EFFECTS OF PHTHALAZINOL, EG 626, ON MEMBRANE AND MECHANICAL PROPERTIES OF SMOOTH MUSCLE CELLS OF RABBIT MAIN PULMONARY ARTERY AND SUPERIOR MESENTERIC ARTERY

Hikaru SUZUKI, Yushi ITO, Hiroshi KURIYAMA and Takio SHIMAMOTO*

*Department of Pharmacology, Faculty of Medicine,
Kyushu University, Fukuoka 812, Japan*

Accepted July 12, 1978

Abstract—Effects of phthalazinol, EG 626, on the membrane and mechanical properties of the vascular smooth muscles, namely superior mesenteric artery and main pulmonary artery of the rabbit were examined. EG 626 (10^{-7} – 3×10^{-5} g/ml) did not modify the membrane potential (-56.5 mV), length constant of the tissue (1.47 mm), and rectifying property of the membrane in the pulmonary artery. The membrane potential of the mesenteric artery (-59.8 mV) was also not modified. By treatment with 10^{-5} g/ml EG 626, the mechanical responses of both muscle tissues induced by either direct muscle stimulation (1 sec pulse) or by nerve stimulation (0.5 msec, 30 Hz) were suppressed. The mechanical response induced by nerve stimulation was more dominantly suppressed than that induced by direct muscle stimulation. Dose-response curve obtained from the relation between noradrenaline and mechanical response in both pulmonary and mesenteric arteries shifted to the right by treatment with 10^{-5} g/ml EG 626. When the depolarization-contraction relationship was observed before and during application of EG 626 (10^{-5} g/ml), using the voltage clamp technique, the application of this agent raised the mechanical threshold to evoke contraction and amplitude of the contraction evoked at any given grade of depolarization was consistently reduced as compared with that evoked in Krebs solution. These observations suggest that EG 626 suppresses the mobilization of Ca^{++} during contraction and also the release of chemical transmitter from the nerve terminals by suppression of Ca^{++} mobilization. EG 626 would thus appear to act on the vascular smooth muscles as a vasodilator.

A newly synthesized compound, phthalazinol (EG 626), has been shown to have cyclic AMP phosphodiesterase inhibiting actions on arteries and platelets (1–3). Moreover, this agent also possesses highly potent, reversible and competitive antagonistic effects on thromboxane A_2 (TXA_2) which is known to induce potent arterial contractions in which platelets participate and arterial spasm follows (4–6).

Since intracellular free Ca^{++} concentration is known to regulate the contraction and relaxation of muscles, investigations were carried out to determine whether or not the anti-spasmodic action of EG 626 on blood vessels is directly produced by relaxation of smooth muscle. To observe the effects of EG 626 on the membrane and mechanical properties of the vascular smooth muscles, the main pulmonary artery and the superior mesenteric artery of the rabbit were used.

*The late T. Shimamoto, Japan Atherosclerosis Institute, Tokyo, Japan.

MATERIALS AND METHODS

Albino rabbits of either sex, weighing 2.0 to 2.5 kg were stunned and bled. The main pulmonary and superior mesenteric arteries were excised and freed of connective tissue using a dissecting microscope. The tissues were cut in a circular direction (main pulmonary artery) and also helically (superior mesenteric artery) at a width of 2.0 mm and a length of about 10 mm for studies on single cell activity and for investigation using the double sucrose gap method. To record the membrane potential, the microelectrode method was applied. To determine the passive properties of the membrane and the tissue, the partition stimulating method described by Abe and Tomita (7) and by Casteels, *et al.* (8, 9) was used.

