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Antagonism of α_1-adrenoceptor agonist-induced responses by rilmenidine in vascular smooth muscle

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Abstract

The effect of the centrally acting antihypertensive agent, rilmenidine, was examined on the contractile properties of isolated rat portal vein strips and on the free cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in isolated myocytes. Rilmenidine (1–30 μM) relaxed strips precontracted with noradrenaline. This effect was not inhibited by the α_2-adrenoceptor antagonist, yohimbine, and was not mimicked by the α_2-adrenoceptor agonist, 5-bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK 14,304). Rilmenidine dose dependently shifted to the right the concentration–response curves to noradrenaline and to phenylephrine but not that to carbachol. Rilmenidine alone (0.1–30 μM) caused a contraction which maximally corresponded to 18% of the maximal noradrenaline-induced contraction. This effect was not produced by UK 14,304, was not affected by yohimbine, but was inhibited by the α_1-adrenoceptor antagonist, prazosin. In isolated myocytes, rilmenidine reduced the noradrenaline-induced $[Ca^{2+}]_i$ increase but alone, it produced a rise in $[Ca^{2+}]_i$, the peak amplitude of which averaged 15% of the noradrenaline-induced transient $[Ca^{2+}]_i$ rise. It is concluded that rilmenidine acts as a partial agonist of α_1-adrenoceptors of vascular smooth muscle, causing relaxation of vessels precontracted by full agonists of α_1-adrenoceptors. © 1998 Elsevier Science B.V.

Keywords: α-Adrenoceptor; Contraction; Ca^{2+}, intracellular; Portal vein

1. Introduction

Rilmenidine, an oxazoline, is an antihypertensive drug that apparently acts via the central nervous system by inhibition of tonic sympathoexcitatory reticulospinal vasomotor neurons of the rostral ventrolateral medulla (Laubie et al., 1985; Koenig-Bérard et al., 1988; Van Zwieten, 1988, 1996). As a consequence, sympathetic nerve activity is reduced and arterial pressure falls. It has been proposed that this hypotensive effect results from activation of imidazoline I_1 sites and/or α_2-adrenoceptors expressed in the rostral ventrolateral medulla (Ernsberger et al., 1992; Sannajust and Head, 1994; Szabo and Urban, 1995).

However, it has been reported that rilmenidine and other centrally acting antihypertensive agents such as clonidine also bind to postjunctional α_1- and α_2-adrenoceptors in the vessels (Kong et al., 1991; Skrbic and Chiba, 1993; Marsault et al., 1996). Although both types of α-adrenoceptors are present, the vasoconstriction induced by noradrenaline is primarily mediated by postjunctional α_1-adrenoceptors. By acting as a partial agonist of vascular α_1-adrenoceptors, clonidine has been shown to selectively interfere with the vasoconstrictor action of full α_1-adrenoceptor agonists such as noradrenaline (Kong et al., 1991).

Therefore, the aim of this work was to define the effects of rilmenidine on vascular smooth muscle, in particular to assess its interaction with α_1-adrenoceptor agonist-induced responses. For this purpose, we studied the effect of rilmenidine on the tension of muscular strips from rat portal vein precontracted with various agents (high-K^+ solution, noradrenaline, phenylephrine, carbachol), and on the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in isolated portal vein myocytes.

2. Materials and methods

Wistar rats (150 g) were stunned and then killed by cervical dislocation. Portal veins were removed and placed into normal physiological solution (PSS, see Section 2.4 for the composition). The veins were cleaned of adherent

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connective tissue and opened longitudinally. The endothelium was carefully removed by gently rubbing the intimal surface with the tip of small forceps. The vessels were then prepared for either tension measurement or isolated cell preparation.

