ELSEVIER

European Journal of Pharmacology 439 (2002) 173–174

www.elsevier.com/locate/ejphar

Rapid communication

Nitric oxide inhibits RhoA/Rho-kinase signaling to cause penile erection

Thomas M. Mills [a,b,*], Kanchan Chitaley [c], Ronald W. Lewis [b], R. Clinton Webb [a]

[a] *Department of Physiology, Medical College of Georgia, Augusta, GA, USA*
[b] *Department of Surgery (Urology Section), Medical College of Georgia, Augusta, GA, USA*
[c] *Department of Physiology, University of Michigan, Ann Arbor, MI, USA*

Received 11 February 2002; accepted 15 February 2002

Abstract

The RhoA/Rho-kinase pathway mediates vasoconstriction in the cavernosal circulation. Inhibition of this pathway leads to penile erection in the in vivo rat model. These studies examined the hypothesis that nitric oxide (NO) inhibits RhoA/Rho-kinase signaling as part of normal erection. The results show that NO causes increased intracavernosal pressure and that this response is potentiated by prior treatment with a threshold dose of the Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-Aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate (Y-27632). These results support the hypothesis that NO inhibits Rho-kinase-induced cavernosal vasoconstriction during erection. © 2002 Published by Elsevier Science B.V.

Keywords: Penile erection; Nitric oxide (NO); RhoA/Rho-kinase

Nitric oxide (NO), released from penile autonomic innervation and endothelial cells is reported to cause vasorelaxation leading to penile erection by lowering intracellular levels of calcium $[Ca^{2+}]_i$ (Andersson, 2001). However, studies in other vascular tissues have shown a transient rise in $[Ca^{2+}]_i$ during agonist-induced vasoconstriction with $[Ca^{2+}]_i$ levels falling to near basal levels despite sustained force generation (DeFeo and Morgan, 1985). Thus, a further lowering of $[Ca^{2+}]_i$ may not be a primary mechanism for NO-mediated vasodilation in vivo. In other vascular beds, NO has been found to cause vasorelaxation by inhibiting the activity of the RhoA/Rho-kinase Ca^{2+} sensitizing pathway (Sauzeau et al., 2000). We have previously demonstrated activity of this pathway in erectile tissue of the rat and reported that inhibition of Rho-kinase leads to erection (Chitaley et al., 2001b).

Male Sprague–Dawley rats (250–300 g) were obtained from Harlan Laboratories and maintained on a 12-h light/dark cycle with rat chow and water available ad libitum. The procedure used to measure the erectile response in the rat has been previously described from this laboratory (Mills et al., 2001). Briefly, the animals were anesthetized with ketamine and xylazine and the left carotid artery cannulated for continuous monitoring of mean arterial blood pressure. The right corpus cavernosum was cannulated to permit continuous monitoring of intracavernosal pressure. The left corpus cavernosum was cannulated for delivery of drugs. Pressure data were collected and analyzed electronically (Polyview, Grass Instrument). Drugs included the Rho-kinase inhibitor (Somlyo and Somlyo, 2000), (+)-(*R*)-*trans*-4-(1-Aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride, monohydrate (Y-27632, Mitsubishi-Pharma, Osaka, Japan, 50 nmol/μl saline) and the NO donor drug (±)-(*E*)-Methyl-2-((*E*)-hydroxyimino)-5-nitro-6-methoxy-3-hexenamide (NOR-1, Biomol, Plymouth, PA, 5 μg/μl in ethanol). Data were analyzed using one-way analysis of variance (ANOVA) with post hoc analysis by Students–Newman–Keul's test. Statistical significance was set at $P < 0.05$.

Fig. 1A and B shows intracavernosal pressure and mean arterial pressure responses to the intracavernosal injection of NOR-1 alone, Y-27632 alone and NOR-1 + Y-27632 in combination. The erectile response (expressed as the intracavernosal pressure relative to the mean arterial pressure, ICP/MAP—Fig. 1B) to the low doses of NOR-1 alone is minimal. Furthermore, injection of Y-27632 fails to increase the erectile response significantly. However, when injected in combination (NOR-1 + Y-27632), the erectile response is significantly greater than the response to either agent alone or to the sum of the individual responses (Fig. 1B).

* Corresponding author. Department of Physiology, Medical College of Georgia, Augusta, GA, USA. Tel.: +1-706-721-7821; fax: +1-706-721-7299.

E-mail address: tmills@mail.mcg.edu (T.M. Mills).

0014-2999/02/$ - see front matter © 2002 Published by Elsevier Science B.V.
PII: S0014-2999(02)01408-5