The double sucrose gap method was used for recording the membrane potential, membrane current and tension development of the cells. The chamber in which the preparation was mounted was a modification of that used originally by Rougier, *et al.* (10) and consisted of five Lucite compartments separated by four diaphragms with holes at the center. Two of the diaphragms between the central and terminal compartments were composed of three close fitting, slotter plates and were utilized for the fixation of the preparation. The other two were rubber membrane, and the muscle bundle was pulled through the tightly closed holes and connected to the strain gauge for the tension measurement. Therefore, the measured tension development was not strictly isometric tension. The central compartment 0.5 mm in width was perfused with Krebs solution or the test solution. The intermediate compartments, each 1 mm in width, were perfused with isotonic sucrose solution. The terminal compartments were relatively large pools, filled with isotonic KCl solution (11). The circuit used for the voltage-clamp experiments was essentially similar to that described by Anderson (12). A high-voltage operational amplifier, Telcdyne-Philbrick 1022, was used as the output amplifier of the feed-back circuit (13–15).

To measure the tension development, the strips excised from the mesenteric artery and pulmonary artery were bathed in the same chamber (2 cc in capacity) and two ring plates coated by silver-chloride were placed on the top and bottom of the organ bath to stimulate the tissues. The perfusing velocity was 2 ml/min at the temperature of 35°C.

Modified Krebs solution (16) was used as the normal solution and was aerated by 97% O₂ and 3% CO₂, and pH of the solution was kept at 7.2. The following drugs were used at the concentrations described in the results; tetrodotoxin (TTX: Sankyo Pharmac. Co. Ltd.), 1 $\pm$ noradrenaline-HCl (Merck Pharmac. Co. Ltd.) and EG 626 (phthalazinol; 7-ethoxycarbonyl-6, 8-dimethyl-4-hydroxy methyl-1(2H)-phthalaxinone). EG 626 was dissolved in propyleneglycol, and final concentration of this solvent was kept at 0.3% in the concentration of 10⁻⁵ g/ml EG 626. The membrane potential and the mechanical activities of both tissues were not affected by 0.3% propyleneglycol.

RESULTS

Effects of EG 626 on membrane properties

The mean membrane potential of circular muscle of the rabbit main pulmonary artery was -56.5 ± 2.3 mV, S.D. (n=54), and that of the superior mesenteric artery was

FIG. 1. Current-voltage relationship of smooth muscle cells of the pulmonary artery observed before and during the application of EG 626 (10^{-5} g/ml). Top records; before and during application (dark thick line) of EG 626, inward and outward current were applied (inkwriting record). Lower record; current-voltage relation curve. The microelectrode was inserted in the same cell throughout the experiment at a distance of 0.3 mm from the stimulating plate.

-59.6 ± 2.4 mV, S.D. ($n=68$). These two tissues showed no spontaneous activity. As the membrane potential of the latter was most sensitive to the stretch, the tissue was kept at the *in vivo* length. Application of EG 626 (10^{-7} – 3×10^{-5} g/ml) did not modify the membrane potential recorded from both tissues (3×10^{-5} g/ml; pulmonary artery was -56.3 ± 2.2 mV, S.D., $n=31$, and mesenteric artery was -60.7 ± 2.4 mV, S.D., $n=23$). Further increased concentrations of EG 626 were not used in the present experiments in order to avoid the actions of the solvent on the tissues.

Fig. 1 shows the current-voltage relationship obtained from the smooth muscles of the main pulmonary artery before and during application of EG 626. The top records show the effects of various intensities of the inward- and outward current pulses (3 sec in duration) on the smooth muscle cell recorded from 0.3 mm distance from the stimulating electrode. Throughout the experiment, the microelectrode was inserted into the same cell. Records were obtained by utilizing an inkwriting recorder. The lower graph shows the current-voltage relationship obtained by the same procedure as that illustrated on the above records, but the data were from the records obtained using the oscilloscope and a camera.

