EFFECTS OF PALYTOXIN ON ISOLATED INTESTINAL AND VASCULAR SMOOTH MUSCLES

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Abstract—Palytoxin (PTX), the most potent marine toxin isolated from the Zoanthid, *Palythoa tuberculosa*, was studied to determine the effect on isolated smooth muscles. In guinea pig taenia coli PTX at above 3×10^{-10} g/ml caused a contraction which slowly subsided under isotonic recording. Under isometric recording PTX at above 1×10^{-10} g/ml caused a contraction which depended on the spontaneous activity. The PTX-induced contraction was not affected by atropine, tripeleennamine or tetrodotoxin but was inhibited by 5 mM Mg, norepinephrine, isoprenaline or papaverine. PTX at above 1×10^{-9} g/ml induced an increase in spike frequency and a slight depolarization accompanied with a contraction when measured using a sucrose gap method. In some cases the spike generation was almost abolished after a long exposure to higher dose of PTX and the developed tension gradually decreased. Under isometric recording, PTX caused a sustained contraction in rabbit aorta, dog mesenteric and coronary arteries at above 1×10^{-10} , 1×10^{-10} and 1×10^{-11} g/ml, respectively, in a dose-dependent manner. The coronary artery was most sensitive among the preparations used. PTX-induced contraction in aorta was irreversible, was not influenced by phentolamine but diminished with 5 mM Mg and disappeared in a D-600 or Ca-free medium. PTX is thus an extremely potent and direct stimulant which acts on smooth muscles.

A highly toxic substance extracted from the Zoanthid, *Palythoa tuberculosa*, by Kimura and Hashimoto (1, 2) is considered to be the cause of ciguatera-like poisoning by a filefish, *Alutera scripta*, in the Ryukyu Islands (1).

On the other hand, Ciereszko et al. (3), and independently Moore and Scheuer (4) found a toxin from *Palythoa* spp.; *P. caribaeorum* and *P. mammosa* in the Caribbean sea, and *P. vestis* and *P. toxica* in the Hawaiian sea, respectively. These toxins were designated as Palytoxin (4) or Palythoatoxin (5). The chemical properties of these substances were similar and it was considered that they were identical or at least closely related substances (2).

Palytoxin (PTX) has an LD₅₀ of 0.53 μ g/kg (in mice; i.v.) (6) and the toxicity is the most potent among the known marine toxins (6, 7).

In a previous report, the general pharmacology of PTX was described (5, 6); in the anesthetized cat i.v. injection of 0.2 μ g/kg PTX caused a rise in the arterial blood pressure followed by a gradually decrease and bradycardia, ectopic beats, bigeminal pulses and T inversion followed by a cardiac arrest were observed in the ECG. PTX caused a positive inotropic effect followed by depression in the atria and papillary muscle of the guinea pig. In the phrenic nerve-diaphragm preparation of the mouse, PTX depressed the contraction

by indirect stimulation accompanied with a contracture. It induced a contracture in the frog rectus muscle, and depolarization in the sartorius muscle which was prevented in a low Na medium and diminished by tetrodotoxin.

Other investigations were performed on the circulatory system (8, 9, 10), however, there is no available data on the effect of PTX on smooth muscle preparations except that PTX causes a contraction in guinea pig ileum (5, 6).

In the present work, the mechanical activity in intestinal and vascular smooth muscles was studied using the Magnus method and for the electrical activity in intestinal smooth muscle, a sucrose gap apparatus was used.

MATERIALS AND METHODS

The ileum and taenia coli were isolated from male guinea pigs weighing 250–300 g. Isotonic shortening of the ileum and the taenia coli were registered on a kymograph with a frontal writing lever or on a pen-writing oscillograph via an isotonic transducer. Taenia coli was preliminarily conditioned with hypertonically added 40 mM K once before PTX was applied for the purpose of obtaining a reproducible response. Isometric contraction of taenia coli was recorded on a pen-writing oscillograph with a strain gauge transducer.

