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Effects of ryanodine on tension development in rat aorta and mesenteric resistance vessels

[1]G. Julou-Schaeffer & J.L. Freslon

Université Louis Pasteur, Laboratoire de Pharmacologie Cellulaire et Moléculaire, UA CNRS 600, BP 24, 67401 Illkirch Cedex, France

1 The effects of ryanodine on contractile responses dependent either on intracellular Ca^{2+} release or on extracellular Ca^{2+} influx were studied in aorta and mesenteric resistance vessels of the rat.

2 In aorta, in the presence of extracellular Ca^{2+}, pretreatment with ryanodine (10^{-5} M) did not modify contractile responses to noradrenaline (NA) (10^{-6} M) whereas in the absence of Ca^{2+}, pretreatment with ryanodine reduced to about 25% the contractile response to NA (10^{-6} M) and totally abolished the transient contraction elicited by caffeine (5×10^{-2} M).

3 In mesenteric resistance vessels, ryanodine (10^{-5} M) had no effects on NA (10^{-5} M)-induced tension in the presence of extracellular Ca^{2+} but totally abolished contractile responses to caffeine (10^{-2} M) in the absence of Ca^{2+}.

4 In K^+-depolarized mesenteric resistance vessels, pretreatment with ryanodine (10^{-5} M) significantly enhanced contractile responses to Ca^{2+} concentrations higher than 10^{-4} M and 10^{-3} M for arteries depolarized with 30 mM and 40 mM K^+ respectively. Concentrations of either diltiazem (6×10^{-7} M) or nifedipine (10^{-8} M) that abolished contractile responses to Ca^{2+} in depolarized arteries (K^+, 40 mM) did not totally inhibit the enhancement of Ca^{2+}-induced contractions obtained in the presence of ryanodine.

5 Ryanodine did not modify the Ca^{2+} concentration-effect relationships in mesenteric resistance vessels exposed to NA or arginine vasopressin.

6 These data are consistent with the hypothesis that ryanodine induces a release of Ca^{2+} from intracellular stores, resulting in a subsequent reduction of the amplitude of contractions dependent upon intracellular Ca^{2+} liberation. Furthermore, the ability of sarcoplasmic reticulum to buffer rises in cytoplasmic Ca^{2+} may be reduced in the presence of ryanodine, thereby accounting for the potentiation of contractile responses to Ca^{2+} in K^+-depolarized mesenteric resistance vessels.

Introduction

Ryanodine is a neutral alkaloid extracted from *Ryana speciosa* (Jenden & Fairhurst, 1969) with variable effects on mechanical activity of muscles. In skeletal muscle, ryanodine generally produces irreversible contracture whereas in cardiac muscle, it causes negative inotropic effects (Edwards *et al.*, 1948; Sleator *et al.*, 1964). Although the mechanism of action of ryanodine is still unclear, considerable evidence suggests that it interferes specifically with Ca^{2+} movements across the sarcoplasmic reticulum of cardiac and skeletal muscles (Sutko *et al.*, 1985; Fabiato, 1985; Feher & Lipford, 1985) and recent studies strongly suggest that ryanodine exerts its effects by either opening or closing the sarcoplasmic reticulum Ca^{2+} channel, depending upon experimental conditions (Meissner, 1986; Rousseau *et al.*, 1987; Nelson, 1987).

In smooth muscle, it is now well established that intracellular Ca^{2+}, sequestered in sarcoplasmic reticulum, may represent an important source of the Ca^{2+} required for contraction (Devine *et al.*, 1972; for review see Johansson & Somlyo, 1980). However, few studies of the effects of ryanodine on mechanical activity in smooth muscle are available and conflicting results have been reported (Steinsland *et al.*, 1973; Ito *et al.*, 1986; Hwang & van Breemen, 1987). In guinea-pig aortic smooth muscle, ryanodine slowed the development of the contractile response to noradrenaline (NA) and significantly decreased peak tension; it was suggested that ryanodine inhibits Ca^{2+} release from sarcoplasmic reticulum (Ito *et al.*, 1986). In contrast, in rabbit aortic smooth

[1] Author for correspondence.

muscle, ryanodine produced a non-competitive inhibition of NA-induced tension in a Ca^{2+}-free medium but enhanced tonic tension elicited by 20 mM K^+ and thus it was proposed that it enhanced rather than inhibited Ca^{2+} release from sarcoplasmic reticulum (Hwang & van Breemen, 1987). These discrepancies prompted us to investigate the effects of ryanodine on contractile responses of rat aorta and mesenteric resistance vessels. Both vessels are suitable for investigating the effects of ryanodine since they contain intracellular Ca^{2+} stores releasable by agonists or caffeine (for review see Godfraind *et al.*, 1986).

