ELSEVIER

European Journal of Pharmacology 323 (1997) 89–91

ejp

Short communication

Differential effects of isoliquiritigenin and YC-1 in rat aortic smooth muscle

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Received 10 February 1997; accepted 11 February 1997

Abstract

We investigated the effects of isoliquiritigenin and YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole) on tension in endothelial-free rat aortic rings precontracted with phenylephrine (3 μM). Both compounds induced a concentration-dependent relaxation (EC_{50} of YC-1 1.9 μM and of isoliquiritigenin 9.4 μM). The effects developed faster with YC-1 than with isoliquiritigenin, and the effects of YC-1 were potentiated by isoliquiritigenin (10 μM). 1*H*-[1,2,4]Oxadiazolo[4,3-*a*]quinoxalin-1-one (30 μM) inhibited the effect of YC-1, but not of isoliquiritigenin. These results suggest that the effects of YC-1 are due to stimulation of soluble guanylyl cyclase activity, whereas the effects of isoliquiritigenin are rather related to inhibition of phosphodiesterase activity. © 1997 Elsevier Science B.V.

Keywords: Smooth muscle tension; Guanylyl cyclase; Isoliquiritigenin; YC-1; ODQ

1. Introduction

Soluble guanylyl cyclase is regarded as the key enzyme in mediating vascular relaxation induced by nitric oxide (NO) and NO-related compounds through increasing cyclic GMP levels (Ignarro, 1990). The use of direct activators and inhibitors of soluble guanylyl cyclase has helped to further clarify the biological role of the guanylyl cyclase-cyclic GMP pathway, independent of NO. Methylene blue and LY 83583 (6-anilino-5,8-quinolinedione), which have been used as inhibitors of soluble guanylyl cyclase are, however, not free from side-effects such as generation of superoxide anions (Marshall et al., 1988; Kontos and Wei, 1993). In addition, methylene blue has been shown to inhibit prostacyclin synthesis (Martin et al., 1989) and NO synthase activity (Mayer et al., 1993). Recently, 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ) has been described as a more potent and specific inhibitor of soluble guanylyl cyclase (IC_{50} = 10–60 nM) thereby inhibiting the NO-dependent cyclic-GMP response to glutamate receptor agonists in rat neuronal tissue (Garthwaite et al., 1995) and inhibiting the relaxation in rat vascular smooth muscle induced by NO donors (Moro et al., 1996). Two substances have recently been described as direct activators of soluble guanylyl cyclase: (1) isoliquiritigenin, isolated from the plant *Dalbergia odorifera T.* (Yu and Kuo, 1995) and (2) YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole; Wu et al., 1995). In this study, we examined the quantitative interaction between inhibition of soluble guanylyl cyclase activity by ODQ and the vasorelaxant activity of both isoliquiritigenin and YC-1 in rat aortic rings precontracted with phenylephrine.

2. Materials and methods

Sprague-Dawley rats (200–300 g) of either sex were anaesthetised with ether and bled from the carotid arteries. The thoracic aorta was removed and immersed in warmed and oxygenated Tyrode's solution. After the connective tissue had been removed, the aorta was cut into rings of 3–5 mm in width and tied with silk ligatures. The endothelium was removed by gently rubbing the luminal surface with a wooden stick.

Preparations were mounted vertically in organ baths (5 ml) containing oxygenated Tyrode's solution at 37 ± 1°C. One end was fixed to a hook of a muscle holder while the other end was connected by means of a steel rod to an inductive force-displacement transducer whose output was fed to a frequency preamplifier (Carrier amplifier/TA2000,

