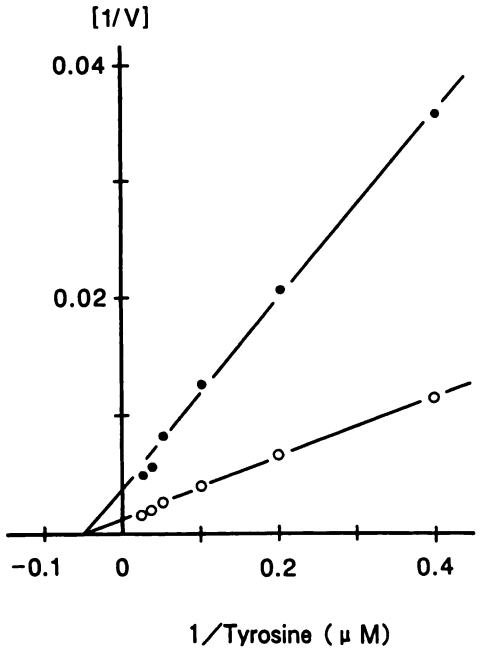

Fig. 6. Kinetic analysis of the inhibitory action of palytoxin on [^{14}C] tyrosine uptake in cultured bovine adrenal chromaffin cells. Cells were incubated with (●) or without (○) 3×10^{-8} M palytoxin in an incubation mixture containing different concentrations of [^{14}C]tyrosine, and the uptake was determined as described in the text. Values are the mean of four separate experiments.

channels on the cell membranes, leading to the reduction of ^{14}C-catecholamine formation observed here. To elucidate this possible mechanism, the direct effect of palytoxin on the activity of tyrosine hydroxylase, which is known to catalyze the rate-limiting step in the catecholamine biosynthetic pathway, was studied *in vitro*, and we found that this toxin failed to cause any significant alteration in the activity of this enzyme

TABLE 1

Effects of palytoxin, VTD, and monensin on [^{14}C]tyrosine uptake into cultured bovine adrenal chromaffin cells

Cells were incubated with palytoxin, VTD, or monensin, and the uptake was determined as described in the text. Values are the mean ± standard deviation of three experiments.

Addition	Tyrosine uptake	
	pmol/well/30 min	*% of control*
None	320.6 ± 9.6	100.0
Palytoxin (3×10^{-8} M)	101.7 ± 4.3	31.7
VTD (5×10^{-5} M)	339.4 ± 26.1	105.9
Monensin (1×10^{-5} M)	327.2 ± 19.7	102.1

TABLE 2

Effect of extracellular Na^+ or Ca^{2+} deprivation on the inhibitory action of palytoxin on [^{14}C]tyrosine uptake into cultured bovine adrenal chromaffin cells

Cells were incubated with various concentrations of palytoxin in the Na^+-free or the Ca^{2+}-free incubation mixture, and the uptake was then determined as described in the text. Values are the mean ± standard deviation of three experiments.

Palytoxin	Tyrosine uptake		
	Normal	Na^+-free	Ca^{2+}-free
M	*pmol/well/30 min*		
0	360.9 ± 12.0	364.5 ± 34.4	358.7 ± 34.8
10^{-9}	287.3 ± 30.1	303.4 ± 11.8	296.9 ± 29.0
10^{-8}	177.8 ± 11.8	194.6 ± 13.8	213.3 ± 22.9
10^{-7}	101.7 ± 10.3	136.2 ± 21.6	114.3 ± 25.3

(Fig. 3). It, therefore, seems conceivable that palytoxin may inhibit ^{14}C-catecholamine formation through its inhibitory action on step(s) in the catecholamine biosynthetic pathway other than tyrosine hydroxylation in adrenal chromaffin cells.

