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Studies on the Mechanism of Spasmolytic Activity of (*O*-Methyl-)-*N*-(2,6-dihydroxybenzoyl)tyramine, a Constituent of *Aniba riparia* (Nees) Mez. (Lauraceae), in Rat Uterus, Rabbit Aorta and Guinea-pig Alveolar Leucocytes

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Abstract—The mechanism of action of a nonspecific smooth muscle relaxant, (*O*-methyl-)-*N*-(2,6-dihydroxybenzoyl)tyramine (riparin), a constituent of *Aniba riparia* (Nees) Mez. (Lauraceae) was studied in relation to Ca^{2+} metabolism in smooth muscle tissues and in guinea-pig alveolar leucocytes. In rat depolarized uterus, riparin inhibited in a reversible and noncompetitive manner $CaCl_2$-induced contraction, a response mediated through voltage-dependent Ca^{2+} channels. The pD_2 value (mean ± s.e.m.) for riparin was 4·98 ± 0·06. When compared with sodium nitroprusside (IC50 2·5 μM), an antagonist of receptor-operated Ca^{2+} channels, riparin was ineffective in suppressing noradrenaline-induced sustained contractions of rabbit aortic strips. However, in the aorta, the compound inhibited intracellular calcium-dependent transient contractions of noradrenaline and riparin (IC50 10·1 μM) was approximately two and a half times more potent than procaine (IC50 25·5 μM) a known inhibitor. In guinea-pig alveolar leucocytes, riparin (IC50 3·2 μM) inhibited intracellular Ca^{2+} accumulation induced by the calcium ionophore A23187. The results suggest that the inhibition of Ca^{2+} influx and of Ca^{2+} release from intracellular stores contribute to the spasmolytic effects of riparin, which may not involve cyclic AMP generation as the levels of this nucleotide were not increased in alveolar macrophages treated with riparin (10–100 μM).

A number of methyl ethers of *N*-(benzoyl) tyramine with broad spectrum antimicrobial activity have been isolated from the unripe fruit of *Aniba riparia* collected from the Amazonas State of Brazil (Barbosa Filho et al 1987). It was reported recently, that one of the above compounds (*O*-methyl-)-*N*-(2,6-dihydroxybenzoyl)tyramine (riparin), obtained synthetically (Barbosa Filho et al 1990) has potent smooth muscle relaxant activity as well (Castelo Branco et al 1991; Castelo Branco 1992). Thus, in concentrations of 8–30 μM, riparin antagonized acetylcholine- and histamine-induced contractions of the guinea-pig ileum, and oxytocin- and bradykinin-induced contractions of the rat uterus. Further, in the guinea-pig trachea, riparin inhibited the spontaneous tone (IC50 7·7 μM) and carbachol-induced contractions (IC50 10 μM).

In the present study, attempts were made to clarify the mode of action of riparin in relation to some aspects of calcium metabolism. $CaCl_2$-induced contractions of rat depolarized uterus (Villar et al 1986) that occur as a result of Ca^{2+} entry through voltage-operated channels were utilized to observe the effect of riparin on these channels. While the tonic component of noradrenaline contraction in the rabbit aorta depends on Ca^{2+} entry through receptor operated channels, the phasic component is due to the release of intracellular Ca^{2+}. These two responses to noradrenaline are inhibited by sodium nitroprusside and procaine, respectively (Karaki et al 1984, 1986), and the effect of riparin was compared with these two agents. The activity of riparin on intracellular Ca^{2+} accumulation induced by the ionophore A23187, which promotes Ca^{2+} entry by forming lipophilic complexes (Pressman 1973), was studied in guinea-pig bronchoalveolar leucocytes. In addition, the ability of riparin to increase cyclic (c) AMP levels in these cells was also investigated as cyclic nucleotides are important regulators of cell calcium (Mathews 1991). Bronchoalveolar leucocytes were used as they contribute to the inflammatory and hyperactivity responses in asthma (Holgate & Finnerty 1988), and it was of interest to observe whether, in addition to its smooth muscle relaxant activity, riparin modifies calcium metabolism in such cells, to assess better the value of riparin in allergic diseases.