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PII S0014-2999(97)00111-8

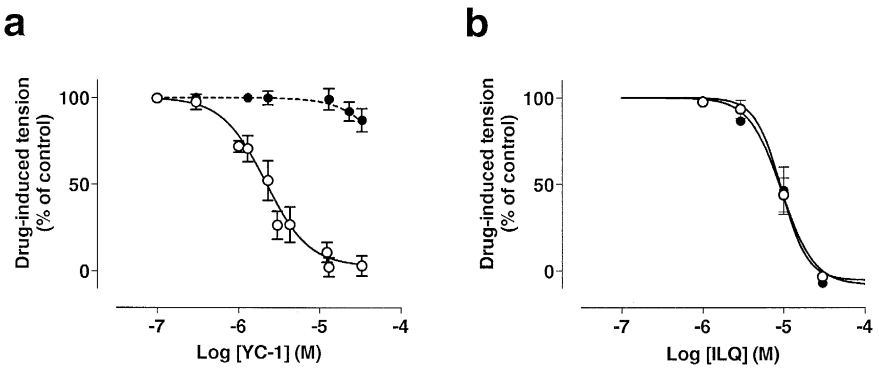

Fig. 1. Concentration-dependent effects of YC-1 (a) and isoliquiritigenin (b) on rat aortic rings precontracted with phenylephrine (3 μM) under control conditions (○) and after preincubation (10 min) with ODQ (30 μM; ●). Data represent means ± S.E.M. ($n \geq 9$ each). Each curve was obtained from individual preparations treated with cumulatively increasing concentrations of either isoliquiritigenin or YC-1.

Gould, Cleveland, OH, USA). Tension was recorded isometrically. Resting tension of the rings was set at 10 mN; the preparations were then precontracted with phenylephrine (3 μM) to produce about 90% of maximal contraction. Removal of endothelium was verified by the lack of any relaxation in response to carbachol (3 μM).

All salts and solvents used were at least p.a. grade and purchased from Sigma (St. Louis, MO, USA), unless otherwise indicated. ODQ was obtained from Tocris Cookson (Bristol, UK). YC-1 was a gift from Hoechst (Frankfurt, Germany). Stock solutions of YC-1, isoliquiritigenin and ODQ were prepared in dimethyl sulfoxide (DMSO) and further diluted to achieve the concentrations as indicated. Drugs were added cumulatively to the organ bath; the final amount of DMSO did not exceed 1% (v/v) and did not affect significantly phenylephrine-induced tension.

Data are expressed as means ± S.D. or means ± S.E.M. Tension was measured as the difference between resting tension and tension induced by phenylephrine (3 μM). Concentration-response curves were fitted to sigmoidal functions (correlation coefficient > 0.99) using GraphPad Prism 2.0 (GraphPad Software, San Diego, CA, USA).

3. Results

Both isoliquiritigenin and YC-1 exerted a concentration-dependent relaxation in endothelium-free aortic rings precontracted with phenylephrine (3 μM). The phenylephrine-induced tension amounted to 10 ± 0.3 mN ($n = 41$; means ± S.D.). The phenylephrine-induced tension was completely abolished by YC-1 or isoliquiritigenin at maximally effective concentrations. Steady-state effects were reached within 15–70 min. The EC_{50} of YC-1 and isoliquiritigenin amounted to 1.9 μM and 9.4 μM, respectively (Fig. 1). ODQ, which is a potent and specific inhibitor of soluble guanylyl cyclase (Garthwaite et al., 1995), was used to inhibit activation of this enzyme. After preincubation with ODQ (30 μM) for 10 min, the relaxant effects of YC-1 were greatly reduced at all concentrations tested (Fig. 1a). In contrast, the concentration-response relationship of isoliquiritigenin remained virtually unchanged by ODQ (30 μM). The EC_{50} of isoliquiritigenin amounted to 9.4 μM under control conditions and to 9.5 μM after preincubation with ODQ (Fig. 1b).