When the current-voltage relationships were compared before and during application of EG 626, identical relation curves were recorded. In both conditions, the rectifying properties of the membrane produced by outward currents appeared at the same depolarization level (6 mV depolarization). These results suggest that the membrane potential and the resistance of the membrane are not affected by treatment with 10^{-5} g/ml EG 626. The length constant of the tissue (λ) was determined from the relationship between the amplitudes of the electrotonic potential against the distances from the stimulating electrode, i.e., as has

been described by Casteels *et al.* (9), the decay of the amplitudes of electrotonic potential plotted by logarithmic scale against the distances from the stimulating partition where the amplitude decayed to e^{-1} was calculated. The values obtained before and after application of EG 626 remained the same i.e., the length constant in Krebs solution was 1.42 ± 0.18 mm, S.D. ($n=5$) and that in the presence of 10^{-5} g/ml EG 626 was 1.43 ± 0.21 mm, S.D. ($n=5$). These observations confirmed the results obtained from current-voltage relation and suggest that the membrane resistance and internal resistance of the myoplasm remain the same before and during application of EG 626 (10^{-5} g/ml).

Effects of EG 626 on the mechanical activities

Fig. 2 shows the effects of EG 626 on the mechanical responses induced by either direct muscle or nerve stimulations. To stimulate the muscle, 1 sec rectangular single pulse was used, and to stimulate the nerve fibres, 0.5 msec pulse duration, 30 Hz in frequency and 10 sec in stimulus duration were used. When TTX (10^{-7} g/ml) was added to the organ bath, the strips excised from both the mesenteric and pulmonary arteries did not generate contraction by short pulse stimulation (b). To avoid effects of the solvent (propyleneglycol) on the tissues, such effects (3×10^{-3} g/ml) were also observed in both tissues, however, such were nil on responses induced by the two different methods of stimulation (d). By application of EG 626 (10^{-5} g/ml), the mechanical responses induced by both stimulus conditions were suppressed (85% of the control in the mesenteric artery and 78% of the control in the pulmonary artery by direct muscle stimulation ($n=5$), and 40% of the control in the mesenteric artery and 30% of the control in the pulmonary artery by nerve stimulation ($n=5$)).

These results indicate that EG 626 suppresses the mechanical responses induced by both stimulating methods and the nerve mediated contraction was more strongly suppressed than the contraction produced by direct muscle stimulation. Therefore, it is postulated that EG 626 suppresses not only the mechanical property but also the release the chemical transmitter from the nerve terminals.

Fig. 3 shows the effects of EG 626 on the noradrenaline induced contraction. The

FIG. 2. Effects of EG 626 (10^{-5} g/ml) on the mechanical responses induced by direct muscle and nerve stimulation. ○; 1 sec pulse stimulation; ●; 0.5 msec in pulse duration, 30 Hz in frequency and 10 sec in stimulation. Effects of tetrodotoxin (TTX) and propyleneglycol were also observed. The final concentration of propyleneglycol in 10^{-5} g/ml EG 626 was 3×10^{-3} g/ml.

FIG. 3. Effects of EG 626 on the noradrenaline-induced contraction obtained from the superior mesenteric and main pulmonary arteries. In comparison to the noradrenaline-induced contracture, contraction was evoked by nerve stimulation (0.5 msec in pulse duration, 30 Hz in frequency and 10 sec in stimulus duration, ●). EG 626 (10^{-5} g/ml) was applied during the generation of contraction.

FIG. 4. Effects of EG 626 (10^{-5} g/ml) on the noradrenaline-induced contraction of pulmonary and mesenteric arteries. The maximum amplitude of the contraction evoked at 10^{-6} g/ml noradrenaline was registered as 100% contraction in both tissues. ●; control. ○; EG 626 treatment.

contraction evoked by nerve stimulation (0.5 msec 30 Hz and 10 sec) is also illustrated. The minimum concentration of noradrenaline required to evoke the mechanical response in the pulmonary artery was 2×10^{-9} g/ml and to evoke the contraction in the mesenteric artery, a 5 times higher concentration was required. In these experiments, the nerve-mediated contraction showed a higher amplitude than 10^{-7} g/ml noradrenaline induced contraction in the mesenteric artery, but the nerve-mediated contraction was lower than the 10^{-8} g/ml noradrenaline induced contraction in the pulmonary artery.