The membrane potential of the taenia coli was measured using the sucrose gap method as described by Bülbring and Burnstock (11), with a modified apparatus. The chamber was divided into three portions; the Tyrode solution portion was 7 mm in diameter except for the bottom portion which was 1 mm to allow for free spontaneous contraction of the muscle. The Tyrode solution was introduced into the chamber from the lower opening and overflowed from the upper edge of the portion so as to maintain the constant level of the Tyrode solution. Artifacts due to level change were thus avoided. The depolarizing KCl portion of the chamber was filled with 160 mM KCl agar and one end of the muscle was fixed herein (12). The central part of the muscle was perfused with sucrose solution which had been passed through a deionizing resin immediately before use. The recording electrode was embedded in KCl-agar of the depolarized portion and the indifferent electrode was inserted into the Tyrode portion.

Rabbits of either sex weighing 1500–2000 g were sacrificed by injecting air to produce embolism and thoracic aortas were then isolated. The adventitia was removed. Mongrel dogs of either sex weighing 9–15 kg were bled under pentobarbital anesthesia and left circumflex of coronary artery and mesenteric artery were excised. Helical strips about 20 mm length were made from each artery and tension was recorded isometrically.

Modified Tyrode solution used contained (mM) NaCl 136.8, KCl 5.4, CaCl_2 2.5, MgCl_2 1.0, NaHCO_3 11.9 and glucose 5.5 aerated with 95% O_2 and 5% CO_2 at 37°C. Hypertonically added 15, 40 or 60 mM potassium (H15K, H40K, H60K) medium was made by adding KCl crystals to Tyrode solution to make a final concentration of 20.4, 45.4 or 65.4 mM KCl.

The Ca-free solution was prepared by omitting CaCl_2 from Tyrode solution with or without 1 mM EGTA.

PTX extracted from *Palythoa tuberculosa* and having an LD₅₀ value of 0.53 $\mu\text{g/kg}$ (i.v. in mice) was kindly donated by Prof. Y. Hashimoto of the University of Tokyo. The toxin was dissolved in distilled water at a concentration of 1×10^{-5} g/ml and kept frozen as stock solution. The stock solution was appropriately diluted just before use.

Drugs used were atropine sulfate (E. Merck AG), chlorpheniramine maleate (Sankyo Co.), tetrodotoxin (Sankyo Co.), tripelennamine hydrochloride (Ciba-Geigy (Japan)), phentolamine mesylate (Ciba-Geigy (Japan)), papaverine hydrochloride (Tokyo Kasei Co.), 1-norepinephrine bitartrate (Wako Pure Chemical Co.), isoprenaline hydrochloride (Tokyo Kasei Co.), D-600 hydrochloride (Knoll AG) and adenosine (Tokyo Kasei Co.),

RESULTS

Effects of PTX on the mechanical activity of ileum and taenia coli of guinea pig

PTX caused a shortening in guinea pig ileum when recorded isotonicly. The shortening was transient, had two peaks each of which was dose-dependent and subsided in several minutes. The response was not affected by 1.5×10^{-5} M atropine or 3.7×10^{-7} M chlorpheniramine. These results confirmed our previous data (6).

Under isotonic recording, taenia coli was previously conditioned with H4OK and 30 min after removal of H4OK PTX was applied. The muscle showed a rapid shortening which was sustained in the presence of H4OK and was rapidly relaxed by removal of H4OK. PTX at above 3×10^{-10} g/ml also induced a contraction but such was slow to develop compared

FIG. 1. Isotonic shortening induced by PTX in taenia coli. The muscle was conditioned preliminarily by H4OK as shown in left of the upper trace. The shortening induced by 1×10^{-9} g/ml PTX was shown at right of the upper trace. The lower trace shows dose-response relation for PTX-induced shortening. The maximal shortening induced by H4OK was expressed 100% in ordinate. 0% in ordinate indicates the Ca-free level and the dotted line indicates the resting level just before PTX adding. Vertical lines in the figure indicate standard error.