2.1. Contraction measurement in intact smooth muscle

The cleaned veins were cut into longitudinal strips (5–7 mm length, 1 mm wide) which were suspended under isometric conditions and connected to a force transducer (Gould, CA) in 3 ml organ baths filled with Krebs–Henseleit solution, maintained at 31°C, and gassed with 95% O_2–5% CO_2. The preparations were initially placed under a resting tension of 200 mg, left to equilibrate for 1 h and washed at 20 min intervals. Successive applications of agonist were separated by a time interval of 20 min. Experiments were done on the tone raised by high-K^+ (40 mM) solution. This protocol was used to stop the spontaneous rhythmic contractile activity which prevented accurate quantifying of the effect of low agonist concentrations. This protocol did not modify the properties of the agents used in this study and all the results described using this protocol were reproduced under basal conditions. Concentration–response curves to agonists were obtained by increasing the concentration in the organ chamber. All contractile responses were expressed as percentages of the maximal response elicited by 10 μM noradrenaline. For measurement of kinetics of the blocking action of rilmenidine and recovery, strips were placed horizontally in a well on a bubble plate (Horiuti, 1988) which allows a rapid change of solutions by sliding of the plate to the adjacent well, and isometric tension was measured with a force transducer (SansoNor, Norway).

2.2. Smooth muscle cell preparation

The portal vein was cut into small pieces, washed for 10 min in low Ca^{2+} (40 μM) PSS and then incubated in low-Ca^{2+} PSS containing 1 mg ml^{-1} collagenase, 0.5 mg ml^{-1} pronase and 1 mg ml^{-1} bovine serum albumin at 37°C for 20 min. After this time, the solution was removed and the pieces of veins were incubated again in a fresh enzyme solution at 37°C for 20 min. The pieces were then placed in enzyme-free solution and triturated using a fire polished Pasteur pipette to separate the cells, which were stored on glass coverslips at 4°C in PSS containing 0.8 mM Ca^{2+} and used on the same day.

2.3. Fluorescence measurement and estimation of $[Ca^{2+}]_i$

Changes in $[Ca^{2+}]_i$ were monitored fluorometrically with the Ca^{2+}-sensitive probe, indo-1, as described previously (Pacaud et al., 1991; Pacaud et al., 1993). Briefly, cells were loaded with indo-1 by incubation in PSS containing 1 μM indo-1 penta-acetoxymethyl ester (indo-1/AM) for 25 min at room temperature. The coverslip with attached cells was then mounted in a chamber and continuously superfused at 30°C. The recording system included a Nikon Diaphot inverted microscope fitted with an epifluorescence attachment (Nikon, Charenton-le-pont). The cell studied was illuminated at 360 nm. Emitted light from a window slightly larger than the cell was counted simultaneously at 405 nm and 480 nm by two photomultipliers (P1, Nikon). Voltage signals at each wavelength were stored in an IBM-PC computer for subsequent analysis. The ratio (405/480) was calculated on-line and displayed with the two voltage signals on a monitor. $[Ca^{2+}]_i$ was estimated from the 405/480 ratio (Grynkiewicz et al., 1985) using a calibration for indo-1 determined within cells (Pacaud et al., 1991).

2.4. Solutions

The PSS contained (in mM): 130 NaCl, 5.6 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 11 glucose, 10 HEPES, brought to pH 7.4 with NaOH. The Krebs–Henseleit solution had the following composition (in mM): 118.4 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 25 $NaHCO_3$, 11 glucose.

2.5. Statistics

All results are expressed as the means ± S.E.M. with n being the sample size. Significance was tested by means of Student's t-test. Probabilities less than 5% ($P < 0.05$) were considered significant. Dose–response curves were fitted to a logistic equation using Origin software.

2.6. Chemicals and drugs

Collagenase was from Worthington Biochemical Corp. (Freehold, NJ). Pronase (type E), bovine serum albumin, noradrenaline, phenylephrine, carbachol, yohimbine, prazosin, idazoxan and agmatine were purchased from Sigma (Saint Quentin Fallavier). 5-Bromo-*N*-(4,5-dihydro-1H-imidazol-2-yl)-6-quinoxalinamine (UK 14,304) was obtained from Research Biochemicals International (Natick, MA). Indo-1/AM was obtained from Calbiochem. Rilmenidine was a gift from Institut de Recherches Internationales Servier (Courbevoie).