Our findings are consistent with the view that ryanodine induces release of Ca^{2+} from rat isolated aorta and mesenteric resistance vessels, thereby depleting the agonist-sensitive Ca^{2+} store and indirectly reducing the ability of sarcoplasmic reticulum to sequester Ca^{2+}.

Methods

All experiments were carried out on vascular preparations obtained from 11–14 week-old female Wistar rats (Iffa credo, L'Arbresle, France).

Rat aorta preparation

After killing the rat by decapitation, the thoracic aorta was removed and rings (5 mm length) were set up under a tension of 2 g in 10 ml baths which contained physiological solution (mM: NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5) maintained at 37°C and aerated with 95% O_2 and 5% CO_2. When necessary, endothelium was physically disrupted by gently rubbing the inside of the rings. Contractile responses were measured with an isometric transducer (Kistler-Morse, Bellevue, Washington, USA) coupled to a recorder.

Effects of ryanodine in a medium containing 1.25 mM Ca^{2+}

After a 60 min equilibration period, successive contractile responses to NA (10^{-6} M) separated by a washout period of 1 h were obtained until the contractions were reproducible. The last contraction was taken as control (100%). The presence of endothelium was shown by the ability of acetylcholine (10^{-6} M) to relax precontracted vessels. After washing the preparation for 1 h, ryanodine (10^{-5} M) was added to the bath for 30 min and a subsequent contraction to NA was elicited. All results are expressed as a percentage of the NA control contraction.

Effects of ryanodine in a Ca^{2+}-free medium

After reproducible contractions to NA (10^{-6} M) had been obtained in physiological solution containing 1.25 mM Ca^{2+}, the contractile effects of NA (10^{-6} M) and caffeine (5×10^{-2} M) were assessed in a Ca^{2+}-free medium (identical to physiological solution except that Ca^{2+} was omitted) as follows: after 40 min in the Ca^{2+}-free medium, during which time the solution was changed 4 times, NA or caffeine was added to the bath. The contractile responses obtained were taken as control (100%). After a washout period of 1 h with Ca^{2+}-containing solution (in order to replenish intracellular Ca^{2+} stores), the same protocol was used except for the addition of ryanodine (10^{-5} M) for the last 30 min in Ca^{2+}-free medium before NA or caffeine were added. In preliminary experiments, it was checked that the contractile effects of NA or caffeine were reproducible. Contractile effects of NA and caffeine in the presence of ryanodine are expressed as percentages of the control contraction.

Rat mesenteric resistance vessels

Third generation branches originating from the superior mesenteric artery were dissected from rats anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). Arterial segments (length $\approx$ 2 mm, internal diameter $\approx$ 100 μm) were prepared and mounted on a myograph (previously described by Mulvany & Halpern, 1977). Briefly, two tungsten wires (30 μm diameter) were inserted through the lumen of the vessel. One was attached to a support on the arm of a force transducer (DSC-6, Kistler-Morse), the other was attached to a support carried by a micromanipulator (MR 50, Micro-Contrôle, Evry, France). After mounting, vessels were equilibrated for 1 h in a physiological salt solution (mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5 and glucose, 5.5), then stretched to a passive wall tension of 1 mN mm^{-1} as calculated below. From preliminary experiments, it was determined that this tension provided the maximal active force development when the vessels were challenged with a test solution containing NA (3×10^{-6} M) and K^+ (124 mM). The presence of endothelium in all preparations was confirmed by the ability of acetylcholine (10^{-7}–10^{-6} M) to relax precontracted vessels.

Effects of ryanodine in a medium containing 2.5 mM Ca^{2+}

Following a 60 min equilibration period, successive contractile responses to NA (10^{-5} M), separated by a washout period of 30 min, were induced in order to

obtain a reproducible contraction which was taken as 100%. A further contractile response to NA (10^{-5} M) was then obtained after a 30 min incubation period with ryanodine (10^{-5} M). This response was expressed as a percentage of the control contraction.