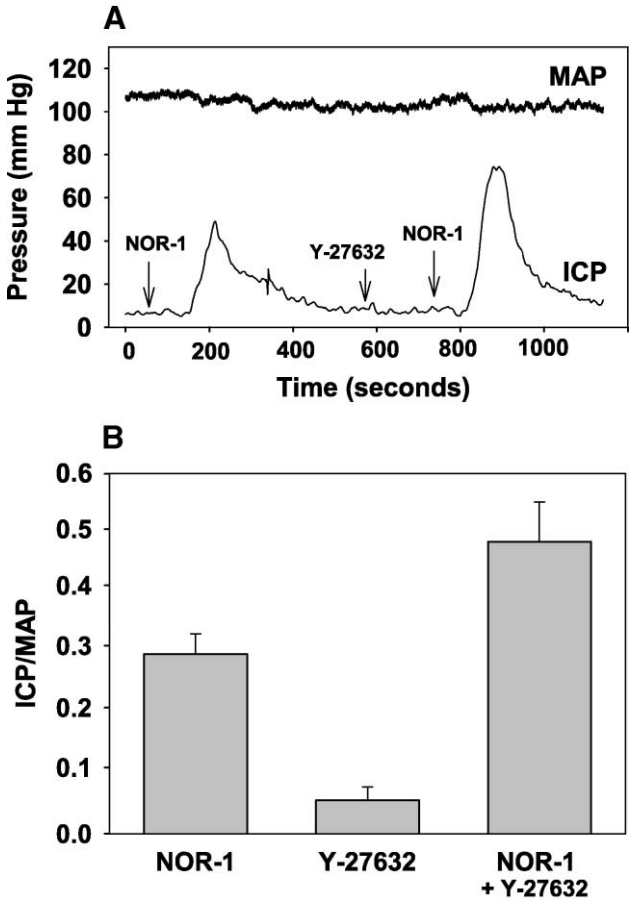

Fig. 1. Tracing of effect of the intracavernosal injection of NOR-1 (2 μg), Y-27632 (<2 nmol) or a combination of the two drugs on intracavernosal pressure (ICP) and mean arterial pressure (MAP) (A). Results of these measurements in six individual animals are expressed as ICP/MAP and summarized in (B). Bars represent the mean ± S.E.M., $n=6$. ANOVA reveals that all means are significantly different (*) from the response to NOR-1 only ($P<0.001$). Furthermore, the sum of the responses to NOR-1 alone and Y-27632 alone is different from the response to combined drug injection ($P<0.05$).

Our results show that increased NO and decreased Rho-kinase activity synergise in their action to elevate intracavernosal pressure in the erectile response. This finding supports the hypothesis that NO mediates the erectile response, in part, via inhibition of the RhoA/Rho-kinase Ca^{2+} sensitizing pathway (Chitaley et al., 2001a). Based on evidence from the literature, the target of NO-mediated inhibition is likely the small, G-protein, RhoA. RhoA-induced stress fiber formation was inhibited by NO stimulated phosphorylation of RhoA (Sauzeau et al., 2000) whereas in another study, translocation of RhoA to the membrane (a step necessary for RhoA activation) was inhibited by NO (Sawada et al., 2001). Furthermore, our prior studies have demonstrated that electrical stimulation of the autonomic innervation leads to a voltage-dependent increase in the erectile response via a NO-dependent mechanism (Chitaley et al., 2001b). When animals are treated with Y-27632 to inhibit Rho-kinase activity, there is a potentiation of the response to electrical stimulation at minimal voltage further demonstrating that inhibition of the RhoA/Rho-kinase pathway enhances the action of NO. Altogether, our findings support the hypothesis that NO initiates penile erection, in part, through the inhibition of endogenous RhoA/Rho-kinase vasoconstrictor activity introducing novel insight into the mechanism of the erectile response. This has particular significance since all previous mechanisms for NO-induced erection have focused on lowering $[Ca^{2+}]_i$, a mechanism that may not be associated with the maintenance of constrictor tone.

References

Andersson, K.E., 2001. Pharmacology of penile erection. Pharmacol. Rev. 53, 417–450.

Chitaley, K., Webb, R.C., Mills, T.M., 2001a. RhoA/Rho-kinase: a novel player in the regulation of penile erection. Int. J. Impotence Res. 13, 67–72.

Chitaley, K., Wingard, C., Webb, R., Branam, H., Stopper, V., Lewis, R., Mills, T., 2001b. Antagonism of Rho-kinase stimulates rat penile erection via a nitric oxide-independent pathway. Nat. Med. 7, 119–122.

DeFeo, T., Morgan, K., 1985. Calcium–force relationships as detected with Aequorin in two different vascular smooth muscles of the ferret. J. Physiol. 369, 269–292.

Mills, T.M., Chitaley, K., Wingard, C.J., Lewis, R.W., Webb, R.C., 2001. Effect of Rho-kinase inhibition on vasoconstriction in the penile circulation. J. Appl. Physiol. 91, 1269–1273.

Sauzeau, V., Le Jeune, H., Cario-Toumaniantz, C., Smolenski, A., Lohmann, S.M., Bertoglio, J., Chardin, P., Pacaud, P., Loirand, G., 2000. Cyclic GMP-dependent protein kinase signaling pathway inhibits RhoA-induced Ca^{2+} sensitization of contraction in vascular smooth muscle. J. Biol. Chem. 275, 21722–21729.

Sawada, N., Itoh, H., Yamashita, J., Doi, K., Inoue, M., Masatsugu, K., Fukunaga, Y., Sakaguchi, S., Sone, M., Yamahara, K., Yurugi, T., Nakao, K., 2001. cGMP-dependent protein kinase phosphorylates and inactivates RhoA. Biochem. Biophys. Res. Commun. 280, 798–805.

Somlyo, A.P., Somlyo, A.V., 2000. Signal transduction by G-proteins, rho-kinase and protein phosphatase to smooth muscle and non-muscle myosin II. J. Physiol. 522 (Pt. 2), 177–185.