Fig. 2a demonstrates the time course of relaxation

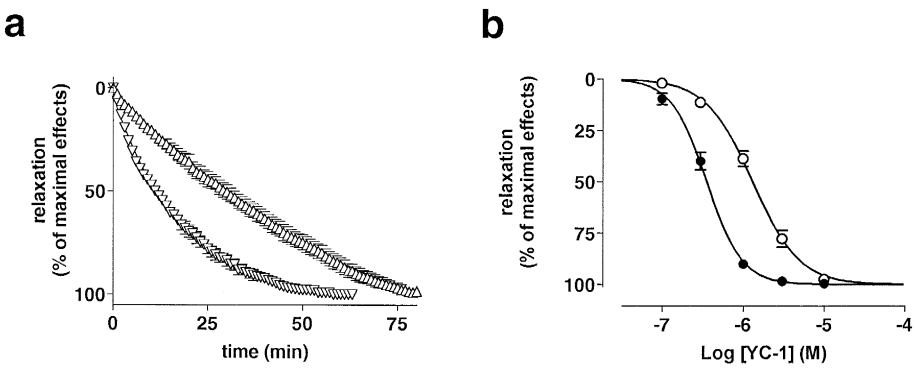

Fig. 2. (a) Time-course of relaxation induced by YC-1 (▽) and isoliquiritigenin (△). YC-1 (10 μM) and isoliquiritigenin (30 μM) induced half-maximal relaxation at 12.5 min and 48 min, respectively. Data represent means ± S.E.M. ($n = 3$ each). (b) Concentration-dependent effects of YC-1 on relaxation under control conditions (○) and after preincubation (20 min) with isoliquiritigenin (10 μM, ●). The EC_{50} values of YC-1 amounted to 1.4 μM in the absence and to 0.35 μM in the presence of isoliquiritigenin. Data represent means ± S.E.M. ($n = 4$ each). Each curve was obtained in parallel from individual rings from the same preparations, each treated with cumulatively increasing concentrations of YC-1.

induced by YC-1 and isoliquiritigenin at maximally effective concentrations. The effect of YC-1 developed faster than the effect of isoliquiritigenin. Half-maximal relaxation was reached within 12.5 min in the presence of YC-1 (10 μM) and within 48 min in the presence of isoliquiritigenin (30 μM).

In another series of experiments, the relaxing effects of YC-1 were investigated comparatively without and in the presence of isoliquiritigenin. In these experiments, isoliquiritigenin (10 μM) relaxed the preparations to 64 ± 3% of control ($n = 4$). Under these conditions, the concentration-response curve of YC-1 was shifted to the left (evaluated as percentage of maximal relaxation; Fig. 2b), indicating that the effects of YC-1 were potentiated by isoliquiritigenin. The EC_{50} of YC-1 amounted to 1.4 μM under control conditions and to 0.35 μM after preincubation with isoliquiritigenin (10 μM).

4. Discussion

We have demonstrated that ODQ, a potent and selective inhibitor of soluble guanylyl cyclase (Garthwaite et al., 1995), depressed the vasorelaxant effects of YC-1 but not of isoliquiritigenin in rat aortic rings precontracted with phenylephrine. Both drugs had been described recently as direct activators of soluble guanylyl cyclase (Yu and Kuo, 1995; Wu et al., 1995). In the study of Yu and Kuo (1995), isoliquiritigenin induced relaxation in rat aortic rings which was abolished by methylene blue used as an inhibitor of soluble guanylyl cyclase. However, since methylene blue exerts also other effects, unrelated to inhibition of soluble guanylyl cyclase activity (Marshall et al., 1988; Martin et al., 1989; Mayer et al., 1993), preference was given in our study to ODQ which is a more potent and more specific inhibitor of this enzyme. ODQ clearly eliminated the relaxant effects of YC-1 but not of isoliquiritigenin. YC-1 and ODQ are therefore regarded as extremely useful tools to investigate the guanylyl cyclase-cyclic GMP pathway, as shown in rat aorta.

The reason why ODQ was ineffective against isoliquiritigenin-elicited relaxation was not clarified in detail, since we did not test the effect of isoliquiritigenin on the isolated enzyme. In physiological conditions, however, isoliquiritigenin seems to induce relaxation by a mechanism independent of guanylyl cyclase activation. This view is supported by the findings that (1) the time course of the effects of YC-1 was much faster than of isoliquiritigenin and (2) the effects of YC-1 and isoliquiritigenin were not simply additive, but the effects of YC-1 were potentiated in the presence of isoliquiritigenin. It is therefore suggested that the effects of isoliquiritigenin are due to inhibition of phosphodiesterase activity for which evidence has also been obtained in cell-free preparations (Kusano et al., 1991) and in rat ventricular heart muscle (Wegener and Nawrath, unpublished observations).

Acknowledgements

We thank Dr. Schönafinger, Hoechst AG (Germany), for providing us with YC-1 and Mrs Johanna Rupp for competent help. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Germany).

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