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Materials and Methods

General

Riparin was solubilized in cremophor EL (Sigma) and was diluted with physiological solutions as required. The final concentration of cremophor never exceeded 0·1% which produced no effect in isolated tissues or cells.

Effect on voltage-dependent Ca^{2+} channels in the rat uterus

Uterine strips were obtained from virgin female rats, 150–200 g, treated with 100 μg kg^{-1} diethylstilboestrol subcutaneously, 24 h before the experiments. The tissues were suspended under a resting load of 1 g in 10-mL organ baths containing De Jalon solution (mM: NaCl 153·8, $NaHCO_3$ 5·95, KCl 5·5, $CaCl_2$ 0·27 and glucose 2·77) maintained at 32°C and bubbled with 95% O_2–5% CO_2. After a period of 20 min, the tissues were exposed for 1 h to high K^+, Ca^{2+}-free

depolarizing solution (mM: KCl 100·0, $MgCl_2$ 2·1, $NaHCO_3$ 5·95, and glucose 2·77). Subsequently, two cumulative concentration-response curves for $CaCl_2$ were obtained, using kymographs, smoked drums and isotonic levers (6- to 7-fold amplification). A third curve for $CaCl_2$ was then obtained in the presence of riparin added 10 min before. Further experiments were conducted with various concentrations of riparin and verapamil using different tissues for the two substances. Antagonism was measured by calculating pD_2' values (Ariens & Van Rossum 1957), defined as the $-\log$ molar concentration to produce a 50% reduction of the maximum contraction.

Effect on receptor-operated Ca^{2+} channels in rabbit aortic strips

Aorta from albino rabbits, 2·0–2·5 kg, was removed and cut into helical strips, and the adventitial layer was removed as described by Karaki et al (1979). The tissues were suspended in Tyrode solution maintained at 37°C and bubbled with 95% O_2–5% CO_2. The composition of the Tyrode solution was (mM): NaCl 136·8, $NaHCO_3$ 11·9, $MgCl_2$ 1·0, KCl 5·4, $CaCl_2$ 2·5, and glucose 5·5. The tissues were allowed to equilibrate for 1 h under a resting tension of 1 g. Force generation was monitored with an isometric transducer (Beckman 410) coupled to a polygraph recorder (Beckman R511A). Two submaximal (65–75% maximal) tonic responses to 3 μM noradrenaline which stabilized in 15 min were registered. A third response was then obtained and riparin was added cumulatively at 10 min intervals in an attempt to obtain a concentration-inhibition curve. The concentrations of riparin used were 1, 3, 10, 30 and 100 μM. In other tissues sodium nitroprusside (0·1, 0·4 and 1·6 μM) was tested as the inhibitor. Some experiments were also conducted in which noradrenaline was added to the tissue and left for at least 2 h to observe whether the tension was maintained during this period. Where possible the IC50 value was obtained graphically from concentration-inhibition curves.

Effect on intracellular Ca^{2+}-dependent noradrenaline response in the aorta

The aortic strip was prepared as indicated before and suspended in Tyrode solution under 1 g tension for 2 h. Two transient phasic contractions to noradrenaline were obtained using the procedure described by Karaki et al (1979) as follows. The strip was washed twice with Ca^{2+}-free Tyrode solution (Tyrode solution with no $CaCl_2$ but containing 1·0 mM EGTA) and left in this solution for 5 min. Noradrenaline (1 μM) was then added and the resultant transient response was recorded using a force displacement transducer coupled to a polygraph as described above. The tissue was washed and suspended in Tyrode solution (calcium loading) for 15 min, then washed twice with Ca^{2+}-free Tyrode solution and resuspended in this solution for 5 min before registering a second noradrenaline response. The procedure was repeated to record a third noradrenaline response which was obtained in the presence of different concentrations of riparin or procaine added 10 min before the noradrenaline. Inhibition of the response was calculated by comparing the response in the absence or presence of the inhibitors. Comparative potencies of riparin and procaine were determined graphically as described earlier.

