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Short communication

Relaxation induced by milrinone and rolipram in human penile arteries and veins

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Abstract

We studied the relaxant effects of milrinone, an inhibitor of phosphodiesterase 3, and rolipram, an inhibitor of phosphodiesterase 4, on contracted human penile dorsal artery and deep dorsal vein. Vascular rings from 12 multi-organ donors were suspended in organ baths for isometric recording of tension. Both milrinone and rolipram inhibited (100%) the contraction induced by noradrenaline and shifted the relaxation–response curves to the cAMP forming agents prostaglandin E_1 and forskolin to the left. The findings indicate that the cAMP pathway appears to be a main determinant of relaxation in human penile vessels.

Keywords: Penile artery; Penile vein; Milrinone; Rolipram; cAMP pathway

1. Introduction

An increase in intracellular levels of cAMP is essential to promote relaxation of erectile tissue in response to different compounds, such as prostaglandin E_1, forskolin or vasoactive intestinal peptide (Andersson and Wagner, 1995). The concentration of cAMP is controlled through the rate of synthesis of adenylate cyclase and the rate of hydrolytic breakdown of cAMP by cyclic nucleotide phosphodiesterases (Thompson, 1991; Beavo, 1995). Rolipram, a cAMP-specific inhibitor of phosphodiesterase 4, and milrinone, an inhibitor of the cGMP-inhibited cAMP phosphodiesterase (phosphodiesterase 3), cause relaxation of human and rabbit isolated corpus cavernosum by increasing the levels of cAMP (Sparwasser et al., 1994; Stief et al., 1998; Bivalacqua et al., 1999). Rolipram elicits an erectile response in the rabbit (Bivalacqua et al., 1999) whereas in patients with erectile dysfunction, intracavernous injection of milrinone induces an erectile response comparable to that obtained with papaverine (Stief et al., 1998). Thus the combination of rolipram and the cAMP forming agent prostaglandin E_1 has been postulated as a potential effective treatment of erectile dysfunction (Sparwasser et al., 1994; Bivalacqua et al., 1999).

Because relaxation of large-diameter penile vessels is a key factor in the process of penile erection, we studied the effects of rolipram and milrinone, given alone or in combination with the cAMP forming agents prostaglandin E_1 and forskolin, on contracted human dorsal artery and deep dorsal vein.

2. Materials and methods

2.1. Sample collection

Penile dorsal arteries and deep dorsal veins were obtained from 12 multiorgan donors during procurement of organs for transplantation (age range, 17–60 years). The study was approved by the ethical committee of our institution. The vessels were immediately placed in cold (4 °C) modified Krebs solution (for composition, see below) aerated with 95% oxygen and 5% carbon dioxide until they were used (within 8 h of collection).

2.2. Organ bath experiments

The vessels were cleaned of adherent connective tissue and cut into rings (3 mm in length). Each ring was

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suspended between two stainless-steel L-shaped pins in 4-ml organ baths containing modified Krebs–Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; $MgCl_2 \cdot 6H_2O$, 1.2; $CaCl_2$, 2.5; $NaHCO_3$, 25; glucose, 11.1; and disodium EDTA, 0.01.

The solution was equilibrated with 95% oxygen and 5% carbon dioxide to give a pH of 7.3–7.4. The temperature was held at 37 °C. One pin was fixed to the organ bath wall, and the other was connected to a strain gauge (Grass FT03, Grass Instruments, Quincy, MA). The changes in isometric force were recorded on a Macintosh computer by use of Chart version 3.4/s software and a MacLab/8e data acquisition system (ADInstruments, East Sussex, UK). The optimal resting tensions were 3.5 g for the dorsal artery and 3 g for the dorsal vein (Segarra et al., 1998). The functional integrity of the endothelium was confirmed routinely by the presence of relaxation induced by acetylcholine (10^{-8}–10^{-7} M) during contraction obtained with noradrenaline (10^{-7}–3×10^{-7} M).

2.3. Relaxation

To study relaxation, rings were contracted with 10^{-7}–10^{-6} M noradrenaline. After a stable contraction was obtained, concentration–response curves were obtained for rolipram (10^{-10}–3×10^{-6} M), milrinone (10^{-10}–3×10^{-6} M), prostaglandin E_1 (3×10^{-11}–10^{-7} M) and forskolin (10^{-10}–3×10^{-6} M). In another series of experiments, relaxation–response curves to prostaglandin E_1 and forskolin were obtained in the presence of either milrinone 10^{-8} M or rolipram 10^{-8} M.