Effects of ryanodine in a Ca^{2+}-free medium

After reproducible contractions to NA had been obtained, vessels were washed for 40 min in Ca^{2+}-free medium. Caffeine (10^{-2} M) was then added to the bath and the elicited contraction was taken as 100%. The vessels were washed with Ca^{2+}-containing medium for 45 min and then with Ca^{2+}-free medium for 40 min. Under these conditions, the contractile effects of caffeine were reproducible. In further experiments, ryanodine (10^{-5} M) was added for the last 30 min in a Ca^{2+}-free medium and before adding caffeine.

Effects of ryanodine on Ca^{2+}-induced tension in depolarized arteries

A first contraction with high K^+-solution (124 mM) containing Ca^{2+} and prepared by equimolar replacement of Na^+ by K^+ was performed in order to activate the vessels maximally. After washing the vessels with either 30 or 40 mM K^+ depolarizing solutions (prepared by equimolar replacement of Na^+ by K^+ and omission of Ca^{2+}) for 30 min, concentration-response curves to Ca^{2+} were obtained by increasing the concentration of Ca^{2+} (10^{-5}–10^{-2} M) in the bath in the presence of phentolamine (10^{-6} M). Phentolamine was added in order to eliminate the influence of depolarization-induced neuronal release of noradrenaline.

Successive Ca^{2+} response curves, separated by a washout of 30 min, were performed. The second and third curves were reproducible. Consequently, the second curve was taken as control in subsequent experiments and ryanodine was incubated with the tissue for 30 min before eliciting the third Ca^{2+} curve. In some experiments, diltiazem (6×10^{-7} M) or nifedipine (10^{-8} M) were added for 20 min before obtaining the second and third curves.

Effects of ryanodine on Ca^{2+}-induced tension in vessels exposed to noradrenaline or arginine-vasopressin

In order to activate the vessels maximally, contractile responses to a maximal concentration of agonist (10^{-5} M for NA and 10^{-7} M for arginine-vasopressin), were first elicited in a medium containing Ca^{2+}. Then arteries were depleted of intracellular-Ca^{2+} stores by inducing successive contractions with the agonist in a Ca^{2+}-free medium containing EGTA (10^{-3} M) until no contractile response occurred. Ca^{2+} concentration-effect relationships were then determined by exposure of the vessel to increasing concentrations of Ca^{2+} (10^{-5}–10^{-2} M) in a Ca^{2+}-free medium in the presence either of NA (3×10^{-6} M) or arginine-vasopressin (10^{-7} M). Preliminary experiments showed that successive Ca^{2+} curves separated by a washout period of 60 min were reproducible. Consequently in further experiments, the first curve was taken as control and the second curve was elicited after an incubation period with ryanodine (10^{-5} M) for 30 min.

Calculations and statistical analysis

The passive wall tension (PWT) was normalized by measuring its length (l, mm) with a micrometer and the strain given by the gauge (F, mN). PWT was calculated as follows (Mulvany & Halpern, 1977)

$$\text{PWT (mN mm}^{-1}) = \frac{\text{F (mN)}}{\text{2l (mm)}}$$

The active wall tension was the increase in tension over the PWT.

All results are expressed as means ± standard error of the mean (s.e.mean), with n representing the number of experiments. The EC_{50} value, the concentration of agonist required to obtain half the maximal contractile response, was obtained by logit/log regression analysis. Results were compared by Student's paired or unpaired t tests.

Drugs

A stock solution (10^{-2} M) of noradrenaline bitartrate (Sigma) was prepared in twice distilled water containing HCl (34 mM) and Na_2SO_3 (7.9 mM). Phentolamine hydrochloride (Ciba-Geigy) and diltiazem hydrochloride (L.E.R.S. Synthelabo), were prepared in twice distilled water. Caffeine (Sigma) was dissolved directly in the Ca^{2+}-free solution. Arginine-vasopressin (Sigma) and (1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 2-(O-methyl) tyrosine) Arg^8 vasopressin ($d(CH_2)_5$ Tyr (Me) AVP; Sigma) were dissolved in twice distilled water containing NaCl (9 g l^{-1}) and bovine serum albumin (Sigma; 1 g l^{-1}) as stock solutions of 10^{-4} M and 10^{-3} M respectively and subsequently kept frozen in small aliquots until used. Ryanodine (Agri-system, Wind Gap, USA) was dissolved in twice distilled water to give a stock solution of 10 mM and this was kept frozen in small aliquots until used. Nifedipine (Bayer) was dissolved in ethanol to give a 1 mM solution before dilution. Nifedipine was kept in the dark and experiments using this drug were performed in light-proofed apparatus to minimize light-induced degradation.

