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J. Pharm. Pharmacol. 1993, 45: 148–150
Communicated April 30, 1992

Absence of desensitization to the relaxant activity of streptozotocin in isosorbide dinitrate-tolerant rat aorta

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Abstract—The effect of in-vitro isosorbide dinitrate (ISDN)-induced tolerance on the vasodilatory actions of streptozotocin, a nitric oxide containing compound, and papaverine was studied in rat aortic strips precontracted by phenylephrine. Aortas made tolerant to ISDN remained fully responsive to streptozotocin but exhibited a greater response to low concentrations of papaverine compared with control strips. Methylene blue produced parallel displacement to the right of the relaxant concentration-effect curves of both ISDN and streptozotocin, whereas responses to only low concentrations of papaverine were significantly antagonized. These results indicate that the relaxant activity of streptozotocin is due to the stimulation of guanylate cyclase and impaired activity of this enzyme is not likely to be the operating mechanism for nitrate tolerance. It is also suggested that the vasodilating action of papaverine is partly dependent on the tissue cGMP level.

Prolonged exposure of blood vessels to high levels of organic nitrates, in-vitro, induces tolerance against the vascular smooth muscle relaxing activity of these drugs. It is generally accepted that tolerance results from reduced biotransformation of organic nitrates (Brien et al 1986; Bennett et al 1989). However, marked desensitization to the vasodilating effect of sodium nitroprusside, an inorganic nitrovasodilator, observed in nitroglycerin-tolerant animals has led some authors to propose that reduced guanylate cyclase activity may also contribute to the development of tolerance (Molina et al 1987; Rapoport et al 1987). Sodium nitroprusside is known to contain a nitric oxide (NO) moiety in its structure which is not spontaneously released in aqueous solution in the absence of light (Marks et al 1991) and the guanylate cyclase-activating property of the drug is thought to result from generation of free NO (Ignarro et al 1981; Rinaldi & Cingolani 1983).

Recently, streptozotocin was shown to elicit relaxation of the rat aorta precontracted by phenylephrine, and this effect has been attributed to the presence of the NO group in the molecule (Thomas & Ramwell 1989). Therefore, it would be reasonable to expect that nitrate tolerance may alter the vasodilating activity of streptozotocin. To test this hypothesis, we conducted the present study by using isosorbide dinitrate (ISDN) as a tolerance-inducing agent in rat aorta. Papaverine was also used to assess the influence of nitrate tolerance on guanylate cyclase-independent vasodilation.

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Materials and methods

Male rats, 250–300 g, were killed by stunning and bleeding. The thoracic aortas were removed and dissected free of fat and connective tissue. Helically cut strips, approximately 2 mm wide and 25–30 mm long were suspended in 10 mL organ baths containing modified Krebs solution at 37°C and were aerated with 95% O_2–5% CO_2, The composition of the Krebs solution was (mM): NaCl 118·2, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2, $NaHCO_3$ 25, glucose 11·1. The aortic strips were attached to an Ugo Basile 7004 force-displacement transducer connected to an Ugo Basile 7070 recorder and were allowed to equilibrate under a resting tension of 1 g for 1·5 h, during which time the tissues were washed every 15 min. Concentration-response curves for relaxant drugs were constructed on strips precontracted with 10^{-7} M phenylephrine. In cumulative concentration-response curves on rat aortic strips, 10^{-7} M phenylephrine produced 60–80% of the maximum attainable contraction. The endothelium integrity was verified by testing the relaxant responses to 10^{-6} M acetylcholine. Strips which failed to respond to acetylcholine were discarded.

After obtaining preincubation concentration-response curves with the relaxant under examination, tolerance was induced by incubating the aortic strips with ISDN at a concentration of 6×10^{-4} M for 2 h. Control strips were incubated with vehicle. After exposure to the tolerance-inducing conditions, strips were washed repeatedly with Krebs solution for 15 min and then exposed to 10^{-7} M phenylephrine. A plateau level of contraction was obtained within 10 min and a single cumulative concentration-effect curve constructed for the vasodilator.

In another series of experiments, concentration-response curves of ISDN, streptozotocin and papaverine were constructed before and after incubation of the aortic strips with methylene blue (5×10^{-6} M) for 30 min.