Inhibition of intracellular Ca^{2+} accumulation induced by ionophore A23187 in guinea-pig alveolar leucocytes

Bronchoalveolar cells, approximately 60–70% of which are macrophages (Bachelet et al 1990), were collected from normal guinea-pigs under general anaesthesia (40 mg kg^{-1} sodium pentobarbitone, i.p.) by 20 successive lavages using 5-mL 0·9% NaCl at room temperature (21°C). The cell suspension was centrifuged at 475 *g* for 10 min and resuspended in a culture medium (RPMI 1640 without phenol red) (Gibco, UK). Loading was started by addition of a small volume of fura-2/acetoxy-methyl ester (fura 2) (Calbiochem, USA) dissolved in dimethylsulphoxide (final concentration of fura 2 was 2 μM). The incubation was continued for 30 min at 37°C in the dark. Bronchoalveolar-loaded cells were then rinsed with fresh RPMI medium and the final suspension was transferred into a stirred thermostated cuvette (37°C) before stimulation with ionophore (1 μM A23187), with increasing concentrations of riparin (0–30 μM) added 1 min before the ionophore. In alveolar leucocytes, the above concentration of ionophore was previously found to produce at least a 100% increase in cytosolic Ca^{2+} which could be used to study the effect of inhibitors. Fluorescent changes were monitored in a Jobin Yvon (JY3D) spectrofluorometer at an excitation wavelength of 340 nm and an emission wavelength of 400 nm. The cells were lysed after the fluorescence measurement by adding 10 μL 100 mg mL^{-1} saponin and the value of F_{max} was determined in the presence of 1 M $CaCl_2$. F_{min} was determined by adding 10 μL 0·5 M EGTA to the cell lysate. $[Ca^{2+}]_i$ concentrations were calculated as described by Tsien (1988).

Effect of riparin on intracellular cAMP levels in guinea-pig alveolar macrophages

Bronchoalveolar cells were collected from normal guinea-pigs as described above. The cell suspensions were centrifuged at 475 *g* for 10 min at room temperature, resuspended in a culture medium (RPMI 1640 containing 3% foetal calf serum) and then plated in 22-mm Petri dishes (Costar, USA, 1 mL/dish containing approximately 10^6 cells). Cells were allowed to adhere for 1 h in a humidified atmosphere containing 5% CO_2. Non-adhering cells were discarded and the remaining monolayers (95% macrophages) were incubated for an additional 1 h in serum-free RPMI medium. To evaluate the intracellular cAMP content, monolayers were incubated in 0·3 mL/well Tris-HCl buffer (50 mM, pH 7·4) containing 0·2 mM IBMX (3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor) (Sigma, USA) and 2 mM EDTA. Incubations were carried out for 3 min at 37°C in the presence of increasing concentrations of riparin (1–100 μM) or of prostaglandin E_2 (2·8 μM). The reaction was stopped by heating the plates in boiling water for 5 min, to empty the cellular content of cAMP into the medium. The medium was transferred to Eppendorf tubes and centrifuged at 12 000 *g* for 2 min, to produce supernatants that were analysed for cAMP using a competitive protein-binding assay (Gilman 1970). Reagents for the cAMP assay were from Amersham, UK.

Statistical analysis

Results were analysed using Student's *t*-test and differences were considered significant for $P < 0.05$.

Results

Effect on voltage-dependent Ca^{2+} channels in the rat uterus

Riparin (3–30 μM) antagonized the cumulative concentration-effect curves to $CaCl_2$ in the depolarized uterus. The antagonism was reversible and was of a noncompetitive nature as there was suppression of the maximal response (Fig. 1A). The values of correlation coefficient (r) and pD_2 were 0.99 ± 0.1 and 4.98 ± 0.06, respectively. Verapamil (0·01–1 μM) also inhibited the $CaCl_2$ responses in a similar manner. The effects of verapamil at 0·1 and 1 μM are shown in Fig. 1B and the values of r and pD_2 were 0.97 ± 0.02 and 6.56 ± 0.1, respectively.