EG 626 (10^{-5} g/ml) consistently suppressed the contraction evoked by various concentrations of noradrenaline (10^{-8} – 3×10^{-7} g/ml) in both tissues. Fig. 4 shows the effects of

noradrenaline on the mechanical response in the presence or absence of EG 626 in the pulmonary and mesenteric arteries. The concentrations of noradrenaline were varied from 10^{-9} to 10^{-6} g/ml, and the maximum amplitude of contraction recorded by treatment with 10^{-6} g/ml noradrenaline was registered as 100% relative tension. Application of EG 626 (10^{-5} g/ml) consistently reduced the amplitude of the tension development and raised the mechanical threshold to evoke contraction by treatment with noradrenaline. There were no differences regarding the effects of EG 626 on the mechanical responses recorded from smooth muscle tissues of the pulmonary and mesenteric arteries.

The mechanical response induced by 118 mM K^+ solution was also partly suppressed by EG 626 but the grade of the suppression was less than that observed on noradrenaline induced contraction.

Effects of EG 626 on the depolarization induced contraction

Effects of EG 626 on the depolarization-induced contraction were observed by voltage clamp technique. As shown in Fig. 5, when the membrane was depolarized in steps, the mechanical response increased up to a certain level. The maximum contraction was evoked by 25 mV depolarization from the resting potential of 40 mV and further depolarization of the membrane did not increase the amplitude of the contraction.

The critical depolarization level of the membrane required to evoke contraction was 5 mV at the resting membrane potential of 43 mV measured by the double sucrose gap method. This value was about 10 mV lower than that measured by the microelectrode

FIG. 5. Depolarization-induced contraction of the main pulmonary artery observed before and during application of EG 626 (10^{-6} and 10^{-5} g/ml). The voltage clamp method in double sucrose gap apparatus was used. Top record, current; middle record, potential; lower record, tension.

method. Following application of EG 626 (10^{-6} g/ml), the mechanical response induced by any given grade of the depolarization was consistently less than that generated in Krebs solution. Moreover, the electrical currents recorded during the application of the various grades of depolarization were not markedly modified by application of EG 626 compared with those measured in Krebs solution.

Fig. 6 shows the relationship between the depolarization of the membrane and the peak amplitude of contraction measured by voltage clamp procedures. Application of 10^{-6} g/ml EG 626 neither raised the critical potential to evoke the contraction nor suppressed the contraction. However, at the concentration of 10^{-5} g/ml, EG 626 raised the threshold to induce contraction from 5 mV to 11 mV, and suppressed the maximum amplitude of contraction to 70% of the control at 40 mV depolarization.

The maximum amplitude of the contraction was evoked at 25 mV depolarization, and further depolarization of the membrane did not enhance the contraction in the presence of EG 626, as observed in Krebs solution. This means that EG 626 suppressed the depolarization-induced contraction and shifted the dose-response curve to the right in a noncompetitive manner with depolarizing action.

DISCUSSION

The passive properties of the smooth muscles of the rabbit main pulmonary artery have been studied extensively, and specific features have been elucidated, i.e. the membrane did not generate spikes by applications of either electrical stimulation or externally applied chemical transmitters, but such did not indicate the inability to generate spikes, since the application of either procaine or TEA generated the spike due to suppression of K-conductance. Moreover, a low concentration of externally applied noradrenaline ($<10^{-8}$ g/ml) produced contraction without depolarization of the membrane, and a higher concentration ($>5 \times 10^{-8}$ g/ml) produced the contraction with depolarization of the membrane. The former is thought to increase the Ca^{++} influx from the extracellular space or to release the loosely bound Ca^{++} on the surface membrane, and the latter to increase the release of the sequestered Ca^{++} in addition of the actions of a low concentration of noradrenaline (8, 9). The membrane properties of smooth muscle cells of guinea pig mesenteric vein have been studied by Nakajima and Horn (16) and the generated spike was postulated to be due to increase in Ca-flux during the activation of the membrane. However, in the rabbit superior mesenteric artery the spontaneously generated spike was not observed.

