Effects of Chronic Oral Administration of Isosorbide Dinitrate on In Vitro Contractility of Rat Arterial Smooth Muscle

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ABSTRACT—In this study, we examined the effects of in vitro and in vivo treatment with isosorbide dinitrate (ISDN) on the in vitro response of isolated rat aorta. The in vitro treatment of isolated aorta with ISDN (100 μM) for 2 hr had no effect on the ISDN-induced relaxation of norepinephrine-induced contraction. In the aorta isolated from the rats treated with a high dose (90 mg/kg) of ISDN for 7–14 days, in contrast, the relaxant effect of ISDN was significantly reduced. However, the relaxant effect of sodium nitroprusside was only slightly attenuated by the treatment with a high dose of ISDN for 14 days; and the relaxant effects of 8-bromo-cGMP, levocromakalim and verapamil were unchanged. These results suggest that tolerance to ISDN was obtained only after the in vivo chronic treatment with a high dose of ISDN. ISDN may desensitize the nitric oxide-generating step rather than inactivate guanylate cyclase or the downstream pathways.

Keywords: Isosorbide dinitrate, Aorta (rat), Relaxation, Chronic treatment

Isosorbide dinitrate (ISDN), one of the organic nitrates, was developed during a search for longer-acting nitro-compounds (1). This compound is widely used for the management of myocardial ischemia, anginal pectoris and congestive heart failure (2–4). Organic nitrates, such as ISDN and nitroglycerin, induce vascular smooth muscle relaxation through a pathway involving the biotransformation from nitrate to nitric oxide (NO), activation of soluble guanylate cyclase, elevation of intracellular cGMP (5, 6) and activation of cGMP-dependent protein kinase (G-kinase) (7). The activated cGMP/G-kinase system hyperpolarizes the membrane by activating K+ channels (8), activates the Ca2+ pump (9), inhibits Ca2+ channels (10), reduces the Ca2+-sensitivity of the contractile element (11) or reduces the turnover of phosphatidylinositol (12) to cause vascular relaxation.

Chronic administration of organic nitrates leads to development of tolerance, which is one of the major limitations to their therapeutic use (13–15). Moreover, exposure of isolated blood vessels to high levels of organic nitrates also induces tolerance against the relaxing activity of these drugs (16–18). Although the mechanism for the development of tolerance to nitrate vasodilators is not fully known (19), at least three mechanisms have been proposed: 1) decrease in biotransformation for NO production or sulfhydryl depletion (20, 21), 2) diminution of guanylate cyclase activity (22, 23), and 3) increment of phosphodiesterase-mediated breakdown of cGMP (24, 25).

There are several reports concerning the effect of in vivo chronic treatment with nitro-compounds on in vivo responses of the blood vessels. For example, Molina et al. (26) reported that subcutaneous injection of nitroglycerin (200 mg/kg/day for 4–7 days) in rats is associated with tolerance not only to nitroglycerin itself but also to other nitrovasodilators such as sodium nitroprusside and the endothelium-dependent vasodilators. Berkenboom et al. (27) found that the same treatment but at lower dosage (30 mg/kg/day for 4 days) decreases only the responses to nitroglycerin. However, there are few reports concerning the effects of chronic administration with nitro-vasodilator on the responses of isolated blood vessels.

The purpose of the present study was to examine the effect of chronic oral administration of ISDN on the contractility of isolated rat aorta. It was found that chronic oral administration with ISDN selectively inhibits the NO-generation step from ISDN.

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MATERIAL AND METHODS

Experimental protocols
The 8-week-old male Wistar rats (Nisseizai, Tokyo) were divided into the non-treated control group, vehicle-control group and ISDN-treated group. Lactose (45 mg/kg in 5% gum arabic solution) and ISDN (1 and 30 mg/kg) were given orally to the vehicle-control and ISDN-treated groups, respectively, 3 times a day at 9:00 a.m., 15:00 p.m. and 21:00 p.m. every day for 3–14 days. All rats received commercial rat diet; food and water were both available ad libitum. Approximately 12 hr after the last administration, rats were killed by stunning at the neck and bleeding. The thoracic aorta was then quickly removed and used for measurement of muscle tension.