3. Results

3.1. Effect of rilmenidine on noradrenaline- and phenylephrine-induced contraction

Rilmenidine (10 μM) relaxed venous strips contracted with 10 μM noradrenaline applied after the tone raised by 40 mM K^+ had reached a steady level (Fig. 1A). This effect was not mimicked with 10 μM UK 14,304 (not

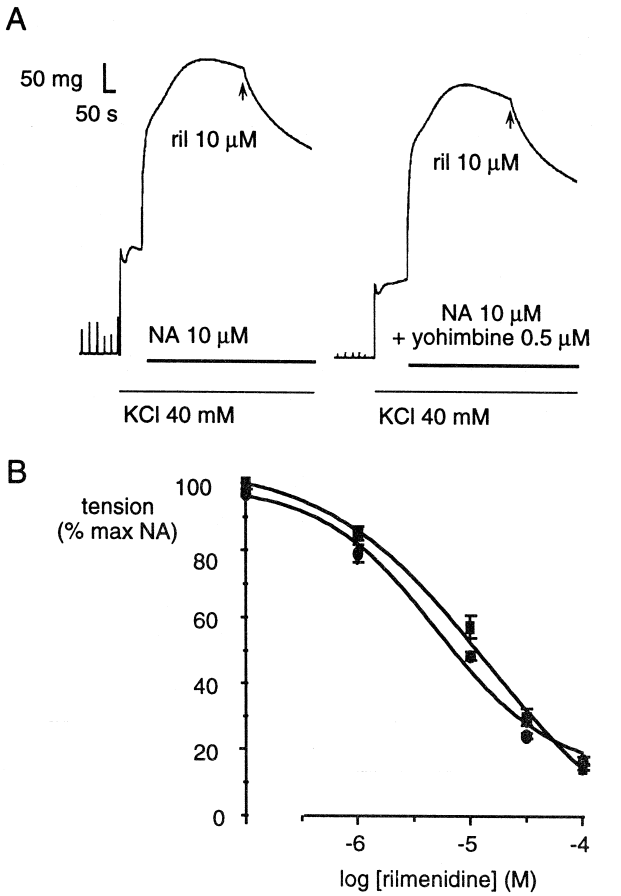

Fig. 1. Relaxing effect of rilmenidine on the noradrenaline-induced contraction. Noradrenaline was applied after the tone raised by high K^+ (40 mM) solution had reached steady state. (A) Rilmenidine (ril, 10 μM) relaxed the noradrenaline-induced contraction (NA, 10 μM) (left). Neither this effect nor the NA-induced contraction was changed in the presence of 0.5 μM yohimbine (right). (B) Concentration-dependence of the relaxation by rilmenidine of the noradrenaline (2 μM) induced-contraction under control conditions (square) and in the presence of 0.5 μM yohimbine (circle). Tension was expressed as percentage of the amplitude of NA-induced tension measured before rilmenidine application, taking the high K^+ contraction as baseline. Each point represents the mean of 4–8 experiments.

shown). The relaxing effect of rilmenidine was not significantly modified by 0.5 μM yohimbine ($n = 4$, $P > 0.3$) (Fig. 1A and B). This experiment also showed that the contraction induced by noradrenaline was not affected by yohimbine ($94.1 \pm 4.6\%$ of control, $n = 4$, $P > 0.2$). The blocking action of rilmenidine was rapid and completely reversible. The time required to reach the half-maximal response ($t_{1/2}$) was 1.10 ± 0.27 min ($n = 7$) and after washout of rilmenidine (10 μM), the $t_{1/2}$ for the recovery was 2.30 ± 0.36 min ($n = 7$). The rilmenidine-induced relaxation depended on the concentration used. The half-maximal relaxation of the noradrenaline (2 μM) induced contraction was obtained with 7.9 μM and 5.0 μM rilmenidine in the control and in the presence of 0.5 μM yohimbine, respectively (4–8 experiments for each of these conditions, Fig. 1B). Pretreatment of venous strips with rilmenidine (1–30 μM) shifted the concentration–response curves of noradrenaline (Fig. 2) and phenylephrine (Fig. 3) to the right. A possible effect on the maximal amplitude of the responses was not investigated as it required the use of high concentrations of agonists (> 100 μM). The noradrenaline concentrations required to produce the half-maximal response were 0.4 μM, 1.2 μM, 6.7 μM and 30 μM under control conditions and in the presence of 1, 10 and 30 μM rilmenidine, respectively (Fig. 2, 4–8 experiments in each case). Phenylephrine concentrations needed to produce contractions with a half-maximal amplitude were 1.2 μM, 2.7 μM, 7.1 μM and 48 μM under control conditions and in the presence of 1, 10 and 30 μM rilmenidine, respectively (Fig. 3, 4–8 experiments under each condition). In contrast, rilmenidine did not relax the tone raised by muscarinic receptor stimulation. The dose–response curve to carbachol was not modi-