2.4. Drugs and solutions

The following drugs were used: acetylcholine chloride, noradrenaline hydrochloride, milrinone, rolipram, forskolin and prostaglandin E_1 (Sigma, St. Louis, MO). Milrinone, rolipram and forskolin were dissolved in dimethylsulfoxide and diluted in water. Noradrenaline and acetylcholine were dissolved in Krebs solution and prostaglandin E_1 was dissolved in ethanol.

2.5. Data analysis

All values are expressed as mean ± S.E.M. Relaxation was expressed as a percentage of inhibition of noradrenaline-induced contraction.

EC_{50} values (concentrations of agonist producing half-maximal relaxation) were determined from individual concentration–response curves by nonlinear regression analysis, and from these values the geometric means were calculated. EC_{50} values were expressed as pD_2 ($-\log EC_{50}$). The pD_2 values were compared by an unpaired *t*-test and an ANOVA with Scheffe's test as post hoc test. The

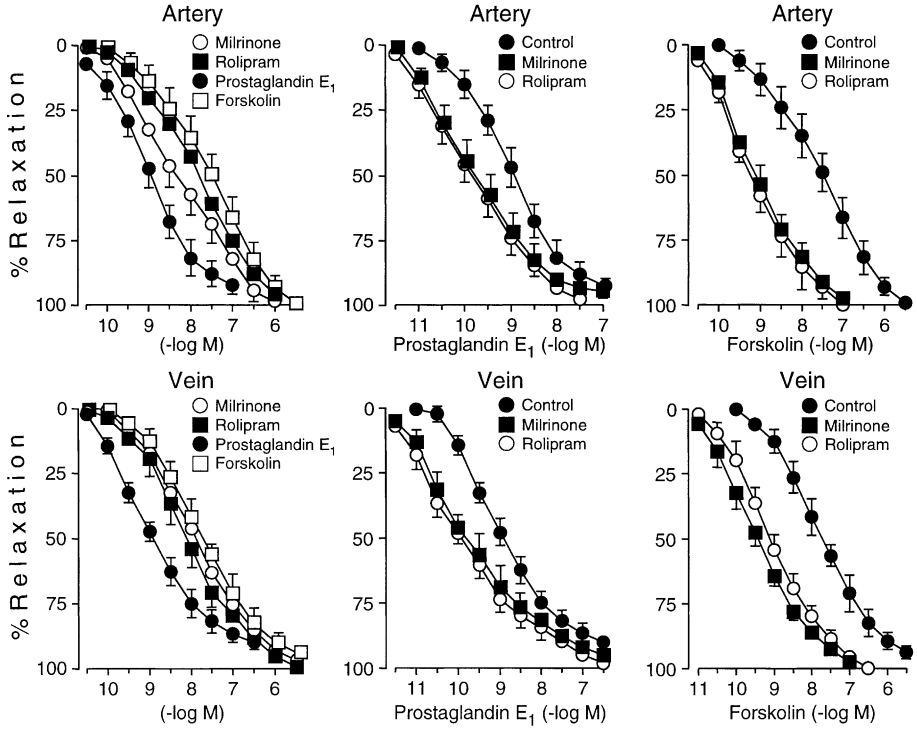

Fig. 1. Concentration–response curves to the tested agents in arteries (top) and veins (bottom). Left: concentration–response curves to milrinone, rolipram, prostaglandin E_1 and forskolin ($n = 7$). Middle: relaxation to prostaglandin E_1 in the absence (control, $n = 7$) and in the presence of milrinone (10^{-8} M, $n = 6$) or rolipram (10^{-8} M, $n = 6$). Right: relaxation to forskolin in the absence (control, $n = 6$) and in the presence of milrinone (10^{-8} M, $n = 5$) or rolipram (10^{-8} M, $n = 5$). Values are mean ± S.E.M. shown by vertical bars.

Table 1
pD_2 values to dilator substances in human penile dorsal artery and deep dorsal vein

	n	Artery	Vein
		pD_2	pD_2
Milrinone	7	8.41 ± 0.26	7.84 ± 0.29
Rolipram	7	7.94 ± 0.18	8.15 ± 0.18
Prostaglandin E_1	7	9.15 ± 0.16	9.10 ± 0.20
+ Rolipram 10^{-8} M	6	9.81 ± 0.22	10.03 ± 0.44
+ Milrinone 10^{-8} M	6	9.82 ± 0.21	9.83 ± 0.24
Forskolin	6	7.62 ± 0.27	7.89 ± 0.13
+ Rolipram 10^{-8} M	5	9.23 ± 0.19	9.11 ± 0.14
+ Milrinone 10^{-8} M	5	9.09 ± 0.15	9.48 ± 0.40

pD_2 indicates $-\log EC_{50}$. Values are mean ± S.E.M.
n, number of subjects.

responses obtained in each subject were averaged to yield a single value. Therefore, all (n) values are presented as the number of subjects. Statistical significance was accepted at $P < 0.05$.