FIG. 6. Relationship between depolarization and contraction before and during application of EG 626 (10^{-6} g/ml and 10^{-5} g/ml). Voltage-clamp method was applied using the double sucrose gap apparatus.

The circular muscle of the pulmonary artery possesses cable-like properties, that is the smooth muscle cells in the tissues are connected to each other as a functional syncytium. However in the mesenteric artery, the relationship between amplitude of the electrotonic potential and the distance from the stimulating electrode was not measured in the present experiments due to technical difficulties. Therefore, it is not yet clear whether the mesenteric artery possesses a cable-like property.

EG 626 suppressed the mechanical responses induced by nerve mediated contraction rather than by direct muscle stimulation. Thus, this agent not only suppresses the Ca^{++} mobilization for the contraction but also suppresses the noradrenaline release from the nerve terminals via suppression of Ca^{++} mobilization as is the case in the neuro-muscular transmission of excitation (17).

In the pulmonary artery, noradrenaline generated the contracture in a concentration of 10^{-9} g/ml, and 10^{-8} g/ml noradrenaline produced a much higher amplitude of contraction than that generated by nerve stimulation. On the other hand, the noradrenaline induced contraction in the mesenteric artery was lower than the contraction evoked by nerve stimulation. The amounts of noradrenaline released from the nerve terminals may differ in both tissues, and as a consequence such a discrepancy may exist regarding contractions induced by two different stimulating methods.

The sources of Ca^{++} required to produce contraction by treatment with noradrenaline differ in vascular tissues (18, 19). EG 626 relaxed to the same extent the contraction produced by high (10^{-7} g/ml) and low (10^{-9} g/ml) concentrations of noradrenaline. Since a low concentration of noradrenaline did not modify the sequestered Ca^{++} in the cells, EG 626 may suppress the mobilization of Ca^{++} distributed in the extracellular space of loosely bound Ca on the surface membrane rather than the suppression of the releasing mechanism from the sequestered sites (8, 9, 14, 15).

The nature of the K-induced contraction also varies with various visceral muscles (20). For example, the smooth muscle of the main pulmonary artery increases Ca-influx with increased ionic conductance of the membrane during application of excess K^+ , and K-induced depolarization is not an essential factor in the production of the contraction (15). As EG 626 suppressed the amplitude of K-induced contracture, this agent probably suppresses the influx of Ca^{++} during increased ionic conductances.

When the effects of EG 626 were observed on the depolarization-contraction relation curve under voltage clamp procedures, the threshold potential level required to generate the contraction was raised without any noticeable change in the membrane potential. Since the amplitude of contraction was not enhanced by stronger depolarization than 25 mV as observed in Krebs solution, EG 626 might also suppress the increase in intracellular free Ca^{++} concentration produced by depolarization.

It is known that verapamil and D 600 suppress the influx of Ca^{++} in the membrane of the smooth muscle and suppress the contraction (19, 21, 22). These agents strongly suppress the membrane activity of spontaneously active smooth muscle rather than the quiescent muscle. However, with application of EG 626 there was no difference in change in resting

membrane properties and contractions of the pulmonary artery and mesenteric artery, and this agent had weak effects on the smooth muscle of the portal vein as compared to that seen in the pulmonary artery (Y. Ito, H. Suzuki and H. Kuriyama; unpublished observations).

