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Penile erectile responses to cavernous nerve stimulation in rats are not affected by nitrate tolerance

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Abstract

The aim of this study was to elucidate whether penile erection induced by electrical stimulation of the cavernous nerve was affected in male rats with nitrate tolerance. Nitrate tolerance to nitroglycerin was induced by oral administration of isosorbide dinitrate (ISDN) at 1000 mg/kg to rats, once or twice a day for 5 or 6 days. The rats were anesthetized with sodium pentobarbital 18–24 h after the last dosing with ISDN or its vehicle. Penile erection induced by electrical stimulation was monitored by measuring the penile diameter sonomicrometrically. After measurement of the penile erectile response, nitroglycerin (3–300 μg/kg) was intravenously (i.v.) injected into eight rats treated with the vehicle or ISDN to examine its hypotensive effect. In the vehicle-treated rats, the maximal developed penile diameter (*D*-max) and the duration of penile erection (*T*50%, period of time from the maximum erection to its 50% decline) produced by electrical stimulation were 509±47 μm and 14.2±1.7 s, respectively. On the other hand, neither *D*-max nor *T*50% in ISDN-treated rats (509±36 μm and 13.1±1.3 s, respectively) was different from those in the vehicle-treated rats. However, the hypotensive effects of i.v. injected nitroglycerin were significantly attenuated in the ISDN-treated group as compared with the vehicle-treated group. It is concluded that nitrate tolerance fails to influence penile erection induced by cavernous nerve electrical stimulation in rats.

Keywords: Penile erection; Penile diameter; Cavernous nerve; Electrical stimulation; Nitrate; Tolerance

1. Introduction

Nitrates are used as a remedy for patients with cardiovascular diseases such as angina pectoris and congestive heart failure. However, long-term administration of nitrates can lead to the development of tolerance, indicating that increasing dosages are required to obtain a pharmacological or therapeutic effect on repeated administration (Ahlner et al., 1991). Development of cross-tolerance to nitrates occurs between related compounds such as nitroglycerin and isosorbide dinitrate (ISDN) (Elkayama, 1991). The precise mechanisms responsible for tolerance to nitrates remain unknown. However, there is substantial evidence to suggest that the tolerance may be associated with a reduction of the content, activation, and vascular bioavailability of nitric oxide (NO) released from parent compounds (Elkayama, 1991; Münzel et al., 2000).

In the mechanism of penile erection, an NO–cyclic guanosine monophosphate (cGMP) pathway plays a critical role in modulating corpus cavernosum smooth muscle relaxation (Bowman and Drummond, 1984; Bush et al., 1992). This finding prompted the current study of the penile erectile response under conditions of nitrate tolerance. However, it is not known whether the penile erectile response in vivo is altered by ISDN, and if cross-tolerance exists between endogenous NO and exogenous nitroglycerin. To date, probably due to technical difficulties with experimental procedures, little information on these issues is available regarding animal experiments except for one in vitro study (Uma et al., 1998).

The objective of the present study was to elucidate whether the penile erection induced by electrical stimulation of the cavernous nerve was affected in male rats with nitrate tolerance. Penile diameter was measured sonomicrometrically with a pair of 10-MHz piezoelectric crystals glued to the opposite surfaces of the adventitia of the penile erectile chamber as reported previously (Adachi et al., 1999). In the present study, hypotensive tolerance to nitrate was induced by oral administration of ISDN to rats for 5 or 6 consecutive

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days, and confirmed by the reduction of the hypotension induced by intravenous (i.v.) injections of nitroglycerin in multiple doses.

2. Materials and methods

All animal procedures were conducted in accordance with company guidelines for animal experimentation (Eisai Research Laboratories, Ibaraki, Japan).

This study used 16 male Sprague–Dawley rats (Charles River Japan, Kanagawa, Japan), weighing 425–502 g. The animals were housed in a temperature (23 °C, permissible limit: 20–26 °C)- and moisture (55%, permissible limit: 40–70%)-controlled room with a 12-h light/dark cycle (lights on at 7:00 AM, lights off at 7:00 PM), and acclimatized for more than 1 week. The animals were fed a commercial diet (MF rat diet, Oriental Yeast, Tokyo, Japan), and tap water was freely available.