Measurement of muscle tension
The isolated aorta was cut into rings (2- to 3-mm-wide) and placed in a normal physiological salt solution that contained: 136.9 mM NaCl, 5.4 mM KCl, 1.5 mM CaCl₂, 1.0 mM MgCl₂, 23.8 mM NaHCO₃, 5.5 mM glucose and 0.01 mM ethylene diamine tetraacetic acid. The endothelium was removed by gently rubbing the intimal surface of the aortic ring with the tip of a forceps moistened with normal solution. High K⁺ solution was made by substituting NaCl with equimolar KCl. Solutions were saturated with a mixture of 95% O₂ and 5% CO₂ at 37°C to maintain the pH at 7.4. Muscle tension was recorded isometrically with a force displacement transducer. Each muscle ring was attached to a holder under a resting tension of 10 mN. After an equilibration for 30 min in a 2-ml muscle bath, each strip was repeatedly exposed to high K⁺ (72.7 mM) solution until the contractile response to high K⁺ solution became stable. In some experiments, muscle rings were blotted with filter paper and weighed on an analytical balance at the end of the experiments. The contractile responses to norepinephrine were expressed as mN/mg wet tissue weight.

Chemicals
Chemicals used were ISDN, 8-bromo-cGMP, carbachol hydrochloride, verapamil hydrochloride (Sigma Chemicals, St. Louis, MO, USA), sodium nitroprusside, norepinephrine bitartrate, gum arabia (Wako Pure Chemicals, Tokyo) and levcromakalim (kindly donated by Smith Kline Beecham Pharmaceutical Co., Surrey, UK). ISDN and levcromakalim were dissolved in dimethyl sulfoxide and 70% ethanol, respectively, and then diluted to the desired concentration with distilled water. Other drugs were dissolved and diluted with distilled water.

Statistics
The results of the experiments are each expressed as the mean±S.E.M. Student’s t-test and analysis of variance (ANOVA, when comparison involved more than two groups) were used for the statistical analysis of the data. A P value less than 0.05 was considered to be significant.

RESULTS

Effects of ISDN treatment of isolated aorta on relaxation
In isolated rat aorta, cumulative addition of various concentrations of ISDN (100 nM–100 μM) inhibited the contraction induced by norepinephrine (100 nM) in a concentration-dependent manner. Following the treatment of muscle with ISDN (100 μM) for 2 hr and subsequent washout with normal solution for 1 hr, addition of 100 nM norepinephrine induced a contraction with similar magnitude as that before the ISDN-treatment. Cumulative addition of ISDN induced relaxation, and the relaxant effect of ISDN was unchanged (Fig. 1).

Effects of chronic oral administration of ISDN on relaxation of isolated aorta
The effects of chronic oral administration of a moderate dose (3 mg/kg/day) or a high dose (90 mg/kg/day) of ISDN on the response of isolated aorta to ISDN itself were studied. As shown in Fig. 2A, treatment with 90 mg ISDN for 3 days did not change the concentration-response curve for the relaxant effect of ISDN on norepinephrine (100 nM)-induced contraction. After treatment with 90 mg/kg/day for 7 days, in contrast, the effect of ISDN on norepinephrine-induced contraction was slightly decreased (IC₅₀ (−log M): 5.9±0.16, n=4, for vehicle-control and 5.5±0.04, n=4, for ISDN-treatment, P<0.05) (Fig. 2B). Treatment with 90 mg/kg/day for 14 days significantly shifted the concentration-response curve for ISDN to the right (IC₅₀ (−log M): 6.0±0.03, n=6, for vehicle-control and 5.5±0.1, n=5, for ISDN-treatment, P<0.01) (Fig. 2C). On the other hand, treatment with a moderate concentration of ISDN (3 mg/kg/day) for 14 days had no effect on the ISDN-induced relaxation of norepinephrine-induced contraction (Fig. 2C).