with that induced by H40K and the peak was reached within 10 min and gradually declined thereafter to the original length as shown in Fig. 1. Removal of PTX during PTX contraction did not abruptly relax the muscle. PTX, 1×10^{-10} g/ml, did not cause significant shortening and 3×10^{-10} g/ml PTX caused a nearly maximum shortening which was about 50% of maximum shortening induced by H40K. The contractile response to PTX between 1×10^{-10} – 1×10^{-8} g/ml is also shown in Fig. 1.

Under isometric recording, taenia coli showed different types of spontaneous activity. The first type was more than one gram in amplitude and appeared at a frequency of 0.1–1/min. Such has been classified by Golenhofen and Loh (13) as minute rhythm contraction. In this preparation 1×10^{-10} – 3×10^{-8} g/ml PTX caused a contraction. The contraction was phasic, that is similar or slightly larger than the spontaneous contraction, followed by a tonic contraction which gradually declined as shown in Fig. 2a. No dose-response relation was obtained and the response depended on the spontaneous contraction as shown in Fig. 3. Correlation coefficient between peak amplitude in the response to PTX and in the spontaneous contraction was 0.908.

Other types of spontaneous activity, less active and not showing minute rhythm contraction, were repetitive contractions of below 0.5 g in amplitude without a quiescent period. In this type preparation, 1×10^{-10} – 1×10^{-7} g/ml PTX caused a contraction which was small as compared with that in a muscle showing the minute rhythm contraction as shown in

FIG. 2. Isometric contraction of guinea pig taenia coli induced by 1×10^{-10} g/ml PTX. Fig. 2-a is a sample record in a preparation showing spontaneous contraction of minute rhythm. Fig. 2-b is a sample record in a preparation not showing minute rhythm contraction.

FIG. 3. Relation between the peak amplitude of PTX response and of spontaneous contraction in the preparations showing minute rhythm contraction. Δ ; 1×10^{-10} – 5×10^{-10} g/ml PTX, $\square$; 1×10^{-9} – 5×10^{-9} g/ml PTX, $\circ$; above 1×10^{-8} g/ml PTX. Regressive line in the figure is calculated from these plots. The correlation coefficient was 0.908. $\blacksquare$; 1×10^{-9} g/ml PTX in the presence of 3×10^{-6} M atropine, $\bullet$; 1×10^{-9} g/ml PTX in the presence of 6×10^{-7} M tetrodotoxin.

Fig. 2b. Some preparations with no spontaneous activity exhibited no response to PTX. The tension development induced by PTX in the preparations not showing minute rhythm contraction was 0.23 ± 0.08 g (mean $\pm$ S.E.; $n=11$) at 1×10^{-10} g/ml, 0.63 ± 0.22 g ($n=7$) at 1×10^{-9} g/ml and 0.87 ± 0.23 g ($n=6$) at 1×10^{-8} g/ml PTX. The difference between the response at 1×10^{-10} g/ml and 1×10^{-9} g/ml was significant ($P < 0.001$).

Removal of PTX during PTX contraction did not restore the tension to the control level. When the contraction with PTX in a concentration higher than 1×10^{-9} g/ml had subsided, a second application of PTX never resulted in a contraction. However, when the concentration of the toxin in the first application was below 1×10^{-9} g/ml, the second application induced a small contraction.

Effects of various drugs and ions on the isometric contraction induced by PTX in taenia coli

Pretreatment with 3×10^{-6} M atropine, 6×10^{-7} M tetrodotoxin or 3×10^{-6} M triptelenamine scarcely modified either the spontaneous contraction of taenia coli or the PTX-induced contraction. Fig. 3 shows the response to 1×10^{-9} g/ml PTX in the presence of atropine or tetrodotoxin.