Results

Effects of ryanodine on rat aorta

Effects of ryanodine in a medium containing Ca^{2+} In a medium containing 1.25 mM Ca^{2+}, ryanodine induced a slowly developing rise in tension (Figure 1) in 5 vessels with the maximal effect obtained after 25 min representing $27 \pm 8\%$ of the maximal response elicited by NA, but in 4 other vessels, ryanodine had no significant contractile effect. Subsequent addition of NA elicited a contractile response with a phasic and a tonic component. The amplitudes of the phasic component elicited by NA in the absence or in the presence of ryanodine were not significantly different. The maximal contractile response in the presence of ryanodine and NA (2.7 ± 0.4 g) was not significantly different from the control NA value (2.7 ± 0.06 g). The contractile effect of ryanodine was not affected by the removal of endothelium.

Effects of ryanodine in a Ca^{2+}-free medium In a Ca^{2+}-free medium, NA (10^{-6} M) elicited a phasic contraction which was reduced to $25 \pm 6\%$ ($n = 9$) of the control value after a preincubation period of 30 min with 10^{-5} M ryanodine (Figure 2a). In these experimental conditions, no contractile effect of ryanodine alone was observed. In a Ca^{2+}-free medium, caffeine also induced a transient phasic contraction which represented $30 \pm 9\%$ of the phasic contraction elicited by NA (10^{-6} M). This contraction was totally abolished by preincubation for 30 min with 10^{-5} M ryanodine (Figure 2b).

Effects of ryanodine in rat mesenteric resistance vessels

Effects of ryanodine in a medium containing 2.5 mM Ca^{2+} After an incubation period of 30 min with ryanodine (10^{-5} M), no contractile effects were observed ($n = 6$). Subsequent addition of NA (10^{-5} M) elicited a contraction with phasic and tonic components (3.02 ± 0.60 and 3.50 ± 0.60 mN mm^{-1} respectively) which were not significantly different from control (respectively 3.05 ± 0.60 and 3.37 ± 0.42 mN mm^{-1}).

Effects of ryanodine in a Ca^{2+}-free medium We were not able to study the effects of ryanodine on phasic contractions elicited by NA in the absence of extracellular Ca^{2+} since contractile responses were not reproducible in these conditions. In a Ca^{2+}-free medium, caffeine (10^{-2} M) induced a transient, phasic contraction which was $66 \pm 6\%$ ($n = 4$) of the phasic contraction elicited by NA (10^{-5} M) in a Ca^{2+}-containing solution. A preincubation period with ryanodine (10^{-5} M) in Ca^{2+}-free medium completely abolished the effects of caffeine ($n = 4$).

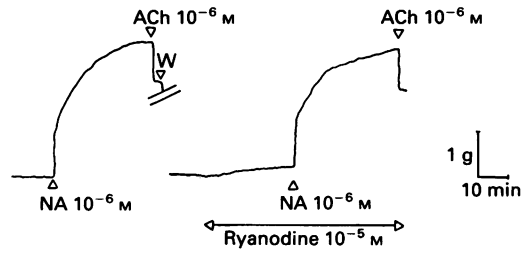

Figure 1 Effects of ryanodine on the contractile response to noradrenaline (NA) of rat aorta in a medium containing Ca^{2+}. A control contraction was obtained with NA (10^{-6} M). The presence of endothelium was confirmed by the ability of acetylcholine (ACh, 10^{-6} M) to relax the precontracted vessel. After a washout period (W) of 1 h, the tissue was exposed to ryanodine (10^{-5} M) for 30 min and then challenged with NA and, subsequently, ACh.

Effect of ryanodine on Ca^{2+}-induced tension in K^+-depolarized mesenteric arteries. The Ca^{2+}-concentration-response curves obtained in 30 mM and 40 mM K^+ depolarizing solutions are shown in