2.1. Establishment of isosorbide dinitrate tolerance in vivo

The animals were divided into two groups: ISDN-treated group (n=8) and vehicle-treated group (n=8). Hypotensive tolerance to a nitrate was induced by oral administration of extremely high doses of ISDN (1000 mg/kg, once a day on Saturday and Sunday, and otherwise twice a day; Eisai, Tokyo, Japan) for 5 or 6 consecutive days. This regimen was adopted based on the results of the preliminary experiment. ISDN (Sigma, St. Louis, MO, USA) was suspended in a 0.5% methyl cellulose solution to make a concentration of 200 mg/ml. The control animals were treated in the same manner with an equivalent volume (5 ml/kg).

2.2. Penile erection induced by electrical stimulation and hypotension induced by nitroglycerin

Rats fasted overnight were anesthetized with sodium pentobarbital (55 mg/kg, intraperitoneally; Abbott Laboratories, North Chicago, IL, USA) 18–24 h after the last dosing with ISDN or its vehicle. The animals were placed in a supine position on a heat-insulated pad and warmed with a heating lamp to prevent hypothermia during the course of the experiment. The right carotid artery and the right jugular vein were exposed in the neck and cannulated with two polyethylene catheters (internal diameter: 0.58 mm; Becton Dickinson, Sparks, MD, USA) for the measurement of systolic and diastolic arterial pressure and the injection of vehicle or nitroglycerin, respectively.

The operation for induction of penile erection and the measurement of rat penile diameter were carried out according to a method described previously (Adachi et al., 1999). A midline incision was made from the umbilicus to the pubis. The rat penis was denuded of skin, the prepuce was circumcised, and the testes were retracted. The scrotum was divided and packed into the upper abdomen along with loops of bowel. The right cavernous nerve was carefully isolated and hooked with a bipolar platinum electrode (MS-001; Unique Medical, Tokyo, Japan) connected to a nerve stimulator (SEN-7103; Nihon Kohden, Tokyo, Japan). A pair of 10-MHz piezoelectric crystals (Murata, Kyoto, Japan) was glued to the opposite surfaces of the adventitia of the penile erectile chamber (the shaft of the corpus cavernosum penis) using a tissue adhesive agent (Vetbond™; 3M, St. Paul, MN, USA).

The penile diameter was measured sonomicrometrically and was used as an index of the penile erection induced by cavernous nerve electrical stimulation. Unilateral electrical stimulation of the cavernous nerve was applied for 30 s using a square wave stimulator. The electrical stimulation voltage was 10 V. The parameters of electrical stimulation were a frequency of 20 Hz and a duration of 2 ms. The penile diameter was measured with an ultrasonic dimension system (UDM-5C; MECC, Fukuoka, Japan), and recorded simultaneously on a multichannel pen-recorder (Polygraph System; Nihon Kohden). As shown in Fig. 1, erectile responses were characterized by the following parameters: D-max, which was defined as the maximal developed penile diameter induced by electrical stimulation; and T50%, which was defined as the period of time from the maximum erection (D-max) to its 50% decline.

After hemodynamic conditions had stabilized, hemodynamic variables such as heart rate and systolic and diastolic arterial pressures were also simultaneously recorded on a multichannel pen-recorder. Mean arterial pressure was calculated as the sum of the diastolic pressure plus 1/3 of the pulse pressure. Heart rate was monitored with a cardiotachometer triggered by the pressure pulse.

The study design for the present experiment was divided into two protocols. In protocol 1, the penile erectile response to cavernous nerve electrical stimulation was examined in the vehicle- and ISDN-treated groups (n=8; for both groups). After recovery of the erectile response, protocol 2 was performed to examine the hypotensive effect of nitro-