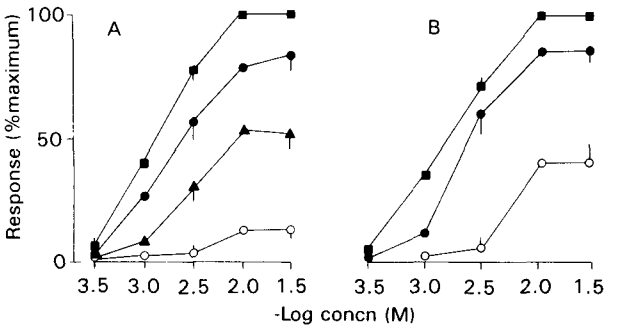

FIG. 1. Effect of riparin (A) and verapamil (B) on cumulative concentration-response curves to $CaCl_2$ in the depolarized rat uterus. A. ■ Control; riparin ● 3×10^{-6} M; ▲ 1×10^{-5} M; ○ 3×10^{-5} M. B. ■ Control; verapamil ● 1×10^{-7} M; ○ 1×10^{-6} M. Values are mean ± s.e.m. of five experiments.

Effect on receptor-operated Ca^{2+} channels in the rabbit aorta

Noradrenaline (30 μM) produced a sustained contraction of the aorta in 15 min which was maintained during the 2 h period of observation. Riparin added cumulatively at 10-min intervals to reach a final concentration of 100 μM to the precontracted aorta failed to relax the tissue. Sodium nitroprusside produced a rapid relaxation that attained the maximum effect in 7–9 min. The relaxation produced by 0·1, 0·4 and 1·6 μM sodium nitroprusside was 32.6 ± 2.2, 59.5 ± 2.4 and 86.9 ± 2.3%, respectively. These results were statistically significant and the IC50 value obtained graphically was approximately 250 nM. Thus, unlike sodium nitroprusside, riparin did not inhibit Ca^{2+} entry through receptor-operated channels in the aorta.

Effect on intracellular Ca^{2+}-dependent transient noradrenaline response in the rabbit aorta

In the absence of extracellular Ca^{2+}, noradrenaline (1 μM) produced a transient contraction of the aorta. The response could be repeated if the intracellular Ca^{2+} levels were maintained by the Ca^{2+}-loading procedure described earlier. Pre-incubation of Ca^{2+}-loaded aorta in Ca^{2+}-free medium with either riparin for 10 min or procaine for 5 min, inhibited the responses to noradrenaline in a concentration-dependent manner. The results are summarized in Table 1. The approximate IC50 values obtained graphically were 10·1 and 25·5 μM for riparin and procaine, respectively.

Table 1. Inhibition of 1 μM noradrenaline-induced transient contractions of rabbit aorta with riparin and procaine.

Compound	Concn (μM)	Inhibition (%) (mean ± s.e.m.)	IC50 (μM)
Riparin	3·0	23·9 ± 1·6*	10·1
	10·0	51·0 ± 2·2*	
	30·0	73·9 ± 2·4*	
	100.0	91·7 ± 4·1*	
Procaine	10·0	20·4 ± 1·4*	25·5
	20·0	45·1 ± 2·0*	
	40·0	64·5 ± 1·9*	
	80·0	80·9 ± 1·9*	

* $P < 0.05$, n = 7.

Table 2. Inhibition of the ionophore A23187-induced increase in cytosolic Ca^{2+} by riparin.

Riparin (μM)	Inhibition % (mean ± s.e.m.)	IC50 (μM)
1·0	31·0 ± 3·0*	3·2
3·0	45·0 ± 7·9*	
10·0	67·4 ± 7·8*	
30·0	94·5 ± 3·5*	

* $P < 0.05$, n = 10.

Effect on intracellular Ca^{2+} accumulation induced by the ionophore A23187 in guinea-pig alveolar leucocytes

Basal values of cytosolic Ca^{2+} concentration in alveolar leucocytes were significantly increased from 185.0 ± 20.1 to 462.5 ± 52.1 nM (mean ± s.e.m., n = 10) by ionophore A23187 in the presence of 0·5 nM calcium in the extracellular medium. This ionophore-induced increase in cytosolic [Ca^{2+}] was dose-dependently antagonized by riparin as shown in Table 2. The approximate IC50 value calculated graphically was 3·2 μM.