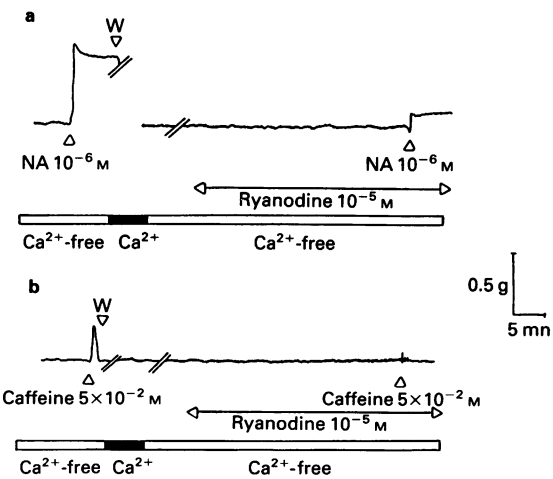

Figure 2 Effect of ryanodine on the contractile response to noradrenaline (NA) (a) and caffeine (b) in rat aorta in a Ca^{2+}-free medium. A control contraction was obtained with NA (10^{-6} M) in Ca^{2+}-free medium. After a washout period (W) of 1 h with physiological Ca^{2+}-containing solution, the tissue was washed with Ca^{2+}-free medium for 10 min then exposed to ryanodine (10^{-5} M) for 30 min and challenged with NA (a). Similarly caffeine (5×10^{-2} M) was tested in the place of NA (b).

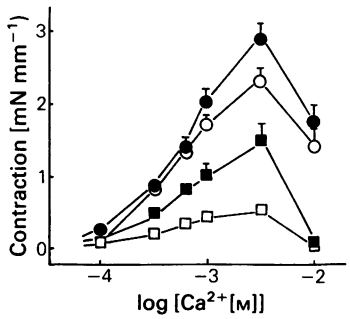

Figure 3 Effects of ryanodine on Ca^{2+}-induced tension in K^+-depolarized mesenteric resistance arteries. Control Ca^{2+} concentration-response curves were obtained in 30 mM K^+ (□) and 40 mM K^+ (○) depolarizing solutions in the absence of ryanodine. After a preincubation period (30 min) with ryanodine (10^{-5} M), the contractile responses to Ca^{2+} were recorded again in 30 mM K^+ (■) and in 40 mM K^+ (●) depolarizing solutions. Amplitudes of contraction are expressed in mN mm^{-1} and each point is the mean of 6 experiments, with vertical bars indicating the s.e. mean. All experiments were performed in the presence of phentolamine (10^{-6} M).

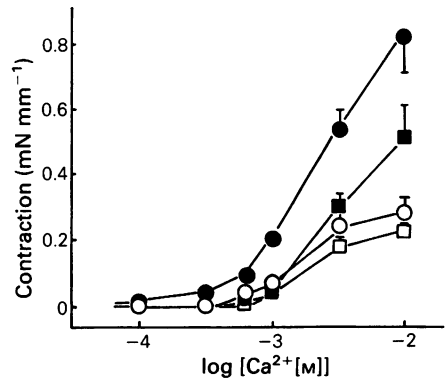

Figure 4 Effects of ryanodine on Ca^{2+}-induced tension in the presence of calcium entry blockers in mesenteric resistance vessels. Control Ca^{2+} concentration-response curves were obtained in 40 mM K^+ depolarizing solution in the presence of nifedipine (10^{-8} M, ○) or diltiazem (6×10^{-7} M, □). After a preincubation period (30 min) with ryanodine (10^{-5} M), the contractile responses to Ca^{2+} were recorded again in the presence of nifedipine (10^{-8} M, ●) or diltiazem (6×10^{-7} M, ■). Nifedipine or diltiazem were preincubated for 20 min before cumulative addition of Ca^{2+}. Amplitudes of contractions are expressed in mN mm^{-1} and each point is the mean of 5 experiments, with vertical bars indicating the s.e.mean. All experiments were performed in the presence of phentolamine (10^{-6} M). Note the different scale as compared to Figure 3.

Figure 3. Ca^{2+} showed a bell-shaped concentration-response relationship in vessels depolarized with 30 mM and 40 mM K^+ (Figure 3). Increasing contractions were obtained with Ca^{2+} concentrations up to 3 mM. On further increasing the concentration of Ca^{2+}, there was a reduction of tension which was greater in 30 mM K^+ than in 40 mM K^+ depolarized vessels ($P < 0.001$), being respectively $93 \pm 5\%$ and $41 \pm 9\%$ ($n = 6$) of the maximal contraction elicited by Ca^{2+}. The maximal active wall tension was about 4 fold greater in arteries depolarized with 40 mM K^+ than in those depolarized with 30 mM K^+, being respectively 2.29 ± 0.21 and 0.58 ± 0.08 mN mm^{-1}. The concentrations of Ca^{2+} giving half maximal contractions (EC_{50}) were $3.89 \pm 0.40 \times 10^{-4}$ M and $4.46 \pm 0.66 \times 10^{-4}$ M in 40 mM and in 30 mM K^+ depolarized vessels respectively.