3. Results

3.1. Relaxation responses to rolipram, milrinone, prostaglandin E_1 and forskolin

All agents tested relaxed noradrenaline-contracted arterial and venous rings in a concentration-dependent manner (Fig. 1). pD_2 values and maximal relaxation are shown in Table 1. Prostaglandin E_1 was the most potent agent in arteries and veins. Removal of the endothelium in arteries ($n = 4$) and veins ($n = 4$) did not modify these responses ($P > 0.05$, $n = 5$, results not shown). There were no significant differences in the concentration–response curves to milrinone and rolipram between arterial and venous rings.

3.2. Effects of phosphodiesterase inhibitors in combination with prostaglandin E_1 or forskolin

In arterial and venous segments, the relaxation response curves to prostaglandin E_1 and forskolin were enhanced by rolipram (10^{-8} M) or milrinone (10^{-8} M), as shown by the displacement of the concentration–response curves to the left (Fig. 1 and Table 1). Higher concentrations of milrinone or rolipram (10^{-7} and 10^{-6} M) did not further potentiate the relaxation induced by prostaglandin E_1 and forskolin.

4. Discussion

The present study describes the responses of human dorsal artery and deep dorsal vein to rolipram and milrinone, two phosphodiesterase inhibitors potentially useful in the treatment of erectile dysfunction (Stief et al., 1998; Sparwasser et al., 1994). We were able to obtain stable results from vessels taken immediately after death, and full concentration–response curves were consistently obtained. The results demonstrate that both rolipram and milrinone induce potent relaxing effects in dorsal artery and deep dorsal vein. Both agents completely inhibited (100%) the contractions induced by noradrenaline. The relaxation appears to be endothelium-independent because mechanical removal of the endothelium did not modify the response to these agents, a finding similar to that found in rat aortic rings (Komas et al., 1991) and in human internal thoracic artery (Liu et al., 1997). The sensitivities to milrinone and rolipram, as assessed by the pD_2 values, were comparable in artery and vein thus suggesting an homogeneous distribution of the phosphodiesterase enzymes in these tissues. This cannot be generalized to other vessels and species because in the rat, the small mesenteric artery is more sensitive to olprinone (a phosphodiesterase 3 inhibitor) than the vein (Fujimoto et al., 1998). The plasma concentrations after administration of clinical doses of milrinone (7×10^{-8}–2×10^{-7} M) to increase left ventricular contractility (Murakami et al., 1995) are within the range of the concentrations tested in the present experiments. Furthermore, the concentrations of milrinone and rolipram used are close to the concentrations that inhibit phosphodiesterase 3 and phosphodiesterase 4, respectively, in rat aorta (Komas et al., 1991). Milrinone and rolipram produce smooth muscle relaxation at significantly lower concentrations than sildenafil and zaprinast, two inhibitors of type 5 cGMP phosphodiesterase which increase the level of cGMP and relax human penile arteries and veins (Medina et al., 2000b).

Because prostaglandin E_1 and forskolin elevate smooth muscle cAMP levels, it is expected that a synergistic action between these two agents and phosphodiesterase 3 and phosphodiesterase 4 inhibitors must exist. Our data indicate that relatively low concentrations of milrinone and rolipram will potentiate the relaxation to prostaglandin E_1 and forskolin. It is assumed that the relaxing effects are due to intracellular accumulation of cAMP. A synergistic action between phosphodiesterase 5 inhibitors (sildenafil and zaprinast) and activators of guanylate cyclase (sodium nitroprusside) has also been observed in the same vessels (Medina et al., 2000b) and in other human arteries (Medina et al., 2000a). Although intracellular levels of cGMP are essential to promote relaxation of the erectile tissue (Rajfer et al., 1992; Trigo-Rocha et al., 1993), the present results reinforce the hypothesis of an intervention of the cAMP system in smooth muscle relaxation of erectile tissues (Sparwasser et al., 1994; Bivalacqua et al., 1999) and extend the observation to human penile vessels.