Effect on intracellular cAMP accumulation in guinea-pig alveolar macrophages

No significant changes were observed in the cellular cAMP accumulation by guinea-pig alveolar macrophages in the presence of riparin (n = 6). Thus, while the mean (± s.e.m.) increase in cAMP levels in untreated cells was 21.6 ± 5.7 pmol/10^6 cells, the values in cells incubated with riparin at 10, 30 or 100 μM were 20.91 ± 4.0, 20.0 ± 5.6 and 12.0 ± 2.3, respectively. The cAMP content of cells treated with prostaglandin E_2 was significantly elevated to 75.2 ± 8.1 pmol/10^6 cells.

Discussion

Riparin was recently shown to have nonspecific and reversible smooth muscle relaxant activity in concentrations ranging from 8 to 30 μM depending on the tissue and the agonist used (Castelo Branco et al 1991; Castelo Branco 1992). The present studies attempt to clarify the mechanism of action of the compound in relation to Ca^{2+}.

In the depolarized uterus, riparin was found to inhibit Ca^{2+} entry through depolarization-dependent Ca^{2+} chan-

nels. The pD_2 value of 4·98 obtained for riparin corresponds to 10·5 μM, which is within the concentration range required to produce spasmolytic effects. Thus, the inhibition of the above Ca^{2+} channels is likely to contribute to the smooth muscle relaxant activity of riparin.

In rabbit aorta, noradrenaline-induced contraction comprises two phases. An initial transient contraction mediated by intracellular Ca^{2+} release (Karaki et al 1984, 1986) has been shown to be due to increased turnover of phosphatidylinositol (Villalobos-Molina et al 1982) and the subsequent production of inositol 1,4,5-trisphosphate (IP_3). Several vasodilators are known to inhibit such agonist-induced phosphoinositide turnover (Challiss et al 1992). The activity of riparin in inhibiting the transient contraction suggests that the IP_3-sensitive intracellular Ca^{2+} stores may also be another site of action. The second sustained phase of noradrenaline response which was not inhibited by riparin depends on Ca^{2+} influx through receptor-operated channels (Karaki et al 1984, 1986), which is blocked by agents such as nitroprusside which activates protein kinase G through cGMP generation (Scott 1991). Riparin does not seem to act in this manner.

It has been proposed (Irvine 1992; Berridge 1993) that the agonist-induced Ca^{2+} influx may be regulated by IP_3, and inositol 1,3,4,5-tetrakis phosphate (IP_4) through their respective plasmalemma receptors in addition to the influence of intracellular Ca^{2+} stores themselves. The two differing results of riparin on the transient and sustained contractions raise the possibility that the compound may act by blocking the IP_3 receptors in the endoplasmic reticulum, which will result in the inhibition of the transient contractions. As riparin may fail to empty the Ca^{2+} stores, modify the generation of IP_3 and IP_4 or block their respective plasmalemma receptors, it will be ineffective in blocking Ca^{2+} influx through receptor-operated channels required for the sustained contraction.

Another explanation for the different effects of riparin on the transient and sustained responses to noradrenaline may be based on the recent proposal (Rasmussen et al 1987; Alkon & Rasmussen 1988) that the two responses depend on different biochemical pathways. Thus, during the sustained but not during the transient response, the intracellular free Ca^{2+} level is only slightly elevated from normal levels. Both the concentration of phosphorylated myosin light chain kinase, and the rate of ATP utilization are also lower during a sustained response and, further, different contractile proteins are phosphorylated in the sustained phase. It is possible that riparin may have little influence on these mechanisms that maintain tone in the rabbit aorta.

Yet another effect of riparin on Ca^{2+} movements was observed in guinea-pig bronchoalveolar leucocytes, 60–70% of which are macrophages (Bachelet et al 1990). Riparin in micromolar concentrations inhibited the ionophore A23187-promoted intracellular Ca^{2+} accumulation. Lipophilic ionophores are known to transport Ca^{2+} across both plasmalemma and intracellular membranes (Sambrook 1990) and their effects are not antagonized by voltage-dependent Ca^{2+}-channel blockers (Gerritsen et al 1987). The above effect of riparin needs further study.