In these preparations, a preincubation period of 30 min with ryanodine (10^{-5} M) induced a potentiation of the contractile responses elicited by Ca^{2+} which was significant ($P < 0.05$) for Ca^{2+} concentrations higher than 10^{-3} M and higher than 10^{-4} M ($P < 0.05$) respectively in 40 mM and in 30 mM K^+ depolarized vessels. The bell-shaped pattern of the Ca^{2+} concentration-effect curves remained evident in the presence of ryanodine. Similar results were obtained when arteries had been previously depleted of intracellular Ca^{2+} with NA in a Ca^{2+}-free medium before determination of the control curve and before preincubation with ryanodine (data not shown). After a preincubation period of 20 min with diltiazem (6×10^{-7} M) or nifedipine (10^{-8} M), the contractile response elicited by Ca^{2+} in 40 mM K^+ depolarizing solution was nearly abolished and the Ca^{2+} concentration-effect relationship was monophasic (Figure 4). After a washout period with 40 mM K^+ depolarizing solution and a preincubation with calcium entry blockers in the presence of ryanodine, the amplitudes of the contractile responses elicited by Ca^{2+} were slightly but significantly enhanced for Ca^{2+} concentrations higher than 6×10^{-4} M ($P < 0.01$) and 10^{-3} M ($P < 0.01$) in nifedipine- and in diltiazem-treated vessels respectively (Figure 4).

Effects of ryanodine on Ca^{2+}-induced tension in vessels exposed to noradrenaline or arginine-vasopressin Preliminary experiments showed that contractions obtained with NA (10^{-6} M) and arginine-vasopressin (10^{-7} M) could be completely abolished by phentolamine (10^{-6} M) and d-$(CH_2)_5$ Tyr (Me) AVP (10^{-8} M), respectively. Increasing Ca^{2+} concentrations in vessels maximally activated with either NA (3×10^{-6} M) or arginine-vasopressin (10^{-7} M) elicited concentration-dependent contractions in the 10^{-4}–10^{-3} M range. Higher Ca^{2+} concentrations either did not further increase tension (3×10^{-3} M) or even induced concentration-depen-

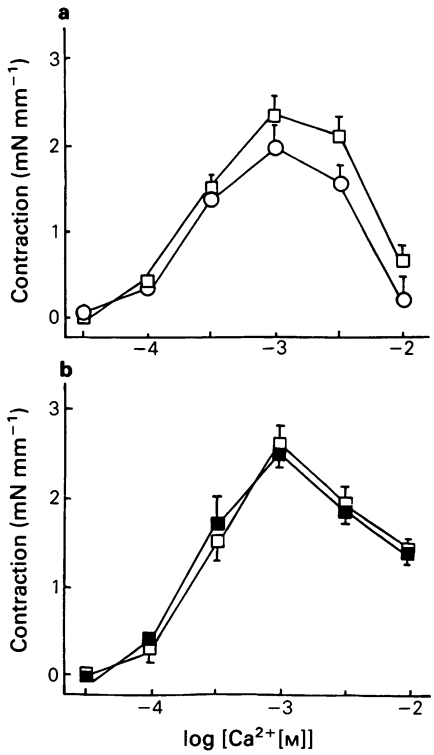

Figure 5 Ca^{2+}-induced tension in mesenteric resistance vessels exposed to noradrenaline (NA, 3×10^{-6} M, □) or arginine-vasopressin (10^{-7} M, ○) (a) and effects of ryanodine in arteries exposed to NA (3×10^{-6} M, ■) (b). Amplitudes of contractions are expressed in mN mm^{-1} and each point is the mean of 6 experiments, with vertical bars indicating the s.e.mean (a and b). The control Ca^{2+} concentration-response curve (□) was obtained in the absence of ryanodine in arteries exposed to NA (3×10^{-6} M). After a preincubation period (30 min) with ryanodine (10^{-5} M), the contractile responses to Ca^{2+} were recorded again (■; $n = 5$). Note the absence of effects of ryanodine (b).