Compared with the relaxing effects of milrinone and rolipram in human (Stief et al., 1998) and rabbit (Sparwasser et al., 1994) cavernous tissue, the pD_2 values obtained in the present study were significantly lower. This might result from differences in the phosphodiesterase activity between species or between cavernous and vascular smooth muscle. Milrinone exhibits a weaker relaxing effect in human internal thoracic artery (Liu et al., 1997) and both milrinone

and rolipram have modest relaxing effects in rat aortic rings (Komas et al., 1991). Thus it appears that human penile smooth muscle is particularly sensitive to rolipram and milrinone.

In conclusion, both milrinone and rolipram have potent relaxant effects on human penile vessels and enhance the relaxation in response to cAMP forming agents such as forskolin and prostaglandin E_1. Because our data are limited to responsiveness of isolated vessels, a link between our results and the potential therapeutic effects in erectile dysfunction cannot be established. The findings, however, support the hypothesis that the cAMP pathway is a main determinant of relaxation in human large penile vessels.

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References

Andersson, K.-E., Wagner, G., 1995. Physiology of penile erection. Physiol. Rev. 75, 191–235.

Beavo, J.A., 1995. Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. Physiol. Rev. 75, 725–748.

Bivalacqua, T.J., Champion, H.C., Rajasekaran, M., Sikka, S.C., Kadowitz, P.J., Doherty, P.C., Hellstrom, W.J., 1999. Potentiation of erectile response and cAMP accumulation by combination of prostaglandin E_1 and rolipram, a selective inhibitor of the type 4 phosphodiesterase (PDE 4). J. Urol. 162, 1848–1855.

Fujimoto, S., Ohashi, M., Hiramoto, A., Inoue, Y., Nagai, K., Shiokawa, H., Itoh, T., 1998. Vasorelaxant effect of olprinone, an inhibitor of phosphodiesterase 3, on mesenteric small artery and vein of rabbits. Eur. J. Pharmacol. 353, 239–246.

Komas, N., Lugnier, C., Stoclet, J.C., 1991. Endothelium-dependent and independent relaxation of the rat aorta by cyclic nucleotide phosphodiesterase inhibitors. Br. J. Pharmacol. 104, 495–503.

Liu, J.J., Doolan, L.A., Xie, B., Chen, J.R., Buxton, B.F., 1997. Direct vasodilator effect of milrinone, an inotropic drug, on arterial coronary bypass grafts. J. Thorac. Cardiovasc. Surg. 113, 108–113.

Medina, P., Segarra, G., Martínez-León, J.B., Vila, J.M., Aldasoro, M., Otero, E., Lluch, S., 2000a. Relaxation induced by cGMP phosphodiesterase inhibitors sildenafil and zaprinast in human vessels. Ann. Thorac. Surg. 70, 1327–1331.

Medina, P., Segarra, G., Vila, J.M., Domenech, C., Martínez-León, J.B., Lluch, S., 2000b. Effects of sildenafil on human penile blood vessels. Urology 56, 539–543.

Murakami, R., Sano, K., Murakami, Y., Shimada, T., Morioka, S., 1995. Effects of intracoronary infusion of an inotropic agent, E-1020 (loprinone hydrochloride), on cardiac function: evaluation of left ventricular contractile performance using the end-systolic pressure–volume relationship. Int. J. Cardiol. 51, 57–63.

Rajfer, J., Aronson, W.J., Bush, P.A., Dorey, F.J., Ignarro, L.J., 1992. Nitric oxide as a mediator of relaxation of the corpus cavernossum in response to nonadrenergic, noncholinergic neurotransmission. N. Engl. J. Med. 326, 90–94.

Segarra, G., Medina, P., Domenech, C., Vila, J.M., Martínez-León, J.B., Aldasoro, M., Lluch, S., 1998. Role of vasopressin on adrenergic neurotransmission in human penile blood vessels. J. Pharmacol. Exp. Ther. 286, 1315–1320.

Sparwasser, C., Drescher, P., Will, J.A., Madsen, P.O., 1994. Smooth muscle tone regulation in rabbit cavernosal and spongiosal tissue by cyclic AMP- and cyclic GMP-dependent mechanisms. J. Urol. 152, 2159–2163.

Stief, C.G., Uckert, S., Becker, A.J., Truss, M.C., Jonas, U., 1998. The effect of the specific phosphodiesterase (PDE) inhibitors on human and rabbit cavernous tissue in vitro and in vivo. J. Urol. 159, 1390–1393.

Thompson, W.J., 1991. Cyclic nucleotide phosphodiesterases: pharmacology, biochemistry and function. Pharmacol. Ther. 51, 13–33.

Trigo-Rocha, F., Aronson, W.J., Hohenfellner, M., Ignarro, L.J., Rajfer, J., Lue, T.F., 1993. Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. Am. J. Physiol. 264, H419–H422.