Overall, the results indicate that the inhibition of Ca^{2+} influx and the release of intracellular Ca^{2+} which result in a reduction of intracellular Ca^{2+} concentration contribute to the spasmolytic effect of riparin. However, the mechanism by which this effect is achieved is not yet clear. Studies with guinea-pig bronchoalveolar macrophages suggest that, at least in these cells, intracellular cAMP concentrations are not affected by riparin, which is supported indirectly by the observation that propranolol does not antagonize the tracheal relaxation produced by the compound (Castelo Branco 1992).

There is increasing evidence that a number of plant-derived substances alter calcium metabolism in cells. These examples include the action of ryanodine (Meissner 1986), jatrophone (Calixto & Santana 1990) and longicaudatine (Medeiros et al 1991) on intracellular Ca^{2+} stores, and the effect of thapsigargin on the enzyme Ca^{2+}-ATPase in the endoplasmic reticulum (Thastrup 1990). Along with other naturally occurring substances with intracellular effects, such as the cardiac glycosides (Noble 1986), forskolin (Seamon & Daly 1983) and the phorbol esters (Stabel & Parker 1991), simple molecules such as riparin may also be useful in investigating cell signal transduction mechanisms, in addition to its possible therapeutic use.

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References

Alkon, D. L., Rasmussen, H. (1988) A spatial-temporal model of cell activation. Science 239: 998–1005

Ariens, E. J., Van Rossum, J. M. (1957) pD_x, pA_x, and pD'_x values in the analysis of pharmacodynamics. Arch. Int. Pharmacodyn. 110: 275–300

Bachelet, M., Lagente, V., Fouque, F., Dumarey, C., Havet, N., Masliah, J., Bereziat, G., Vargaftig, B. B. (1990) Antigen-dependent activation of alveolar macrophages from ovalbumin-sensitized guinea-pigs: relevance of the route of administration and the amount of antigen provided. Clin. Exp. Allergy 20: 693–699

Barbosa Filho, J. M., Yoshida, M., Gottlieb, O. R., Barbosa, R. C. S. B. C., Giesbrecht, A. M., Young, C. M. (1987) Benzoyl esters and amides, styrylpyrones and neolignans from the fruits of *Aniba riparia*. Phytochemistry 26: 2615–2617

Barbosa Filho, J. M., Yoshida, M., Gottlieb, O. R. (1990) The tyramines of *Aniba riparia*: transformation into models of natural products. Rev. Latinoamer. Quim. 21: 5–7

Berridge, M. J. (1993) Inositol phosphates and calcium signalling. Nature 361: 315–325

Calixto, J. B., Santana, A. E. G. (1990) Evidence for the mechanism of the inhibitory action of jatrophone in the isolated rat uterine muscle. Gen. Pharmacol. 21: 117–122

Castelo Branco, U. J. V. (1992) Estudos Farmacológicos do Éter Metílico de *N*-(2,6-dihydroxibenzoyl)-tiramine. MSc Thesis, Universidade Federal de Paraíba, Brazil

Castelo Branco, U. J. V., Thomas, G., Araújo, C. C., Barbosa Filho, J. M. (1991) Atividade espasmolítica de benzamidas isoladas de *Aniba riparia* (Parte 1). Resume VI Reunião Anual de Federação de Sociedades de Biologia Experimental, Abstr. 6–69, 302

Challiss, R. A. J., Patel, N., Arch, J. R. S. (1992) Comparative effects of BRL38227, nitrendipine and isoprenaline on carbachol- and histamine-stimulated phosphoinositide metabolism in airway smooth muscle. Br. J. Pharmacol. 105: 997–1003