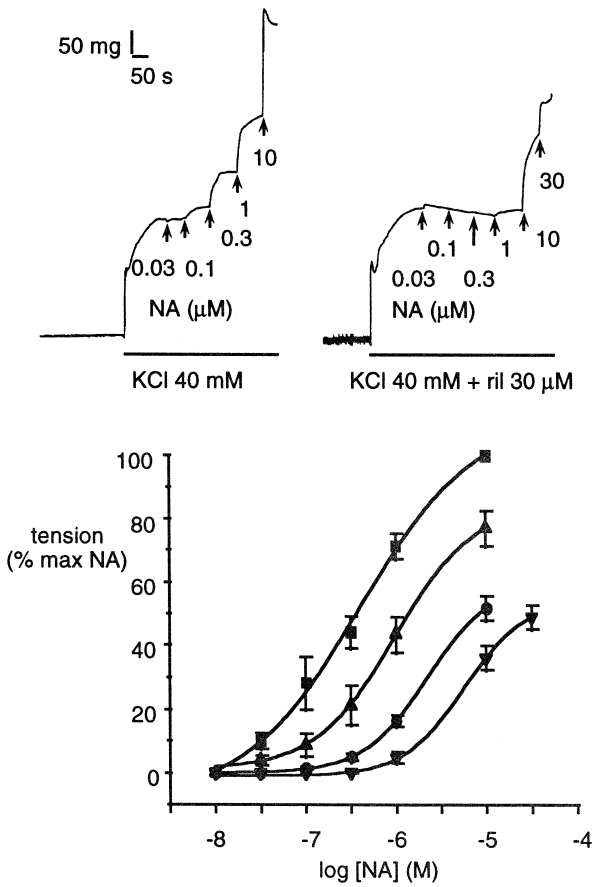

Fig. 2. Effect of rilmenidine on the concentration–response curve to noradrenaline applied after the tone raised by high K^+ (40 mM) solution had reached steady state. Cumulative dose–response curves to noradrenaline (NA) were obtained under control conditions (square), in the presence of rilmenidine 1 μM (up triangle), 10 μM (circle) and 30 μM (down triangle). All contractile responses were expressed as percentages of the maximal response to 10 μM noradrenaline measured under control conditions, taking the high K^+ contraction as baseline. Each point represents the mean of 4–8 experiments. Typical responses to noradrenaline (NA) in the absence (left) and in the presence of 30 μM rilmenidine (ril, right) are shown above the curves.