2.5×10^{-5} M papaverine abolished the spontaneous contraction, and the application of 1×10^{-9} g/ml PTX after 30 min preincubation with papaverine caused no contraction. Application of 3×10^{-7} M norepinephrine or 5×10^{-7} M isoprenaline after the tension development by 1×10^{-9} g/ml PTX rapidly lowered the tension to the control level.

Pretreatment of the muscle with 5 mM Mg for 15 min which abolished the spontaneous contraction prevented or depressed a contraction due to 1×10^{-9} g/ml PTX. After removal of PTX and excess Mg, a contraction occurred as shown in Fig. 4. Adding 5 mM Mg after the tension development by 1×10^{-9} g/ml PTX lowered the muscle tension to the control level. A contraction was restored by the removal of excess Mg and PTX from the bathing medium.

FIG. 4. Inhibition of 1×10^{-9} g/ml PTX-induced contraction by treatment of 5 mM Mg and appearance after removal of excess Mg and PTX.

Pretreatment of the muscle with 1×10^{-6} M D-600, an organic Ca antagonist (14), which abolished the spontaneous contraction prevented a contraction due to 1×10^{-9} g/ml PTX. In the Ca-deficient medium, the spontaneous contraction disappeared after a transient contraction and application of 1×10^{-9} g/ml PTX after 30 min induced no contraction.

In H60K medium, the cell membrane of taenia coli was depolarized and the muscle exhibited the sustained tension development. Application of 1×10^{-9} g/ml PTX in the presence of H60K did not induce further contraction.

Changes in membrane potential of taenia coli muscle by PTX

The membrane potential and mechanical activity in the taenia coli were simultaneously observed with the sucrose gap method.

FIG. 5. Changes in membrane potential and tension of guinea pig taenia coli showing spontaneous contraction of minute rhythm. Mechanical activity was recorded isometrically. Upper trace shows the membrane potential and lower trace shows the tension. At the triangle mark 1×10^{-8} g/ml PTX was added.

FIG. 6. Changes in membrane potential and tension of taenia coli not showing minute rhythm contraction. Upper trace indicates the membrane potential and lower trace indicates the tension.

In the muscle showing the minute rhythm contraction, 1×10^{-9} or 1×10^{-8} g/ml PTX induced an increase in spike frequency and depolarization accompanied with tension development as shown in Fig. 5. The magnitude of depolarization due to PTX was similar or slightly larger than that observed during spontaneous contraction, which was usually 5–10 mV in the present experiment. In many cases, the spike discharges were then decreased while depolarization remained. At a higher dose of PTX, 1×10^{-9} g/ml, the decrease of spike activity was pronounced. In some cases the spike configuration became small and complex or aborted and eventually spike generation ceased. After a long exposure to PTX, the membrane potential gradually increased accompanied with a tension decline. The second application of PTX caused no change either in membrane potential or in mechanical response.

In the muscle showing repetitive spike activity, 1×10^{-8} g/ml PTX increased the spike frequency and depolarized the membrane slightly as shown in Fig. 6.

When H15K was added to the medium the membrane of muscle was depolarized by 10–18 mV and spike frequency increased. The depolarization induced by PTX was always smaller than that induced by H15K in the same preparation.

When the mechanical activity was recorded isotonicly, PTX caused an increase of spike discharge and depolarization in all preparations used followed by occasional decrease in spike activity.