It has been reported that EG 626 inhibits cyclic AMP phosphodiesterase activity (3). As the result of various investigations, a possible role for intracellular cyclic AMP in the regulation of the smooth muscle tone and responsiveness to physiological stimuli has been suggested, i.e. activation of adenylate cyclase by β -adrenergic agonist, resulting in inhibition of contractility (23–25). In fact, a good correlation could be demonstrated in a comparative study of the effects of noradrenaline on the uterine relaxation and adenylate cyclase activation, thereby providing evidence for regulatory role of cyclic AMP in uterine motility. On the other hand, findings obtained with application of prostaglandin E rule out the hypothesis that cyclic AMP controls relaxation, as prostaglandin E induces contraction and increases cyclic AMP levels (26). As the action of EG 626 on cyclic AMP levels was not examined herein, the obtained results were not discussed in relation to the enzymatic activity.

It is thus concluded that EG 626 possesses a vasodilative action, i.e. this agent suppressed the mechanical activity with no noticeable change in the membrane properties. These responses of vascular muscles were similar to those obtained by treatment with diltiazem *in vitro* (27, 28). Presumably this agent suppresses the Ca^{++} mobilization to activate the contractile machine and also the chemical substance releasing mechanism from the nerve terminals. Whether EG 626 actually has a specific action on the vascular system should be clarified by further investigations on the tissues *in vivo* and *in vitro*.

REFERENCES

- 1) ADACHI, K. AND NUMANO, F.: Phosphodiesterase inhibitors: Their comparative effectiveness *in vitro* in various organs. *Japan. J. Pharmacol.* **27**, 97–103 (1977)
- 2) ASANO, T., OCHIAI, Y. AND HIDAKA, H.: Selective inhibition of separated forms of human platelet cyclic nucleotide phosphodiesterase by platelet aggregation inhibitors. *Mol. Pharmacol.* **13**, 400–406 (1977)
- 3) SHIMAMOTO, T., TAKASHIMA, Y., KOBAYASHI, M., MORITA, K. AND TAKAHASHI, T.: A thromboxane A_2 -antagonistic effect of pyridinolcarbamate and phthalazinol. *Proc. Japan Acad.* **52**, 591–594 (1976)
- 4) HAMBERG, M., SVENSSON, J. AND SAMUELSSON, B.: Thromboxanes; a new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994–2998 (1975)
- 5) NEEDLEMAN, P., MONCADA, S., BUNTING, S., VANE, J.R., HAMBERG, M. AND SAMUELSSON, B.: Identification of an enzyme in platelet microsomes which generates thromboxane A_2 from prostaglandin endoperoxides. *Nature* **261**, 558–560 (1976)
- 6) SHIMAMOTO, T., TAKANO, T., TAKENO, K. AND NUMANO, F.: Treatment of amyotrophic lateral sclerosis with phthalazinol, a cyclic AMP phosphodiesterase inhibitor. *Proc. Japan Acad.* **52**, 78–81 (1976)
- 7) ABE, Y. AND TOMITA, T.: Cable properties of smooth muscle. *J. Physiol.* **196**, 87–100 (1968)
- 8) CASTEELS, R., KITAMURA, K., KURIYAMA, H. AND SUZUKI, H.: The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.* **271**, 41–61 (1977)
- 9) CASTEELS, R., KITAMURA, K., KURIYAMA, H. AND SUZUKI, H.: Excitation-contraction coupling in the smooth muscle cells of the rabbit main pulmonary artery. *J. Physiol.* **271**, 63–79 (1977)
- 10) ROUGIER, O., VASSORT, G., GARNIER, D., GARGOUILI, Y.M. AND CORABOEUF, E.: Existence