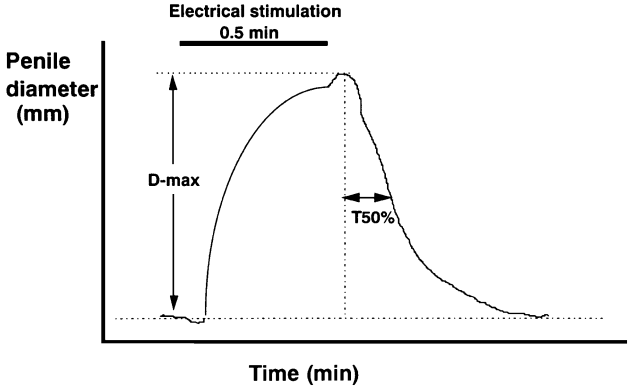

Fig. 1. Parameter values calculated from penile erectile response to cavernous nerve electrical stimulation. D-max, maximal developed penile diameter induced by the stimulation; T50%, period of time from the maximum erection to a 50% decline from the maximum.

glycerin. Nitroglycerin (Nihon Kayaku, Tokyo, Japan) at doses of 3, 10, 30, 100, and 300 μg/kg was incrementally injected i.v. over 15 s via the right jugular vein followed by flushing with saline 0.2 ml. The next injection was conducted at a 5-min interval or after the animals had recovered from the hemodynamic changes induced by the previous dose of nitroglycerin.

2.3. Statistical analysis

All data are expressed as means±S.E.M. The values for erectile response parameters and hemodynamics in the ISDN-treated group were compared with those obtained in the vehicle-treated group, using an unpaired Student's *t*-test. *P* values of less than 0.05 (two-sided) were considered significant. Statistical analysis was conducted using the software package, SAS 6.12 (SAS Institute Japan, Tokyo, Japan).

3. Results

3.1. Erectile response to electrical stimulation in vehicle- and ISDN-treated groups

The baseline values of penile diameters of rats before erection were 3.371±0.075 and 3.269±0.116 mm in the vehicle- and ISDN-treated groups, respectively, as summarized in Table 1. Representative tracings of penile erection produced by electrical stimulation in a vehicle-treated rat and an ISDN-treated rat are shown in Fig. 2A and B, respectively. Cavernous nerve electrical stimulation caused a marked increase in the penile diameter in the ISDN-treated rat, as well as in that of the vehicle-treated rat. In the vehicle-treated group, the *D*-max and *T*50% produced by electrical stimulation were 509±47 μm (*n*=8) and 14.2±1.7 s (*n*=8), respectively. On the other hand, in the ISDN-treated group, the *D*-max and *T*50% produced by electrical stimulation were 509±36 μm (*n*=8) and 13.1±1.3 s (*n*=8), respectively. Neither *D*-max nor *T*50% was significantly different between the two groups.

Table 1
Penile erectile response to cavernous nerve electrical stimulation in the vehicle- and isosorbide dinitrate-treated groups

	Vehicle (*n*=8)	Isosorbide dinitrate (*n*=8)
Baseline penile diameter (mm)	3.371±0.075	3.269±0.116
D-max (μm)	509±47	509±36
*T*50% (s)	14.2±1.7	13.1±1.3

All values are the means±S.E.M. for eight animals. *D*-max, maximal developed penile diameter; *T*50%, period of time from maximum response to a 50% decline of the maximum response. The values for the two parameters were calculated from the penile erectile response to cavernous nerve electrical stimulation performed before the injection of nitroglycerin. Neither parameter was significantly different between the two groups.