Effects of PTX on the isolated rabbit aorta

A strip of rabbit aorta was preliminarily conditioned by H60K and PTX was then applied. PTX induced a slowly developing contraction in a dose dependent manner (Fig. 8) in rabbit aorta. The rate of rise in PTX-induced contraction was slower than that of H60K as shown in Fig. 7a. At a dose of PTX above 3×10^{-9} g/ml, the muscle attained the peak tension within 15 min and sustained this level for more than 1 hr. The highest dose, 3×10^{-8} g/ml, of PTX contracted the muscle to the level induced by H60K followed by a slow decline in the presence of PTX, in some cases. At the lower doses, a longer time was needed for the

FIG. 7. Contractions induced by 1×10^{-9} g/ml PTX in rabbit aorta (a), dog mesenteric artery (b) and dog coronary artery (c). The muscles were preliminarily conditioned with H6OK which was shown at left for comparison. (W) indicates washing out of stimulants. In rabbit aorta and dog mesenteric artery PTX caused a sustained contraction lasting for more than 1 hr. In dog coronary artery 1×10^{-9} g/ml PTX caused a contraction which reached the peak in 10 min followed by slow relaxation. Each contraction was not altered by washing out of PTX (W) from the bath.

FIG. 8. Dose-response relation for the contraction in rabbit aorta ■—■, dog mesenteric artery ●—● and dog coronary artery ▲—▲. Vertical bars represent the standard error. 100% in ordinate represents the maximal contraction in each artery induced by H6OK.

muscle to reach the peak of contraction, i.e., about 1 hr at a concentration of 1×10^{-10} g/ml. The aorta did not readily relax with removal of PTX from the bath.

Post-treatment with 2×10^{-6} M phentolamine, phenoxybenzamine or 3×10^{-6} M atropine did not affect 3×10^{-9} g/ml PTX-induced contraction. Addition of 5 mM Mg during the 1×10^{-8} g/ml PTX contraction lowered the muscle tension by 25%. The degree of inhibition of PTX-induced contraction by 5 mM Mg was similar to that of H6OK-induced

contraction. Pretreatment of the muscle with 1×10^{-6} M D-600 for 15 min inhibited a contraction due to 3×10^{-9} g/ml PTX. Addition of 1×10^{-6} M D-600 during the 3×10^{-9} g/ml PTX contraction reduced the tension to the control level in 20 min.

Addition of 3 mM EGTA to the bath in the course of tension development by 3×10^{-9} g/ml PTX lowered the tension to the control level in 30 min. In the Ca-deficient medium with or without 1 mM EGTA, the aorta did not exhibit contraction in response to 3×10^{-9} g/ml PTX and by adding 2.5 or 3.5 mM Ca to the medium 20 min after the addition of PTX, tension developed in the aorta.

Effects of PTX on isolated coronary and mesenteric arteries of dogs

Coronary and mesenteric arteries of the dog were preliminarily conditioned by H60K and then PTX was applied. PTX induced contractions in dog mesenteric and coronary arteries are shown in Fig. 7b and 7c. Dose-response relation was observed in both muscles (Fig. 8). The rate of rise of PTX contraction in coronary artery was faster than that in the mesenteric artery or rabbit aorta at a concentration above 1×10^{-10} g/ml but slightly slower than that by H60K. PTX-induced contraction reached the peak tension in 10–15 min followed by a slow relaxation at 3×10^{-9} g/ml, or in some cases, 1×10^{-9} g/ml in the coronary artery and at 3×10^{-9} g/ml in mesenteric artery (Fig. 7b, 7c). At a concentration below 1×10^{-10} g/ml, contractile response in the coronary artery was slow and continued to rise for more than 1 hr. The minimum concentration of PTX required for contraction was 1×10^{-11} g/ml in the coronary artery which was the most sensitive to PTX among the vascular muscles used.

Adenosine at a concentration of 5×10^{-5} g/ml depressed 3×10^{-10} g/ml PTX contraction in the coronary artery by 50% but never completely even with a higher adenosine concentration (1×10^{-4} g/ml).