- and role of a slow inward current during the frog atrial action potential. *Pflügers Arch.* **308**, 91–110 (1969)
- 11) GOTO, M., WADA, Y. AND SAITO, M.: Tension components and tension fall of the bullfrog atrial muscle during depolarization. *Japan. J. Physiol.* **24**, 359–375 (1974)
 - 12) ANDERSON, N.C.: Voltage-clamp studies on uterine smooth muscle. *J. gen. Physiol.* **54**, 145–165 (1969)
 - 13) OHBA, M., SAKAMOTO, Y. AND TOMITA, T.: The slow wave in the circular muscle of the guinea-pig stomach. *J. Physiol.* **253**, 505–516 (1975)
 - 14) ITO, Y., KURIYAMA, H. AND SUZUKI, H.: Effects of caffeine and procaine on the rabbit pulmonary artery. *Japan. J. Physiol.* **27**, 461–481 (1977)
 - 15) ITO, Y., KURIYAMA, H. AND SUZUKI, H.: On the roles of calcium ion during potassium-induced contracture in the smooth muscle cells of the rabbit main pulmonary artery. *Japan. J. Physiol.* **27**, 755–770 (1977)
 - 16) NAKAJIMA, A. AND HORN, L.: Electrical activity of single vascular smooth muscle fibres. *Am. J. Physiol.* **213**, 25–30 (1967)
 - 17) DEL CASTILLO, J. AND KATZ, B.: Statistical factors involved in neuromuscular facilitation and depression. *J. Physiol.* **124**, 574–585 (1954)
 - 18) HURWITZ, L.: Characterization of calcium pools utilized for contraction in smooth muscle. *Smooth Muscle Pharmacology and Physiology*, Edited by WORCEL, M. AND VASSORT, G., p. 369–380, INSERM Paris (1975)
 - 19) GOLENHOFEN, K.: Theory of P and T systems for calcium activation in smooth muscle. *Physiology of Smooth Muscle*, Edited by BÜLBRING, E. AND SHUBA, M.F., p. 197–202, Raven Press, New York (1976)
 - 20) KURIYAMA, H., OSA, T., ITO, Y. AND SUZUKI, H.: Excitation-contraction coupling mechanism in visceral smooth muscle. *Adv. Biophys.* **8**, 115–190 (1975)
 - 21) FLECKENSTEIN, A.: Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. *A. Rev. Pharmacol. Toxicol.* **17**, 149–166 (1977)
 - 22) FLECKENSTEIN, A., DÖRING, H.J. AND KAMMERMEIER, H.: Einfluss von Beta-Rezeptorenblockern und verwandten Substanzen auf Erregung, Kontraktion und Energiestoffwechsel der Myokardfaser. *Klin. Wschr.* **46**, 343–351 (1968)
 - 23) ROBISON, G.A., BUTCHER, R.W. AND SUTHERLAND, E.W.: *Fundamental Concepts in Drug-receptor Interactions*, p. 59–91, Academic Press, N.Y. (1970)
 - 24) HARBON, S. AND CLAUSER, H.: Cyclic adenosine 3',5'-monophosphate levels in rat myometrium under the influence of epinephrine, prostaglandins and oxytocin, correlations with uterus motility. *Biochem. biophys. Res. Commun.* **44**, 1496–1503 (1971)
 - 25) POLACEK, I. AND DANIEL, E.E.: Effect of α - and β -adrenergic stimulation on the uterine motility and adenosine 3',5'-monophosphate level. *Canad. J. Physiol. Pharmacol.* **49**, 988–998 (1971)
 - 26) HARBON, S., VESIN, M. AND DOKAC, L.: The effects of epinephrine and prostaglandins of cAMP formation and binding to its intracellular receptors. Correlations with myometrial activity. *Smooth Muscle Pharmacology and Physiology*, Edited by WORCEL, M. AND VASSORT, G., INSERM, Paris, **50**, 83–100 (1975)
 - 27) SATO, M., NAGANO, T., YAMAGUCHI, I., NAKAJIMA, H. AND KIYOMOTO, A.: Pharmacological studies on a new 1,5-benzothiazepine derivative (CRD-401). *Arzneim.-Forsch.* **21**, 1338–1343 (1971)
 - 28) MAGARIBUCHI, T., NAKAJIMA, H. AND KIYOMOTO, A.: Effects of diltiazem and Lanthanum ion on the potassium contracture of isolated guinea pig smooth muscle. *Japan. J. Pharmacol.* **27**, 333–339 (1977)