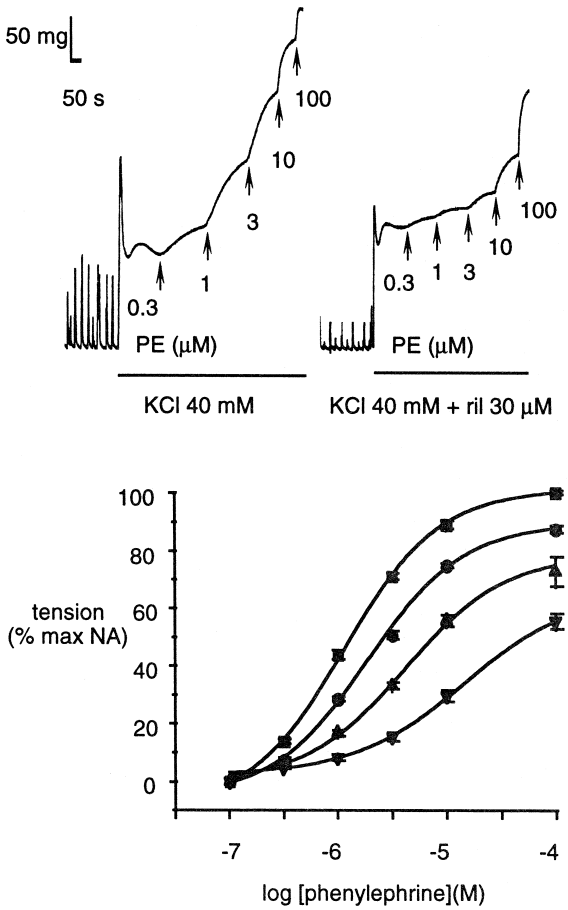

Fig. 3. Effect of rilmenidine on the concentration–response curve to phenylephrine applied after the tone raised by high K^+ (40 mM) solution had reached steady state. Cumulative dose–response curves to phenylephrine were obtained under control conditions (square), in the presence of rilmenidine 1 μM (circle), 10 μM (up triangle) and 30 μM (down triangle). All contractile responses were expressed as percentages of the maximal response to 10 μM noradrenaline measured under control conditions, taking the high K^+ contraction as baseline. Each point represents the mean of 4–8 experiments. Typical responses to phenylephrine (PE) in the absence (left) and in the presence of 30 μM rilmenidine (ril, right) are shown above the curves.

fied in the presence of rilmenidine (30 μM) (Fig. 4, 8 experiments). The concentration of carbachol required to produce a half-maximal effect was 2.8 μM and 3.3 μM in the control and in the presence of 30 μM rilmenidine, respectively.

3.2. Contracting effect of rilmenidine

Fig. 5A shows the effect on tension of various agonists applied on portal vein strips after the tone raised by 40 mM K^+ had reached a steady level. Noradrenaline, phenylephrine and rilmenidine produced a concentration-dependent increase in tension whereas UK 14,304 (0.1–10 μM) was ineffective. Maximal contractions were obtained in response to 10 μM noradrenaline or 100 μM phenylephrine. The maximal rise in tension induced by rilmenidine (10 and 30 μM) was only 18% of the maximal noradrenaline-induced contraction. Comparison of the EC_{50} gives an order of potency for the three agonists: noradrenaline (0.4 μM) > phenylephrine (1.2 μM) > rilmenidine (2.3 μM). The concentration–response curve to rilmenidine was not significantly modified in the presence of 0.5 μM yohimbine ($n = 4$, $P > 0.7$) (Fig. 5B) (EC_{50} was 2.9 μM in the control and 3.3 μM in the presence of yohimbine). In contrast, 0.03 μM prazosin completely inhibited the effect of rilmenidine.

3.3. Effect of rilmenidine on $[Ca^{2+}]_i$ and on noradrenaline-induced rise in $[Ca^{2+}]_i$ in smooth muscle cells isolated from portal vein

Rilmenidine (30 μM) applied to myocytes loaded with indo-1 induced a rise in $[Ca^{2+}]_i$, the maximal amplitude of

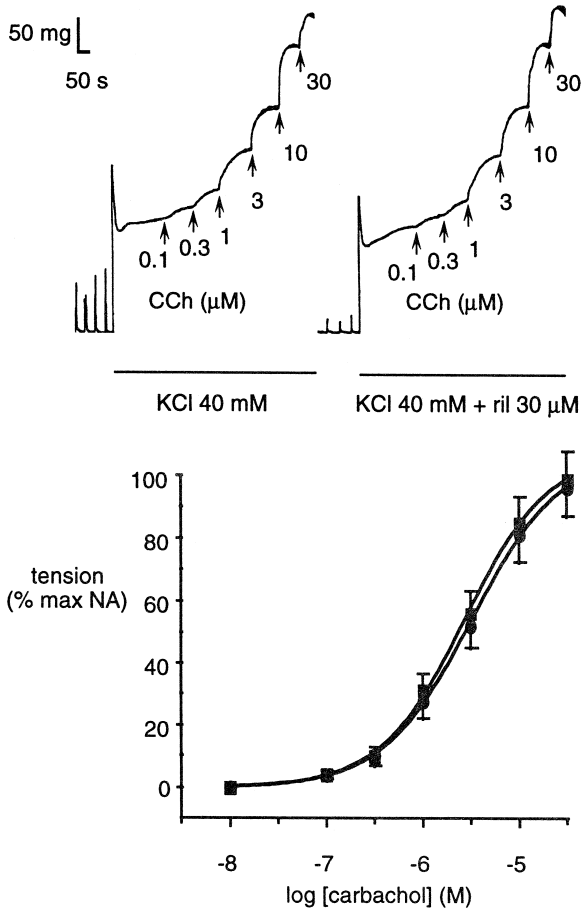

Fig. 4. Effect of rilmenidine on the concentration–response curve to carbachol applied after the tone raised by high-K^+ (40 mM) solution had reached a steady state. Cumulative dose–response curves to carbachol were obtained under control conditions (square) and in the presence of rilmenidine 30 μM (circle). Responses were expressed as percentages of the maximal response to 10 μM noradrenaline measured under control conditions, taking the high K^+ contraction as baseline. Each point represents the mean of 8 experiments. Typical responses to carbachol (CCh) in the absence (left) and in the presence of 30 μM rilmenidine (ril, right) are shown above the curves.