DISCUSSION

In the present experiment we examined the pharmacological effects of PTX extracted from *Palythoa tuberculosa* on isolated smooth muscles. PTX induced contractions in both intestinal and vascular smooth muscles. The concentration of PTX required for the contraction in ileum (above 1×10^{-10} g/ml), taenia coli (above 1×10^{-10} g/ml) or blood vessels (above 1×10^{-11} – 1×10^{-10} g/ml) was extremely low as compared to other stimulants; acetylcholine on intestinal smooth muscles (1×10^{-9} g/ml), norepinephrine or epinephrine on vascular smooth muscles (5×10^{-9} g/ml), histamine (1×10^{-8} g/ml), serotonin (1×10^{-7} g/ml), nicotine (5×10^{-8} g/ml) in our experiment or prostaglandins (1×10^{-9} g/ml) (15), on both preparations.

The coronary artery of dog was the most sensitive to PTX, and contracted at a concentration of 1×10^{-11} g/ml. Deguchi et al. (6) reported that PTX had a potent cardiotoxic effect and their data on ECG in cat showed bradycardia, ectopic beats, bigeminy, ventricular arrhythmias, fall in ST segment and ensuing T inversion. Fall in ST segment and T inversion suggested the possibility of myocardial damage as a result of coronary insufficiency. Kaul et al. (8) reported that 5×10^{-10} g/ml PTX depressed coronary flow in isolated perfusing heart of guinea pig and suggested that the lethality of PTX was due to a profound coronary vaso-

constriction. Further, Vick and Wiles (10) suggested that death by PTX was due to profound coronary vasoconstriction and cardiac failure. The highest sensitivity of coronary artery strip in this experiment is consistent with the suggestions of these authors.

Ineffectiveness of atropine, tripeleennamine or tetrodotoxin on the PTX contraction of taenia coli and ileum and of phentolamine, phenoxybenzamine or atropine on rabbit aorta suggests that PTX acts directly on the smooth muscles.

Removal of PTX from the bathing medium during PTX-induced contraction did not alter the contraction, indicating the irreversibility of the PTX response. Further, the fact that the second application of PTX produced no contraction or only a weak contraction indicates the lack of reproducibility of PTX response.

Electrophysiological experiments showed that PTX depolarized the cell membrane of taenia coli and increased spike discharge. The magnitude of depolarization induced by PTX was similar or slightly larger than that of spontaneous activity. The excitatory effect of PTX in taenia coli was small when compared with that of H15K. During the prolonged PTX treatment, both the spike discharge and the contractile tension were gradually depressed. Magnitude of depolarization by PTX at this stage was less than that induced by H15K which induced both depolarization and increase in spike activity followed by a tension development. From these data, the inhibition of spike generation by PTX could not be a depolarization block.

The depolarizing effect of PTX was also observed in frog sartorius muscle (6). PTX appeared to have a depolarizing action in both skeletal and smooth muscles. Sometimes a small and complex or an abortive spike appeared after a long exposure to PTX at higher dose (1×10^{-8} g/ml). Burnstock (16) reported that the small and complex spike was due to asynchronous excitation and an abortive spike was due to decrease in the number of firing cells when measured with the sucrose gap method. These results together with the absence of reproducible response by the second application of PTX suggest that after a long exposure to PTX, the excitation factor of the cell membrane is altered.

In taenia coli, the PTX-induced contraction was dependent on the spontaneous activity under isometric recording. Changes due to PTX in membrane potential also depended on the pre-existing spontaneous activity. On the other hand, the response to PTX under isotonic recording was independent on the spontaneous activity. This mode of action in taenia coli was different from other stimulants. In this regard, it is noteworthy that according to Bose (17) and Lüllmann et al. (18), ouabain also exhibited a contraction which was dependent on spontaneous contraction. Furthermore, Casteels (19) reported that the initial increase of spike frequency produced by ouabain was related to the spontaneous spike discharge of the tissue. In isotonic recording, Schatzmann and Ackermann (20) observed a dose-dependent contraction by ouabain in taenia coli. From these data, the response of taenia coli to ouabain was similar to PTX. We cannot explain why the spontaneously active muscle solely responded to PTX or ouabain. It is also unclear whether or not the mechanisms of action of PTX and ouabain on taenia coli are similar. It is speculated that the action of PTX on taenia coli is related to activities of the muscle, especially to the resting

membrane potential or metabolic activity of the muscle.