Effects of chronic oral administration of ISDN on relaxation of isolated aorta induced by other vasodilators
The effects of chronic administration with ISDN (90 mg/kg/day) for 14 days on the response of isolated aorta to sodium nitroprusside, 8-bromo-cGMP, levcromakalim and verapamil were studied. As shown in Fig. 3A, the concentration-response curve for sodium nitroprusside on norepinephrine (100 nM)-induced contraction was only slightly shifted to the right by the ISDN-treatment.
for 14 days (IC$_{50}$ (-log M): 8.4±0.04, n=6, for vehicle-control and 8.2±0.1, n=6, for ISDN-treatment, P<0.05). In contrast, treatment with ISDN (90 mg/kg/day) for 14 days did not change the concen-

Fig. 1. Effects of in vitro treatment with ISDN on the subsequent relaxation to ISDN in isolated rat aorta. A: Muscles had been pretreated with ISDN (100 μM) for 2 hr. After exposure to ISDN, strips were washed repeatedly with normal PSS for 1 hr and then exposed to norepinephrine (100 nM). After the contraction reached a plateau, ISDN (0.1–100 μM) was cumulatively added. B: Concentration-response relationship for the inhibitory effect of ISDN on norepinephrine-induced contraction (■: control, □: ISDN-treated). Results are each expressed as the mean±S.E.M. of 5–6 experiments.

Fig. 2. Effects of chronic treatment with ISDN on ISDN-induced relaxation in vitro (A: 90 mg/kg for 3 days, B: 90 mg/kg for 7 days, C: 3 and 90 mg/kg for 14 days; p.o.). After the norepinephrine (100 nM)-induced contraction reached a steady state, ISDN (0.1–100 μM) was cumulatively added. ■: Aorta isolated from vehicle-treated rat, □: Aorta isolated from ISDN (90 mg/kg)-treated rat, ○: Aorta isolated from ISDN (3 mg/kg)-treated rat. Results are each expressed as the mean±S.E.M. of 4–6 experiments. *,**: Significantly different from the control with P<0.05 and P<0.01, respectively (Student's t-test for panels A and B and ANOVA for panel C).
tration-response curve for 8-bromo-cGMP (concentration which inhibits contraction by 50\% \((-\log M)\): 3.7±0.07, \(n=5\), for vehicle-control and 3.6±0.03, \(n=6\), for ISDN-treatment) (Fig. 3B).

As shown in Fig. 4 (A and B), the concentration-response curves for levocromakalim (10 nM - 1 \(\mu\)M) (concentration that inhibits contraction by 50\% \((-\log M)\): 6.8±0.2, \(n=4\)) and verapamil (10 nM - 10 \(\mu\)M) (concentration that inhibits contraction by 50\% \((-\log M)\): 5.8±0.2, \(n=6\)) were also unaffected by the chronic ISDN (90 mg/kg/day)-treatment (6.9±0.1, \(n=4\), for levocromakalim and 6.1±0.08, \(n=6\), for verapamil).

Effects of chronic oral administration of ISDN on contraction of isolated aorta induced by norepinephrine

The effects of chronic administration with ISDN (90 mg/kg/day) for 14 days on the contractile response of isolated aorta to norepinephrine were studied. Cumula-

Fig. 3. Effects of chronic treatment with ISDN (90 mg/kg/day, p.o., 3 times a day for 14 days) on in vitro relaxation of norepinephrine-induced contraction induced by sodium nitroprusside (\(n=6\)) (A) and 8-bromo-cGMP (\(n=6\)) (B). After the norepinephrine (100 nM)-induced contraction reached a steady state, sodium nitroprusside (0.1-300 nM) and 8-bromo-cGMP (10-300 \(\mu\)M) were cumulatively added. □: Aorta isolated from vehicle-treated rat, ■: Aorta isolated from ISDN-treated rat. Results are each expressed as the mean±S.E.M. *: Significantly different from the control with \(P<0.05\) and \(P<0.01\), respectively (Student's t-test).

