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Effects of BAY 41–2272 on smooth muscle tone, soluble guanylyl cyclase activity and NADPH oxidase activity/expression in corpus cavernosum from wild-type, neuronal and endothelial NOS null mice

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Background

Nitric oxide (NO) is considered to play a critical role in the control of erectile function, by activating soluble guanylyl cyclase (sGC) in cavernosal smooth muscle to generate cGMP, which, in turn, promotes relaxation leading to penile erection. The pyrazolopyridine 5-cyclopropyl-2-[1-(2-fluorobenzyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]pyrimidin-4-ylamine (BAY 41–2272) sensitizes sGC to NO, lowers mean arterial pressure in spontaneously hypertensive rats and increases survival in a low-NO rat model of hypertension. In vitro, BAY 41–2272 causes potent relaxation of vascular smooth muscle, rat anococcygeus muscle as well as human and rabbit corpus cavernosum (CC).

Purpose

The aims of the present work were to characterize the mechanisms involved in the relaxant responses induced by BAY 41–2272 and the pharmacological interactions between this compound and NO in the CC from wild-type (WT), eNOS-/- and nNOS-/- mice. We also assessed the effect of BAY 41–2272 on superoxide formation and NADPH oxidase expression in cavernosal smooth muscle treated with the thromboxane A_2 mimetic U46619.

Methods

In functional studies, cavernosal strips were mounted under a resting tension of 2 mN in a myograph for isometric force recording, coupled to a PowerLab 8/SP™ data acquisition system. Cavernosal strips were stimulated for 10 min with BAY 41–2272 (1 μM), SNP (1 μM), or their combination. Cyclic GMP was extracted and quantified using commercially available kits. sGC activity in the presence of BAY 41–2272 was determined in the supernatant fractions of the cavernosal samples by the conversion of GTP to cGMP. Thirty μg of each sample was incubated for 10 min at 37°C in a total volume of 100 μl containing 50 mM Tris-HCl (pH 7.4), 1 mM 3-isobutyl-1-methylxantine, 3 mM $MgCl_2$, 0.5 mM GTP, 3 mM DTT, 5 mM phosphocreatine and 0.25 mg/ml creatine kinase. The reaction was terminated by inactivation of sGC at 95°C for 10 min, and cGMP measured. Segments of CC were incubated with U46619 with or without BAY 41–2272 for 1 or 8 h at 37°C. Superoxide dismutase-inhibitable superoxide formation was assessed using the reduction of ferricytochrome c measured spectrophotometrically, and NADPH oxidase subunits assessed using Western blot analysis.

Results

BAY 41–2272 (0.01–10 μM) relaxed CC with pEC_{50} values of 6.36 ± 0.07, 6.27 ± 0.06 and 5.88 ± 0.07 in WT, nNOS-/- and eNOS-/- mice, respectively (Fig. 1). L-NAME (100 μM) rightward shifted the curves to BAY 41–2272 by 3-fold in CC from WT and nNOS-/-, but not eNOS-/-. ODQ (10 μM) reduced BAY 41-2272-evoked relaxations as evi-