Vasodilator-induced relaxation was expressed as percentage of reversal of the phenylephrine-induced contraction. Vasodilator potency (EC50) was defined as the concentration required to cause 50% relaxation.

The data are expressed as the means ± s.e. Statistical differences between two means were determined by the Wilcoxon test.

Drugs. Phenylephrine, acetylcholine, streptozotocin, methylene blue and papaverine were dissolved in distilled water. ISDN was dissolved in dimethylsulphoxide and dilutions were made in distilled water. All drugs were purchased from Sigma Chemical Company.

Table 1. Comparative responsiveness of ISDN-tolerant and control rat aortic strips to selected vasodilators. ISDN-tolerant strips were pre-exposed for 2 h to 6×10^{-4} M ISDN. Values shown are the means (μM) for 5–7 experiments. Numbers in parentheses are 95% confidence limits.

	EC50		Ratio
Vasodilator	Control	Tolerant	(tolerant/control)
ISDN	1·75 (0·86–2·64)	7·26 (2·86–11·66)	3·90* (1·68–6·12)
Streptozotocin	8·24 (5·82–10·66)	11·70 (4·48–18·92)	1·41 (0·29–2·51)
Papaverine	0·99 (0·49–1·50)	0·50 (0·23–0·78)	0·53* (0·25–0·81)

*$P < 0.05$.

Results

The response of the rat aortic strips to 10^{-7} M phenylephrine was not significantly different before and after incubation with either ISDN (0·68 ± 0·12 vs 0·74 ± 0·07 g, n = 18) or vehicle (0·66 ± 0·11 vs 0·70 ± 0·08 g, n = 12). In addition, no tension increment was observed during either of the incubation conditions.

Incubation of strips of rat thoracic aorta with 6×10^{-4} M ISDN for 2 h, induced a significant tolerance to the relaxant effect of ISDN ($P < 0.05$) (Fig. 1A). The ISDN concentration-response curve for ISDN-incubated aortic strips was shifted approximately 4-fold to the right compared with vehicle-incubated control strips (Table 1). Pretreatment of the aortic strips with 5×10^{-6} M methylene blue also shifted the concentration-response curve of ISDN to the right (Fig. 1A).

It is evident that the induction of ISDN tolerance had no influence on the relaxant effect of streptozotocin (Fig. 1B). ISDN incubation did not significantly alter streptozotocin-induced relaxation when compared with vehicle-incubated strips (Table 1). However, methylene blue (5×10^{-6} M) treatment caused rightward displacement of the streptozotocin concentration-effect curve (Fig. 1B).

Unexpectedly, ISDN incubation increased the responsiveness of aortic strips to papaverine (Fig. 1C) ($P < 0.05$). ISDN-tolerance produced approximately a 2-fold shift to the left of the papaverine-concentration response curve (Table 1). This leftward displacement was manifest at papaverine concentrations between 3×10^{-7} and 3×10^{-6} M, while no significant change was observed at higher concentrations. In contrast, the effects of the same low concentrations of papaverine were markedly inhibited in the presence of methylene blue with no significant change of those induced by the higher concentrations (Fig. 1C).

Incubation of the aortic strips with methylene blue did not alter the sensitivity to phenylephrine-evoked contractions.

Discussion

There is a considerable amount of evidence indicating that organic nitrates induce vascular smooth muscle relaxation by activation of soluble guanylate cyclase (Axelsson et al 1979; Kukovetz et al 1979; Ignarro et al 1981). In support of this, we have demonstrated that ISDN-induced vascular relaxation was significantly antagonized by methylene blue, an inhibitor of the enzyme, in rat aorta. Methylene blue also diminished the relaxant responses to streptozotocin, thus indicating that streptozotocin too may exert its effect by activating guanylate cyclase. This is in good agreement with the study of Vesely et al (1977) who have shown that streptozotocin is a powerful activator of guanylate cyclase in many rat tissues. However, ISDN tolerance was not associated with an attenuation of vascular responsiveness to the vasodilator action of streptozotocin. Our results do