Gerritsen, M. E., Nganele, D. M., Rodrigues, A. M. (1987) Calcium ionophore (A23187) and arachidonic acid stimulated prostaglandin release from microvascular cells: effect of calcium antago-

nist and calmodulin inhibitors. J. Pharmacol. Exp. Ther. 240: 837–846

Gilman, A. G. (1970) A protein binding assay for adenosine 3′:5′-cyclic monophosphate. Proc. Natl. Acad. Sci. USA 67: 305–312

Holgate, S. T., Finnerty, J. P. (1988) Recent advances in understanding the pathogenesis of asthma and its clinical implications. Quant. J. Med. 66: 5–19

Irvine, R. F. (1992) Inositol phosphates and Ca^{2+} entry: toward a proliferation or a simplification? Fed. Am. Soc. Exp. Biol. J. 6: 3085–3091

Karaki, H., Kubota, H., Urakawa, N. (1979) Mobilization of stored calcium for phasic contraction induced by norepinephrine in rabbit aorta. Eur. J. Pharmacol. 56: 237–245

Karaki, H., Nakagawa, H., Urakawa, N. (1984) Comparative effects of verapamil and sodium nitroprusside on contraction and ^{45}Ca uptake in the smooth muscle of rabbit aorta, rat aorta and guinea-pig taenia coli. Br. J. Pharmacol. 81: 393–400

Karaki, H., Murakami, K., Urakawa, N. (1986) Mechanism of inhibitory action of sodium nitroprusside in vascular smooth muscle of rabbit aorta. Arch. Int. Pharmacodyn. 280: 230–240

Mathews, G. (1991) Ion channels that are directly activated by cyclic nucleotides. Trends Pharmacol. Sci. 12: 245–247

Medeiros, C. L. C., Thomas, G., Mukherjee, R. (1991) The source of Ca^{2+} for the spasmolytic actions of longicaudatine, a bisindole alkaloid isolated from *Strychnos trinervis* (Vell.) Mart. (Loganiaceae). Phytother. Res. 5: 24–28

Meissner, G. (1986) Ryanodine activation and inhibition of the Ca^{2+} release channel of sarcoplasmic reticulum. J. Biol. Chem. 261: 6300–6306

Noble, D. (1986) Sodium-calcium exchange and its role in generating electric current. In: Nathan, R. D. (ed.) Cardiac Muscle: The Regulation of Excitation and Contraction. Academic Press, New York, pp 121–200

Pressman, B. C. (1973) Properties of ionophores with broad range cation selectivity. Proc. Fed. Am. Soc. Exp. Biol. 32: 1698–1703

Rasmussen, H., Takuwa, Y., Park, S. (1987) Protein kinase C in the regulation of smooth muscle contraction. Fed. Am. Soc. Exp. Biol. J. 1: 177–185

Sambrook, J. F. (1990) The involvement of calcium in transport of secretory proteins from the endoplasmic reticulum. Cell 61: 197–199

Scott, J. D. (1991) Cyclic nucleotide-dependent protein kinases. Pharmacol. Ther. 50: 123–145

Seamon, K. B., Daly, J. W. (1983) Forskolin, cyclic AMP and cellular physiology. Trends Pharmacol. Sci. 4: 120–123

Stabel, S., Parker, P. J. (1991) Protein kinase C. Pharmacol. Ther. 51: 71–95

Thastrup, O. (1990) Role of Ca^{2+}-ATPases in the regulation of cellular Ca^{2+} signalling, as studied with the selective microsomal Ca^{2+}-ATPase inhibitor, thapsigargin. Agents Actions 29: 8–15

Tsien, R. Y. (1988) Fluorescence measurement and photochemical manipulation of cytosolic free calcium. Trends Neuro. Sci. 11: 419–424

Villalobos-Molina, R., Uc, M., Hong, E., Garcia-Sainz, J. A. (1982) Correlation between phosphatidylinositol labelling and contraction in rabbit aorta: effect of alpha-1 adrenergic activation. J. Pharmacol. Exp. Ther. 222: 258–261

Villar, A., Ivora, M. D., D'Ocon, M. P., Anselmi, E. (1986) Effects of sulphonylureas on spontaneous motility and induced contractions in rat isolated uterus. J. Pharm. Pharmacol. 38: 778–780