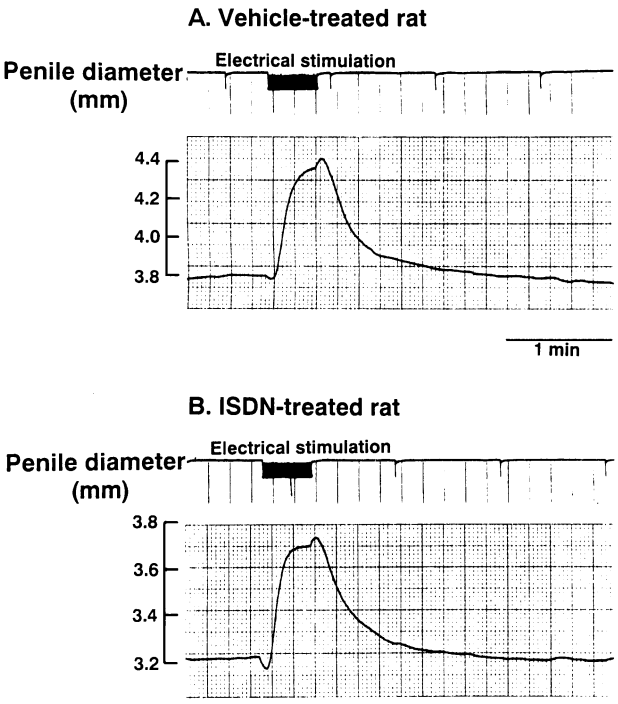

Fig. 2. Representative tracings of penile erectile response to cavernous nerve electrical stimulation obtained from (A) a vehicle- or (B) isosorbide dinitrate-treated rat. Electrical stimulation was applied for 30 s at the time indicated by the black bar.

3.2. Hypotensive effect of nitroglycerin in vehicle- and ISDN-treated groups

The baseline values of heart rate or mean arterial pressure in the vehicle- and ISDN-treated groups are shown in Table 2.

In the vehicle-treated group, i.v. injection of nitroglycerin at 3–300 μg/kg decreased the mean blood pressure in a dose-dependent manner. The maximum hypotensive response to nitroglycerin at 30 μg/kg was 53±5 mm Hg (Fig. 3). On the other hand, in the nitrate tolerant group, the depressor effect of nitroglycerin at doses of 3, 10, 30, and 100 μg/kg was attenuated significantly as compared with that in the vehicle-treated group (Fig. 3). However, the hypotensive effect of nitroglycerin at 300 μg/kg was restored to the level of that in the vehicle-treated group and was not statistically significant. The lessened hypotension induced by i.v. injection of nitroglycerin in the ISDN-

Table 2
Baseline values of heart rate and mean arterial pressure before nitroglycerin injection in the vehicle- and isosorbide dinitrate-treated groups

	Vehicle (*n*=8)	Isosorbide dinitrate (*n*=8)
Heart rate (beats/min)	363±13	375±15
Mean arterial pressure (mm Hg)	115±4	127±4

All values are the means±S.E.M. for eight animals. Measurements were made at baseline just before the initial injection of nitroglycerin. Neither parameter was significantly different between the two groups.

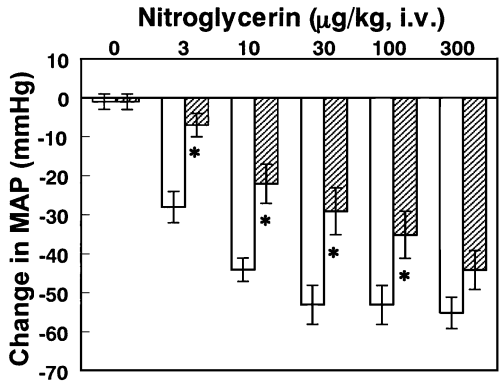

Fig. 3. Acute hypotensive effects of intravenous injection of nitroglycerin in vehicle (white bars)- or isosorbide dinitrate (hatched bars)-treated rats. MAP: mean arterial pressure. All values are the means±S.E.M. for eight animals. *P<0.05 vs. vehicle-treated group.

treated group was evidence for cross-tolerance of nitrate with administration of large amounts of ISDN for 5 or 6 days.

4. Discussion

The major finding of the present study was that the decrease in blood pressure induced by i.v. injection of nitroglycerin was attenuated by long-term treatment with large amounts of ISDN, whereas the penile erection produced by cavernous nerve electrical stimulation was not affected. These results suggest that ISDN-induced tolerance in the systemic circulation may result in an abnormal NO metabolism or NO–cGMP pathway as reported previously by numerous researchers (Needleman, 1970; Fung and Bauer, 1994), but does not lead to impairment in the penile erectile response to cavernous nerve electrical stimulation.

Possible mechanisms for the development of nitrate tolerance are as follows: (1) impaired biotransformation to NO via sulfhydryl groups, (2) desensitization of one or more of the intracellular pathways, such as soluble guanylyl cyclase activated by NO, and (3) superoxide generation by vascular endothelium, relating to NO inactivation (Münzel et al., 1995; De la Lande et al., 1999).