Fig. 4. Effects of chronic treatment with ISDN (90 mg/kg/day, p.o., 3 times a day for 14 days) on in vitro relaxation of norepinephrine-induced contraction induced by levocromakalim (\(n=4\)) (A) and verapamil (\(n=6\)) (B). After the norepinephrine (100 nM)-induced contraction reached a steady state, levocromakalim (0.1-300 nM) and verapamil (10 nM - 10 \(\mu\)M) were cumulatively added. ■: Aorta isolated from vehicle-treated rat, □: Aorta isolated from ISDN-treated rat. Results are each expressed as the mean±S.E.M.
tive addition of norepinephrine (0.1 nM–1 μM) induced contractions in a concentration-dependent manner in either vehicle-treated or ISDN-treated aortic rings. Treatment with ISDN did not change the contractile responses to norepinephrine (EC50 (-log M): 8.6±0.02, n=6, for vehicle-control and 8.6±0.05, n=5, for ISDN-treatment) (Fig. 5). Moreover, the maximum contraction (expressed as mN/mg wet weight tissue) induced by norepinephrine was not affected by the ISDN-treatment (9.9±1.0 mN for vehicle-control and 9.5±1.4 mN for ISDN-treatment).

DISCUSSION

It has been reported that treatment with nitroglycerin for 1 hr induced marked desensitization to the vasodilating effect of nitroglycerin in vitro. This effect is considered to be due to reduced sulfhydryl availability (16, 28, 29). In contrast, treatment with 100 μM ISDN, the maximum concentration soluble in water, for 2 hr did not cause desensitization. These results are consistent with the observation that among the nitrates tested, ISDN induced the least tolerance (20).

Treatment with moderate dose of ISDN (3 mg/kg/day) for 14 days and a high dose of ISDN (90 mg/kg/day) for 3 days did not change the relaxant effects of ISDN itself on norepinephrine-induced contraction in isolated aorta. However, treatment with a high dose of ISDN (90 mg/kg/day) for 7–14 days reduced the ISDN-induced relaxation. Moreover, treatment with the high dose of ISDN for 14 days only slightly decreased the relaxant responses to sodium nitroprusside. The inorganic nitrates, such as sodium nitroprusside, spontaneously release NO (30–32), while the organic nitrates, such as ISDN, must be enzymatically biotransformed for the production of NO (6, 33, 34). Results that the chronic ISDN-treatment more strongly attenuated the ISDN-induced relaxation than sodium nitroprusside-induced relaxation suggest that the chronic oral administration of ISDN causes desensitization mainly at the NO-generating step.

In contrast to the results with ISDN, the relaxant effect of 8-bromo-cGMP, which directly activates the cGMP-dependent kinase (35), was not inhibited by the chronic ISDN-treatment. Furthermore, the relaxant effect of sodium nitroprusside was only slightly inhibited by the chronic ISDN-treatment as compared with the effect of ISDN itself. These results suggest that the down-stream pathways after the NO-generation is not desensitized; i.e., the activities of guanylate cyclase, cGMP production and cGMP-mediated phosphorylation steps may be intact. These results are consistent with the previous observation showing that the relaxant effect of 8-bromo-cGMP in the isolated blood vessels was not adversely affected by chronic oral administration of nitroglycerin for 4–7 days (26). Furthermore, the present study showed that the chronic treatment with a high dose of ISDN did not change the inhibitory effects of levromakalim, a K+ channel opener, and verapamil, an L-type Ca2+ channel blocker in isolated aorta, suggesting that the chronic ISDN-treatment does not affect the relaxation mediated through the K+ channel opening or Ca2+ channel blocking. Recently, we have observed that chronic treatment of rats with a high dose of levromakalim not only inhibited the relaxation induced by levromakalim but also augmented the contractility to norepinephrine and high K+ in the isolated aorta (36). In contrast, the present study showed that the contractile response to norepinephrine was not changed after the ISDN-treatment. These results suggest that the contractility of vascular smooth muscle is not changed after the ISDN-treatment and the mechanism of tolerance occurred mainly at the NO-generating step.

In summary, the results of the present study showed that the aortic strips isolated from the rats administrated a high dose of ISDN for 7–14 days exhibited desensitization to ISDN but not to other vasodilators. It is suggested that the tolerance is mainly due to the inhibition of NO-generation from ISDN.

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REFERENCES


35. Schultz KD, Bohme E, Kreye VAW and Schulz G: Relaxation of hormonally stimulated smooth muscular tissues by the 8-
bromo derivative of cyclic GMP. Naunyn Schmiedeb ergs Arch Pharmacol 306, 1–9 (1979)