dent relaxations (Figure 5a). Maximal active wall tension and EC_{50} values were not significantly different in the two experimental conditions, being respectively 2.35 ± 0.22 mN mm^{-1}, 1.94 ± 0.27 mN mm^{-1} and $2.24 \pm 0.21 \times 10^{-4}$ M, $1.95 \pm 0.05 \times 10^{-4}$ M in vessels exposed to noradrenaline- or arginine-vasopressin. Ryanodine did not modify Ca^{2+}-induced tension in vessels maximally activated by 3×10^{-6} M NA (Figure 5b). When similar experiments were performed with either arginine-vasopressin (10^{-7} M) or lower agonist concentrations (NA 10^{-6} M; arginine-vasopressin 6×10^{-9} and 10^{-8} M), ryanodine also did not modify Ca^{2+} concentration-effect curves (data not shown).

Discussion

The aim of this study was to assess the effects of ryanodine on contractile responses in vascular smooth muscle depending either on intracellular Ca^{2+} release or on extracellular Ca^{2+} influx. To this end, the effects of ryanodine were first studied on contractions elicited by NA and caffeine in a Ca^{2+}-free medium since responses to these agonists are dependent upon intracellular Ca^{2+} release (Somlyo *et al.*, 1971; Godfraind & Kaba, 1972) from sarcoplasmic reticulum (Weber & Herz, 1969; Casteels & Droogmans, 1981; Itoh *et al.*, 1982; Bond *et al.*, 1984; for review see Johansson & Somlyo, 1980). Note first that, in this study, contractions elicited by caffeine in rat aorta were about 3 times smaller than phasic contractions induced by NA, whereas in mesenteric resistance vessels the ratio between the two types of contractions was less than 2. The smaller magnitude of the contractions elicited by caffeine in the rat aorta may be related to the thickness of this vessel as compared to mesenteric resistance arteries since the contractile effects of caffeine have been shown to be greater in thin preparations (Leijten & van Breemen, 1984). An alternative explanation could be that caffeine mobilises a greater part of the Ca^{2+} stored in the sarcoplasmic reticulum of resistance vessels than of the aorta.

Pretreatment with ryanodine greatly decreased the contractile responses elicited by NA in Ca^{2+}-free medium in rat aorta and the contractile effects of caffeine were totally abolished both in mesenteric resistance vessels and in the aorta. These data are in agreement with previous studies in guinea-pig aorta and rabbit ear artery (Ito *et al.*, 1986; Hwang & van Breemen, 1987) and suggest that ryanodine in some way interferes with intracellular Ca^{2+} release either by a stimulation of Ca^{2+} release (and therefore depletion of Ca^{2+} stores) or a direct inhibition of release. However, the observation that ryanodine induced small contractions in some aortae (Figure 1) in the presence of extracellular Ca^{2+} would rather suggest that this drug stimulates release of intracellular stored Ca^{2+}. Since this action of ryanodine was not observed in all preparations, it may be that some heterogeneity among vessels exists with respect to the importance of Ca^{2+} stores. In Ca^{2+} free-medium where the cytosolic free Ca^{2+} level (Ca^{2+}_i) is lower (Kobayashi *et al.*, 1986) and a greater Ca^{2+} release may therefore be necessary to elicit a contractile response, ryanodine had no contractile effect (Figure 2). The effects of ryanodine could however also be explained by a direct stimulant effect of the drug on Ca^{2+} entry. Although this second hypothesis cannot be entirely excluded in our preparations, there is considerable evidence indicating that ryanodine does

not in any way directly interfere with plasma membrane Ca^{2+} channels. Actually, in cardiac and skeletal muscle, it has been shown that [^{3}H]-ryanodine does not bind to, or affect $^{45}Ca^{2+}$ influx into, t-tubule preparations, where Ca^{2+} channels are abundant (Lattanzio *et al.*, 1987). Also in cardiac tissue, ryanodine was found to be unable to displace [^{3}H]-nitrendipine from its binding sites (Pessah *et al.*, 1985). Furthermore, in cultured heart cells, initial $^{45}Ca^{2+}$ uptake was not affected or even slightly decreased by ryanodine (Rasmussen *et al.*, 1987).

In Ca^{2+}-containing medium, ryanodine did not modify the contractile effects of NA either in rat aorta or in mesenteric resistance vessels. These results do not appear inconsistent with a release of intracellular Ca^{2+} in the presence of ryanodine, since contractile responses elicited by NA still display a phasic and a tonic component when Ca^{2+}-depleted vessels are exposed to the agonist in the presence of extracellular Ca^{2+} (unpublished data). This observation suggests that the phasic component of the NA-induced contractile response in these vessels is not necessarily entirely dependent upon intracellular Ca^{2+} release when extracellular Ca^{2+} is present and is thus less sensitive to ryanodine.