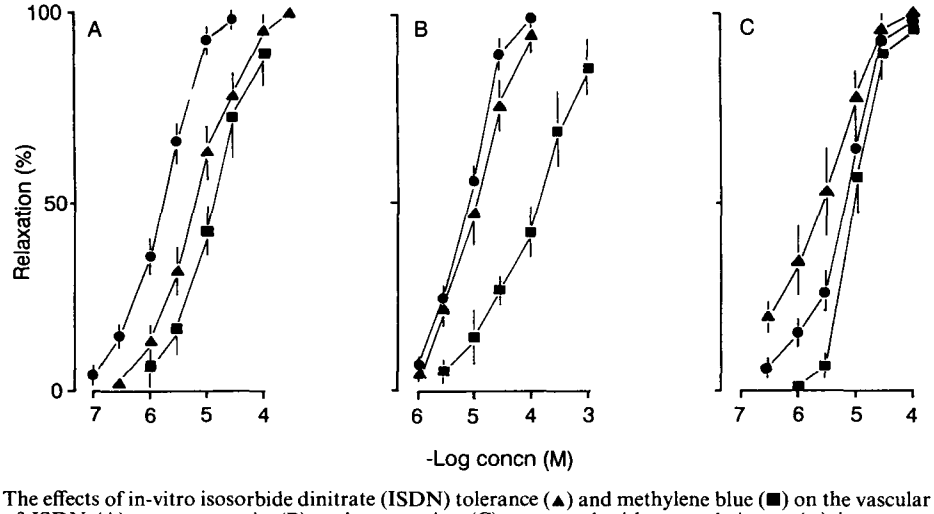

FIG. 1. The effects of in-vitro isosorbide dinitrate (ISDN) tolerance (▲) and methylene blue (■) on the vascular relaxant effects of ISDN (A), streptozotocin (B) and papaverine (C) compared with control tissues (●) in rat aortic strips precontracted by phenylephrine (10^{-7} M). Each curve represents the mean ± s.e. for 5–7 experiments.

not show whether streptozotocin indeed releases NO under the present conditions, although Thomas & Ramwell (1989) have stated that streptozotocin gives chemical reactions characteristic of NO/NO_2^-. It can also be argued that the retained activity of streptozotocin in ISDN-tolerant aorta may be due to the presence of an additional pathway for streptozotocin. However, there is no evidence, at present, that this drug elicits relaxation by a mechanism other than guanylate cyclase activation (Thomas & Ramwell 1989). On the other hand, in our preliminary experiments we observed that ISDN-tolerant rat aortic strips remained fully responsive to the relaxant effect of sodium nitroprusside (data not shown), another NO-containing compound, which is in agreement with several reports (Keith et al 1982; Kowaluk et al 1987). Therefore, we suggest that streptozotocin relaxes vascular smooth muscle by a mechanism similar to that of sodium nitroprusside, where initial site of action of both drugs is probably distinct from that of organic nitrates, as suggested by Henry et al (1989). Furthermore, the lack of cross-tolerance between ISDN and streptozotocin appears to be consistent with the proposal of reduced metabolic activation of organic nitrates (Brien et al 1986; Fung & Polizczuk 1986; Bennett et al 1989), rather than guanylate cyclase impairment, as a mechanism for ISDN tolerance.

In the current study, tolerance-inducing conditions with ISDN increased the sensitivity of rat aortic strips to the relaxant effects of low concentrations of papaverine. It has been shown that sodium nitroprusside enhances isoprenaline-induced relaxation of rat aorta (Maurice & Haslam 1990) and this synergistic interaction between nitrovasodilators and activators of adenylyl cyclase is attributed to the ability of cGMP to increase the accumulation of cAMP by inhibiting a specific cGMP-inhibited cAMP phosphodiesterase (Maurice et al 1991). This seems likely to be the operating mechanism for the interaction between ISDN and papaverine observed in our experiments since Keith et al (1982) have shown increased basal levels of cGMP in nitroglycerin-tolerant rat aortas. The effects of higher concentrations of papaverine ($> 10^{-5}$ M) were not affected by incubation either with methylene blue or ISDN, indicating that additional mechanisms may be involved in the vasodilatory action of the drug.

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