Excess Mg and D-600 inhibited the PTX-induced contraction and PTX did not induce a contraction in Ca-free medium in taenia coli. Further, PTX-induced contraction was inhibited by D-600 or in a Ca-free medium in the aorta. From these results, Ca in the medium was required for PTX to induce a contraction. In rabbit ventricle, Rayner et al. (9) reported an increase in ^{45}Ca uptake by PTX. We have also observed that PTX increased the intracellular ^{45}Ca uptake in rabbit aorta (unpublished data). Thus it would appear that PTX-induced contraction is due to Ca entry into the cell.

REFERENCES

- 1) KIMURA, S., HASHIMOTO, Y. AND YAMAZATO, K.: *Toxicon* **10**, 611 (1973)
- 2) KIMURA, S. AND HASHIMOTO, Y.: *Pub. Seto. Mar. Biol. Lab.* **20**, 713 (1973)
- 3) CIERESZKO, L.S. AND ATTAWAY, D.H.: *Res. Abst. The Petroleum Res. Fund. Amer. Chem. Soc.*, p. 107, cited from reference 8 (1961)
- 4) MOORE, R.E. AND SCHEUER, P.J.: *Science* **172**, 495 (1971)
- 5) DEGUCHI, T., AOSHIMA, S., SAKAI, Y., TAKAMATSU, S. AND URAKAWA, N.: *Japan. J. Pharmacol.* **24**, Suppl. 116 (1974)
- 6) DEGUCHI, T., URAKAWA, N. AND TAKAMATSU, S.: *Animal, Plant and Microbial Toxins*, Edited by OHSAKA, A., HAYASHI, K. AND SAWAI, Y., Vol. **2**, p. 379, Plenum Publishing Corporation, New York (1976)
- 7) WILES, J.S., VICK, J.A. AND CHRISTENSEN, M.K.: *Toxicon* **12**, 427 (1974)
- 8) KAUL, P.N., FARMER, M.R. AND CIERESZKO, L.S.: *Proc. West. Pharmacol. Soc.* **17**, 294 (1974)
- 9) RAYNER, M.D., SANDERS, B.J., HARRIS, S.M., LIN, Y.C. AND MORTON, B.E.: *Res. Commun. Chem. Path. Pharmacol.* **11**, 55 (1975)
- 10) VICK, J.A. AND WILES, J.S.: *Toxic. appl. Pharmacol.* **34**, 214 (1975)
- 11) BÜLBRING, E. AND BURNSTOCK, G.: *Brit. J. Pharmacol.* **15**, 611 (1960)
- 12) WASHIZU, Y.: *Experiments in Physiology and Biochemistry*, Edited by KERKUT, G.A., Vol. **2**, p. 177, Academic Press, London (1969)
- 13) GOLENHOFEN, K. AND LOH, D.V.: *Pflügers Arch.* **314**, 312 (1970)
- 14) FLECKENSTEIN, A., TRITTHART, H., FLECKENSTEIN, B., HERBST, A. AND GRUEN, G.: *Arch. Pharmacol.* **264**, 227 (1969)
- 15) HORTON, E.W.: *Physiol. Rev.* **49**, 122 (1969)
- 16) BURNSTOCK, G.: *J. Physiol.* **143**, 165 (1958)
- 17) BOSE, D.: *Brit. J. Pharmacol.* **55**, 111 (1975)
- 18) LÜLLMANN, H., URAKAWA, N. AND ZIEGLER, A.: *Arch. Pharmacol.* (in press)
- 19) CASTEELS, R.: *J. Physiol.* **184**, 131 (1966)
- 20) SCHATZMANN, H.J. AND ACKERMANN, H.: *Helv. physiol. pharmacol. Acta* **19**, 196 (1961)