In further experiments, we assessed the effects of ryanodine on Ca^{2+}-induced tension in K^+-depolarized mesenteric resistance vessels where contractile responses depend entirely upon extracellular Ca^{2+} influx and are independent of any intracellular Ca^{2+} release (Skärby *et al.*, 1984; Cauvin & Malik, 1984). However, it has been recently proposed that the sarcoplasmic reticulum in these preparations takes up a fraction of the Ca^{2+} which enters the cell before it is able to activate myofilaments, and thus indirectly reduces the Ca^{2+} sensitivity of the vessels (van Breemen *et al.*, 1986). This buffer function of sarcoplasmic reticulum in depolarized arteries, proposed by van Breemen *et al.* (1986), may explain the potentiation of the contractile response to Ca^{2+} observed in the presence of ryanodine in arteries depolarized with 30 mM K^+, which cannot be explained by an increase in Ca^{2+} entry since this drug has been shown not to affect depolarization-dependent Ca^{2+} entry from the external medium (Ito *et al.*, 1986). Thus, ryanodine-induced Ca^{2+} release could disable the buffer function of sarcoplasmic reticulum so that a greater proportion of the stimulated influx of Ca^{2+} would be able to activate myofilaments leading to the enhancement of contractile responses to K^+.

The absence of effects of ryanodine on contractile responses elicited by Ca^{2+} in mesenteric resistance vessels exposed to maximal or submaximal concentrations of NA or arginine-vasopressin supports this hypothesis and is also in agreement with the view that ryanodine has no direct stimulant effect on Ca^{2+} entry. Actually, if ryanodine had Ca^{2+} entry activating properties, it would have been expected to increase contractile force in these preparations, especially in submaximally activated vessels. The finding that ryanodine was inactive in these arteries can more easily be explained by the observation that occupation of receptors by agonists reduces Ca^{2+} uptake into intracellular stores (Karaki *et al.*, 1979; Loutzenhizer & van Breemen, 1983) and accordingly precludes any additional Ca^{2+} release by ryanodine.

However, in 40 mM K^+ depolarizing solution, no effects were observed for ryanodine at Ca^{2+} concentrations lower than 10^{-3} M and only slight effects occurred at higher concentrations. The slight effects at higher Ca^{2+} concentrations could be due to a physiological limitation of contraction since depolarization with 40 mM K^+ has been shown to activate these vessels maximally (Julou & Freslon, 1986). Furthermore, it must be emphasized that the contractile responses elicited by all Ca^{2+} concentrations in 30 mM K^+ depolarizing solution in the presence of ryanodine were still lower than contractions elicited by the same Ca^{2+} concentrations in 40 mM K^+ depolarizing solution in the absence of ryanodine. This suggests that the increase in cytoplasmic Ca^{2+} concentration caused by the indirect inhibitory effects of ryanodine on Ca^{2+} accumulation in sarcoplasmic reticulum is quantitatively not as important as the influx of Ca^{2+} through voltage-operated channels. Moreover, the refilling process may occur at least partly through a pathway independent of Ca^{2+} channels, either by the so-called leak channels, as proposed by Casteels & Droogmans (1981), or, possibly, by another mechanism activated at high extracellular Ca^{2+} e.g. the Na^+–Ca^{2+} exchange (Ashiba & Blaustein, 1987) in rat mesenteric resistance vessels (Hogestätt, 1984; Julou & Freslon, 1986). This is consistent with the fact that Ca^{2+} still elicited contractions after preincubation with ryanodine in the presence of concentrations of nifedipine and diltiazem which almost abolished the contractile effects of Ca^{2+} in 40 mM K^+ depolarizing solution. This may also explain why, in arteries depolarized with 40 mM K^+, the increase of Ca^{2+} release by ryanodine had no potentiating effect at low external Ca^{2+} concentrations.

In conclusion, our results support the view that ryanodine leads to a liberation of intracellular Ca^{2+}, thereby depleting the NA-sensitive intracellular Ca^{2+} store and indirectly reducing the ability of sarcoplasmic reticulum to sequester Ca^{2+} in rat aorta and mesenteric resistance vessels.

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