The earlier observations that the precursor supply may play a role as a possible factor regulating the biosynthesis of various neurotransmitters in the brain have already been summarized (24, 25). It, therefore, seems reasonable to presume that palytoxin may be able to cause the inhibition of catecholamine biosynthesis as a result of reducing tyrosine supply into the cells. In fact, the present study indicated that the formation of ^{14}C-catecholamines was markedly inhibited by palytoxin under the conditions in which the inhibition of [^{14}C]tyrosine uptake by the toxin was observed, and this suggested that the inhibition of ^{14}C-catecholamine formation might be closely related to that of [^{14}C]tyrosine uptake into the cells. However, it is still questionable whether palytoxin may cause the apparent reduction of ^{14}C-catecholamine formation as a consequence of lowering the specific activity of [^{14}C]tyrosine in the intracellular pools or may actually reduce catecholamine production through the inhibition of precursor supply to the pathway of catecholamine biosynthesis within the cells. Because the uptake of tyrosine into the cells was shown to be dramatically inhibited by palytoxin, the intracellular tyrosine level is presumed to be lowered by treatment of the cells with this toxin. It, therefore, seems conceivable that palytoxin actually causes the reduction of catecholamine production as a consequence of reducing the precursor supply to the catecholamine biosynthetic pathway in the adrenal chromaffin cell.

To obtain further information on a possible mechanism of the inhibitory action on tyrosine uptake, the effect of palytoxin on [^{14}C]tyrosine uptake into the cells was investigated under different experimental conditions. The inhibitory action of this toxin on tyrosine uptake was observed to be in noncompetitive

(Fig. 6). Although both VTD and monensin are also known to cause an increase in the intracellular Na^+ concentration, neither of them were shown to exert any significant effect on the uptake under the conditions in which the inhibitory action of palytoxin was observed (Table 1). This inhibitory action on the uptake was, furthermore, shown to be independent of extracellular Na^+ and Ca^{2+} (Table 2). These findings, therefore, seem to indicate that neither the stimulation of Na^+ influx into the cells nor the elevation of intracellular Na^+ level is connected with the inhibitory action of palytoxin on tyrosine uptake into the cells. Thus, the possibility that the inhibition of tyrosine uptake observed here may be attributed to the stimulation of tetrodotoxin-insensitive Na^+ channels seems to be excluded.

In addition to the stimulation of tetrodotoxin-insensitive Na^+ channels, palytoxin has been suggested to modulate the mobilization of various ions across the plasma membranes, probably through its direct action on Na^+/K^+-ATPase in different types of cells (26). Although the inhibition of tyrosine uptake by palytoxin was shown to be independent of extracellular Na^+, the possibility that the inhibitory action of palytoxin on tyrosine uptake may be related to its action on cation transport through the plasma membranes is considered to be not completely excluded. However, preliminary studies on the tyrosine transport mechanism have shown that tyrosine uptake itself is also independent of extracellular Na^+ and insensitive to ouabain or various metabolic inhibitors.[1] Even though palytoxin causes the complete inhibition of Na^+/K^+-ATPase in plasma membranes, the inhibition of this enzyme is presumed to be not connected with the inhibition of tyrosine uptake observed here. Thus, it seems quite possible that palytoxin may be able to inhibit tyrosine uptake into the cells, presumably through its novel action on the tyrosine transport mechanism, rather than its action on the cation transport system in plasma membranes. To obtain a conclusive answer to this critical question, the properties of the tyrosine transport system in cultured bovine adrenal chromaffin cells are being more precisely investigated.