which reached 63 ± 15 nM ($n = 8$) (Fig. 6A). The peak amplitude of the noradrenaline (1 μM) induced $[Ca^{2+}]_i$ rise measured in the same cells averaged 803 ± 115 nM ($n = 8$). This $[Ca^{2+}]_i$ response to noradrenaline consisted of a transient component followed by a plateau phase which has already been defined and involves both inositol 1,4,5-trisphosphate-induced Ca^{2+} store release and Ca^{2+} entry (Pacaud et al., 1991, 1993). The $[Ca^{2+}]_i$ increases induced by rilmenidine or noradrenaline were both abolished by 0.03 μM prazosin (not shown). In the presence of rilmenidine (30 μM), the peak of the response to noradrenaline (1 μM) was strongly reduced (Fig. 6B). The relative amplitude of the $[Ca^{2+}]_i$ responses expressed as percentages of the noradrenaline-induced increase in $[Ca^{2+}]_i$ is shown in Fig. 6C. In addition, application of rilmenidine (30 μM) during the plateau phase of the noradrenaline-induced $[Ca^{2+}]_i$ rise reversibly decreased its amplitude to $15.6 \pm 4.2\%$ ($n = 7$) (Fig. 6D).

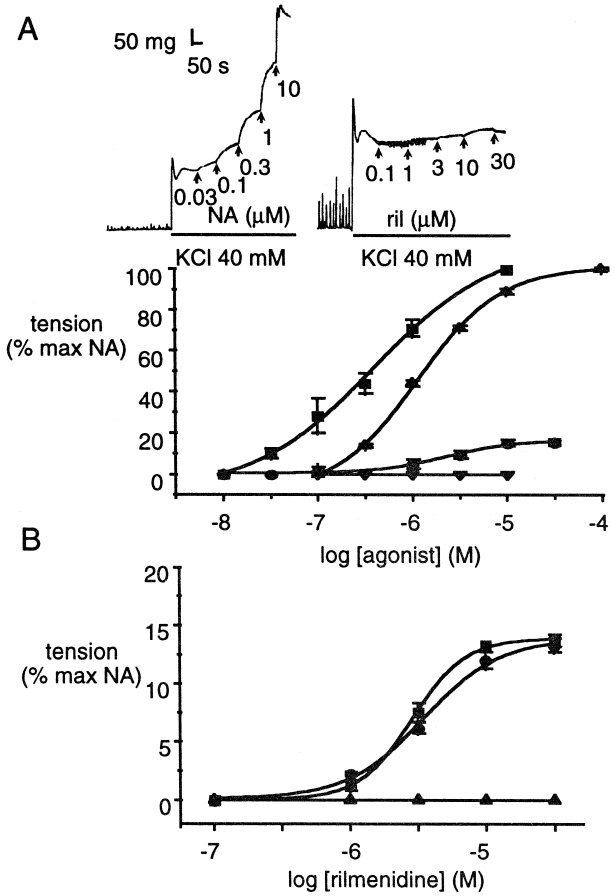

Fig. 5. Effect of noradrenaline, phenylephrine, rilmenidine and UK 14,304 on portal vein strips precontracted with 40 mM K^+. (A) Concentration–response curves to noradrenaline (square), phenylephrine (up triangle), rilmenidine (circle) and UK 14,304 (down triangle) were obtained by increasing the concentration in the organ chamber. All contractile responses were expressed as percentages of the maximal response to 10 μM noradrenaline measured under control conditions, taking the high K^+ contraction as baseline. Each point represents the mean of 4–8 experiments. Typical responses to noradrenaline (NA) and rilmenidine (ril) are shown above the curves. (B) Effect of yohimbine and prazosin on the concentration–response curve to rilmenidine. Concentration–response curves were obtained by increasing the concentration of rilmenidine in the organ chamber under control conditions (square), in the presence of 0.5 μM yohimbine (circle) and 0.03 μM prazosin (triangle). Tension was expressed as a percentage of the maximal response elicited by 10 μM noradrenaline, taking the high K^+ contraction as baseline. Each point represents the mean of 4 experiments.