In the present study, we treated rats with ISDN (1000 mg/kg, p.o.) once or twice a day for 5 or 6 days as indicated by the study protocol to evoke nitrate tolerance in the systemic circulation of rats. Large amounts of ISDN (1000 mg/kg) were administered to animals via the oral route as used clinically. After oral administration of ISDN at a lower dose of 2 mg/kg, complete absorption from the gastrointestinal tract occurred, and the absolute bioavailability after oral administration was about 40%. After oral dosing of ISDN, the dose was metabolized mainly to 5-isosorbide mononitrate, a pharmacologically active metabolite, which is also one of the nitrates and circulates in the body (Morrison and Fung, 1984). Therefore, rats treated with ISDN were considered to be subjected to excessive NO released from ISDN and its metabolite. This hypothesis was confirmed by a reduction in the hypotensive response to i.v. injection of nitroglycerin at multiple doses as shown in Fig. 3.

Activation of the peripheral nerves, which innervate the penis, such as nonadrenergic–noncholinergic, cholinergic, vasoactive intestinal peptide- and calcitonin gene-related peptide-containing nerves, seems to play a trigger role in the peripheral mechanism of penile erection (Andersson and Wagner, 1995). NO released from the nonadrenergic–noncholinergic nerve causes dilatation of the helicine arterioles and relaxation of smooth muscle in the corpus cavernosum. Shear stress and acetylcholine receptor activation on the corpus cavernosal endothelium stimulate the production of NO via an increase in intracellular calcium and activation of endothelial NO synthesis. NO diffuses into the smooth muscle and enhances its relaxation (Moreland et al., 2001). In a previous study using the same rat model (Adachi et al., 1999), an NO synthase inhibitor, N^{ω}-nitro-L-arginine methyl ester, significantly depressed the maximum penile diameter (D-max) increase obtained after the termination of electrical stimulation, without changes in its recovery time (T50%). Additionally, a well-known phosphodiesterase 5 inhibitor, zaprinast, prolonged the recovery time. These findings strongly suggest that an NO–cGMP pathway contributes to the mechanism of penile erection, and the animal model used in the present study may be useful for detecting abnormal penile erectile responses to cavernous nerve electrical stimulation when the amount of NO or its availability in the peripheral tissue including nerves and smooth muscle of the corpus cavernosum is reduced.

In the present study, although repeated administration of ISDN caused the development of nitrate tolerance characterized by a reduction in the hypotensive effect of nitroglycerin, penile erection induced by electrical nerve stimulation was unaltered despite sustained exposure of the systemic circulation of rats to ISDN. To date, there is little information on the effects of nitrate tolerance on penile erection. Uma et al. (1998) have previously reported that prolonged exposure of rabbit corpus cavernosum strips to high concentrations of ISDN caused significant desensitization to its relaxant effect, while the strips made tolerant to ISDN relaxed fully in response to electrical field stimulation or sodium nitroprusside. This result suggests that the weakened response to ISDN may be due to an abnormal NO metabolism, which may link to a reduced amount of available NO generated in the penile tissue, without impairment of stimulating soluble guanylate cyclase and then of increased cGMP formation. These present findings provide the first evidence supporting the above hypothesis in a physiological in vivo study of penile erection.

Epidemiological studies suggest an association between impotence and cardiovascular disease, as well as other vascular risk factors including cigarette smoking (Moreland et al., 2001). Nitrate therapy for angina pectoris and heart failure is effective, safe, and economical, and is chronically prescribed. Accordingly, unlike other pharmacological treat-

ments, such as antihypertensive drugs (β-adrenergic blockers), diuretics, and serotonin re-uptake inhibitors (Meinhardt et al., 1997), organic nitrates are a useful remedy in view of avoiding the possibility of male erectile dysfunction.

In conclusion, nitrate tolerance does not influence penile erection induced by electrical stimulation of the cavernous nerve in rats treated with ISDN. There may be a different mechanism related to NO for the hypotension induced by nitroglycerin and for the penile erectile response to electrical nerve stimulation. The present results suggest that desensitization of guanylate cyclase activity is not associated with the operating mechanism for nitrate tolerance, and penile erectile dysfunction does not necessarily occur in animals, including humans, with nitrate tolerance.

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