References

1. Kilpatrick, D. L., R. Slepetis, and N. Kirshner. Ion channels and membrane potential in stimulus-secretion coupling in adrenal medulla cells. *J. Neurochem.* **36**:1245–1255 (1981).
2. Kilpatrick, D. L., R. J. Slepetis, J. J. Corcoran, and N. Kirshner. Calcium uptake and catecholamine secretion by cultured bovine adrenal medulla cells. *J. Neurochem.* **38**:427–435 (1982).
3. Amy, C., and N. Kirshner. $^{22}Na^+$ uptake and catecholamine secretion by primary cultures of adrenal medulla cells. *J. Neurochem.* **39**:132–142 (1982).
4. Kilpatrick, D. L. Ion channels and membrane potential in stimulus-secretion coupling in adrenal paraneurons. *Can. J. Physiol. Pharmacol.* **62**:477–483 (1984).
5. Narahashi, T. Chemicals as tools in the study of excitable membranes. *Physiol. Rev.* **54**:813–889 (1974).
6. Catterall, W. A. Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annu. Rev. Pharmacol. Toxicol.* **20**:15–43 (1980).
7. Fuhrman, F. A. Recent advances in marine pharmacology. *Trends Pharmacol. Sci.* **1**:187–189 (1980).
8. Honerjäger, P. Cardioactive substances that prolong the open state of sodium channels. *Rev. Physiol. Biochem. Pharmacol.* **92**:1–74 (1982).
9. Dubois, J. M., and J. B. Cohen. Effect of palytoxin on membrane potential and current of frog myelinated fibers. *J. Pharmacol. Exp. Ther.* **201**:148–155 (1977).
10. Ito, K., H. Karaki, and N. Urakawa. Effects of palytoxin on mechanical and electrical activities of guinea-pig papillary muscle. *Jpn. J. Pharmacol.* **29**:467–476 (1979).
11. Pichon, Y. Effects of palytoxin on sodium and potassium permeabilities in unmyelinated axons. *Toxicon* **20**:41–47 (1982).
12. Muramatsu, I., D. Uemura, M. Fujiwara, and T. Narahashi. Characteristics of palytoxin-induced depolarization in squid axons. *J. Pharmacol. Exp. Ther.* **231**:488–494 (1984).
13. Ito, K., N. Saruwatari, K. Mitani, and Y. Enomoto. Characterization of depolarization induced by palytoxin and grayanotoxin-I in isolated cardiac tissues from dogs and guinea-pigs. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **330**:67–73 (1985).
14. Ohizumi, Y., and S. Shibata. Mechanism of the excitatory action of palytoxin and *N*-acetylpalytoxin in the isolated guinea-pig vas deferens. *J. Pharmacol. Exp. Ther.* **214**:209–212 (1980).
15. Tatsumi, M., M. Takahashi, and Y. Ohizumi. Mechanism of palytoxin-induced [^{3}H]norepinephrine release from a rat pheochromocytoma cell line. *Mol. Pharmacol.* **25**:379–383 (1984).
16. Nakanishi, A., M. Yoshizumi, K. Morita, Y. Murakumo, H. Houchi, and M. Oka. Palytoxin: a potent stimulator of catecholamine release from cultured bovine adrenal chromaffin cells. *Neurosci. Lett.* **121**:163–165 (1991).
17. Ungar, A., and J. H. Phillips. Regulation of the adrenal medulla. *Physiol. Rev.* **63**:787–843 (1983).
18. Morita, K., S. Ishii, H. Uda, and M. Oka. Requirement of ATP for exocytotic release of catecholamines from digitonin-permeabilized adrenal chromaffin cells. *J. Neurochem.* **50**:644–648 (1988).
19. Levine, M., K. Morita, and H. B. Pollard. Enhancement of norepinephrine biosynthesis by ascorbic acid in cultured bovine chromaffin cells. *J. Biol. Chem.* **260**:12942–12947 (1985).
20. Morita, K., K. W. Brocklehurst, S. M. Tomares, and H. B. Pollard. The phorbol ester TPA enhances A23187- but not carbachol- and high K^+-induced catecholamine secretion from cultured bovine adrenal chromaffin cells. *Biochem. Biophys. Res. Commun.* **129**:511–516 (1985).
21. Morita, K., M. Levine, E. Heldman, and H. B. Pollard. Ascorbic acid and catecholamine release from digitonin-treated chromaffin cells. *J. Biol. Chem.* **260**:15112–15116 (1985).
22. Morita, K., A. Nakanishi, H. Houchi, M. Oka, K. Teraoka, K. Minakuchi, S. Hamano, and Y. Murakumo. Modulation by basic polypeptides of ATP-induced activation of tyrosine hydroxylase prepared from bovine adrenal medulla. *Arch. Biochem. Biophys.* **247**:84–90 (1986).
23. Morita, K., K. Teraoka, and M. Oka. Interaction of cytoplasmic tyrosine hydroxylase with chromaffin granule: *in vitro* studies on association of soluble enzyme with granule membranes and alteration in enzyme activity. *J. Biol. Chem.* **262**:5654–5658 (1987).
24. Wurtman, R. J., and J. D. Fernstrom. Control of brain neurotransmitter synthesis by precursor availability and nutritional state. *Biochem. Pharmacol.* **25**:1691–1696 (1976).
25. Milner, J. D., and R. J. Wurtman. Catecholamine synthesis: physiological coupling to precursor supply. *Biochem. Pharmacol.* **35**:875–881 (1986).
26. Habermann, E. Palytoxin acts through Na^+,K^+-ATPase. *Toxicon* **27**:1171–1187 (1989).

[1]Manuscript in preparation.

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