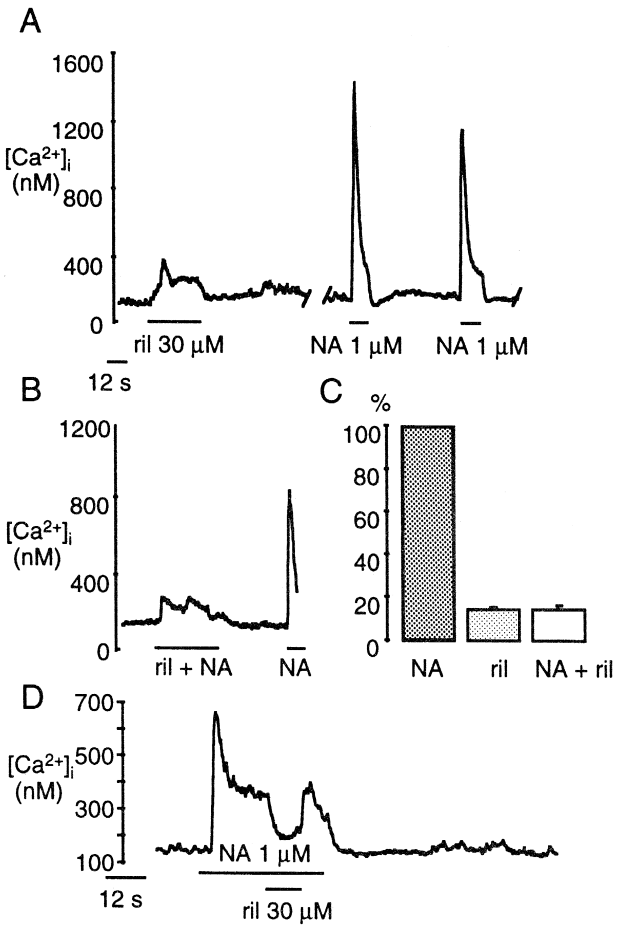

Fig. 6. Effect of rilmenidine (ril) and noradrenaline (NA) on $[Ca^{2+}]_i$ in isolated portal vein cells. (A) Rilmenidine (30 μM) induced a small increase in $[Ca^{2+}]_i$ compared to those induced by consecutive applications of noradrenaline (1 μM). (B) The $[Ca^{2+}]_i$ rise induced by noradrenaline (1 μM) was strongly reduced when it was applied in the presence of rilmenidine (30 μM). Consecutive application of noradrenaline alone was still able to induce a large transient $[Ca^{2+}]_i$ rise. (C) Amplitude of the transient $[Ca^{2+}]_i$ rise induced by noradrenaline (1 μM), rilmenidine (30 μM) and both together. Results are expressed as percentages of the amplitude of the transient component of the noradrenaline (1 μM) induced $[Ca^{2+}]_i$ rise ($n = 8$). (D) Effect of rilmenidine on the plateau phase of the noradrenaline-induced $[Ca^{2+}]_i$ rise. Rilmenidine (ril, 30 μM) reversibly inhibited the sustained increase in $[Ca^{2+}]_i$ induced by noradrenaline (NA, 1 μM).

4. Discussion

This study showed that rilmenidine had a dual effect in smooth muscle from rat portal vein. It relaxed muscular strips precontracted with noradrenaline or phenylephrine

and decreased the noradrenaline-induced $[Ca^{2+}]_i$ rise in isolated cells. Added alone, it produced a contraction of muscle strips and increased $[Ca^{2+}]_i$ in isolated myocytes.