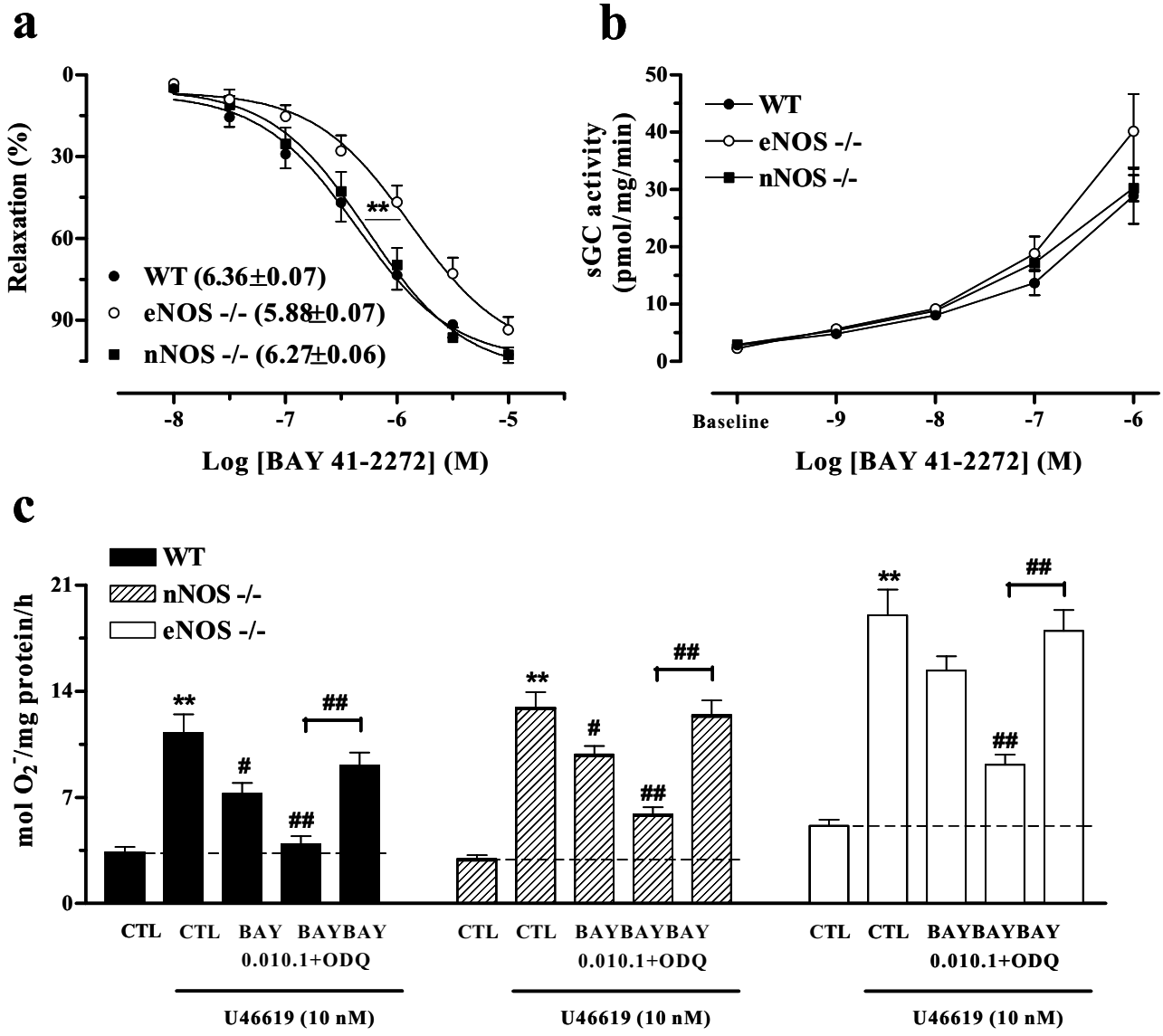

Figure 1

(a) Concentration-response curves to BAY 41–2272 (0.01–10 μM) in CC from wild-type (WT), eNOS and nNOS knockout animals (n = 6); **(b)** Formation of cGMP under basal conditions and after stimulation by BAY 41–2272 (0.001–1 μM) in crude protein extracts of cavernosal tissue; **(c)** Inhibitory effect of BAY 41–2272 (0.01–0.1 μM) on superoxide formation elicited by incubation of CC with U46619 over a 8 h period.

denced by the rightward shifts of 5-, 4- and 2.5-fold in CC from WT, nNOS-/- and eNOS-/- mice. Sildenafil (0.1 μM) potentiated the relaxations induced by BAY 41–2272 in all groups. BAY 41–2272 (0.01–0.1 μM) enhanced SNP-induced CC relaxations in all groups in a concentration-dependent manner. In addition, BAY 41–2272 potentiated acetylcholine (ACh, 0.01–1 μM)- and electrical field stimulation (EFS, 1–16 Hz)-induced relaxations. At 1 μM, BAY 41–2272 caused a 30-fold increase in cGMP concentration in WT and nNOS-/- samples *versus* an 18-fold increase in eNOS-/- CC. Co-incubation with SNP (1 μM) resulted in a synergistic increase in cGMP levels in an ODQ-sensitive manner. Expression and activity of sGC did not significantly differ in CC from WT, nNOS-/- and eNOS-/-. Superoxide formation was significantly greater in tissues incubated with U46619 after 1 and 8 h incubation than in controls, an effect blocked by NADPH oxidase inhibitors. These effects of U46619 were inhibited by BAY 41–2272 (0.01–0.1 μM), which in turn were negated by the guanylyl cyclase inhibitor, ODQ (10 μM). BAY 41–2272 also inhibited $p22^{phox}$ and $gp91^{phox}$ expression induced by U46619.

Conclusion

Our results demonstrated that BAY 41–2272 potently relaxes the CC, synergistically with NO released from the endothelium and/or nitrergic nerves. In addition, BAY 41–2272 is a potent inhibitor of superoxide formation in the penis. This effect is mediated through the accumulation of cGMP, which in turn inhibits NADPH oxidase expression and activity. Therefore, sGC activation in the penis by BAY 41–2272 directly or via enhancement of NO effects may provide a novel treatment for erectile dysfunction, particularly in the event of an increased intrapenile oxidative stress.