The contraction induced by rilmenidine was concentration-dependent and was observed under basal conditions (not shown) or when the tone was increased by 40 mM K^+. The α_2-adrenoceptor antagonists (idazoxan (not shown) and yohimbine) did not have any effect on the contraction in response to rilmenidine. In contrast, the contraction induced by rilmenidine was completely abolished by prazosin, indicating that this effect of rilmenidine was associated with the stimulation of α_1-adrenoceptors. The rilmenidine-induced prasozin-sensitive $[Ca^{2+}]_i$ rise observed in isolated cells indicated that the α_1-adrenoceptors involved were located in the membrane of vascular smooth muscle cells. Both the amplitude of maximal contractions and $[Ca^{2+}]_i$ rises induced by rilmenidine were weak in comparison to those obtained in response to noradrenaline. Taken together, these results thus suggest that rilmenidine acts as a partial agonist of α_1-adrenoceptors in vascular smooth muscle.

The effects of rilmenidine as a ligand for α_1-adrenoceptors were further investigated by assessing its effects as an antagonist versus the α_1-adrenoceptor agonists noradrenaline or phenylephrine. Rilmenidine produced a concentration-related antagonism of the noradrenaline- and phenylephrine-induced contractions and decreased the noradrenaline-induced $[Ca^{2+}]_i$ rise. The inhibitory effect of rilmenidine on both tension and $[Ca^{2+}]_i$ was quick, and after the removal of rilmenidine, recovery was rapid and complete. The time course of the blocking action and of recovery appeared to be faster in single cells than in strips. This difference probably resulted from the presence of intercellular spaces and connective tissue which slowed down the access to smooth muscle cells in multicellular preparations. The relaxing effect of rilmenidine was not affected by yohimbine. Rilmenidine did not relax the high K^+- or carbachol-induced rise in tension. Carbachol-induced contraction in rat portal vein was mediated via activation of the G protein/phospholipase C-coupled m3-muscarinic receptors (Pfaffendorf and Van Zwieten, 1993) and thus used the same intracellular pathways as do α_1-adrenoceptors. Therefore, the absence of effect of rilmenidine on the carbachol-induced contraction suggests that the antagonism of the α_1-adrenoceptor activation-induced responses did not involve inhibition of intracellular coupling mechanisms. The relaxing effect of rilmenidine seems thus only related to its interaction with the smooth muscle α_1-adrenoceptors. Our results thus demonstrate that rilmenidine, being a partial agonist of α_1-adrenoceptors, has both agonist and antagonist actions.

Although rilmenidine is known as a ligand of imidazoline I_1 binding sites (Ernsberger et al., 1992; Sannajust and Head, 1994), we have no experimental evidence supporting the involvement of these sites in the effects of rilmenidine on portal vein smooth muscle since: (i) the imidazoline I_1 site ligand, agmatine (Li et al., 1994; Regunathan and Reis, 1996), had neither contracting nor relaxing effects in rat portal vein (not shown) and (ii) idazoxan, which has a high affinity for imidazoline binding sites did not prevent the rilmenidine-induced contraction or relaxation.

The effects of rilmenidine on vascular smooth muscle were similar to those previously described for the imidazoline, clonidine (Kong et al., 1991; Skrbic and Chiba, 1993; Silva et al., 1996). Clonidine induces vasoconstriction but also has a vasodilator effect when applied to a vascular bed precontracted with noradrenaline. Indeed, we found that clonidine had qualitatively the same effects as rilmenidine on rat portal vein. According to Kong et al. (1991), clonidine acts as a partial agonist on the vascular smooth muscle α_1-adrenoceptors and thereby interferes selectively with the vasoconstrictor action of α_1-adrenoceptor agonists. More generally, it appears that some α_1-adrenoceptor agonism is always found for imidazolines (McGrath et al., 1995). According to our results, this property is not restricted to imidazolines but could extend to non-imidazoline agonists of I_1-sites, which include rilmenidine. The physiological consequence of this property is that these agents could have a direct effect on the vascular smooth muscle in which α_1-adrenoceptors are coupled to contraction. The more interesting outcome is that their affinity for vascular α_1-adrenoceptor could cause these agents to attenuate, rather than potentiate, a response to α_1-adrenoceptor agonists. Agents such as rilmenidine, which have only weak agonist activity, are thus able to have potent relaxing effects.

In conclusion, this study showed that, in addition to its well known centrally mediated hypotensive effect, rilmenidine has a direct action on vascular smooth muscle. Our results indicate that rilmenidine acts as a partial agonist of α_1-adrenoceptors of vascular smooth muscle, causing relaxation of vessels precontracted by α_1-adrenoceptor stimulation.

Acknowledgements

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