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Zaprinast, but not dipyridamole, reverses hemodynamic tolerance to nitroglycerin in vivo

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Abstract

Hemodynamic tolerance to nitroglycerin was developed in spontaneously hypertensive rats following 2–3 days of pretreatment with 100 mg/kg of nitroglycerin administered s.c. 3 times/day. Tolerance was evaluated both in vivo, by administering ascending bolus doses of nitroglycerin of 1–300 μg/kg i.v., and ex vivo in isolated, denuded aortic vascular rings by exposure to ascending concentrations of nitroglycerin of 0.0003–100 μM. Tolerance was observed as a significant blunting of the hypotensive and vasorelaxant effect of nitroglycerin. Co-incubation of tolerant aortic rings and pretreatment of tolerant SHR with 10 μM and 0.1–10 mg/kg zaprinast, respectively, resulted in full restoration of the vasorelaxant and hypotensive effect of nitroglycerin. Zaprinast partially reversed hemodynamic tolerance at 0.01 mg/kg. Conversely, dipyridamole (10 μM) reversed tolerance ex vivo, but was ineffective in reversing tolerance in vivo at pretreatment doses of 30 and 60 mg/kg. Following a 100-μg/kg i.v. challenge dose of nitroglycerin, aortic cyclic guanosine monophosphate (cGMP) levels were lower in nitroglycerin tolerant SHR when compared to non-tolerant SHR. Pretreatment of tolerant SHR with 10 mg/kg zaprinast restored the increase in cGMP levels to nitroglycerin to that seen in non-tolerant SHR. Conversely, dipyridamole (30 mg/kg) pretreatment was not effective in restoring cGMP levels. These data therefore suggest that reversal of hemodynamic tolerance in vivo is related to restoration of changes in vascular cGMP levels. Zaprinast, a selective cGMP phosphodiesterase inhibitor, effectively reverses tolerance and dipyridamole, a rather non-selective inhibitor, does not.

Keywords: Nitroglycerin; Tolerance; Phosphodiesterase inhibitor; cGMP monophosphate; Blood pressure; Spontaneously hypertensive rat (SHR); Zaprinast; Dipyridamole

1. Introduction

It is postulated that the mechanism by which organic nitrates are activated is via biotransformation to a reactive nitrosothiol which then activates soluble guanylate cyclase in vascular smooth muscle cells (Kowaluk et al., 1992; Ignarro et al., 1981; Needleman and Johnson, 1973). Intracellular cyclic guanosine monophosphate (cGMP) levels then rise and vasorelaxation ensues (Waldman and Murad, 1987; Ignarro and Kadowitz, 1985). The clinical utility of nitrate vasodilators is well recognized for the treatment of congestive heart failure and coronary vascular diseases (Lopez et al., 1993; Ahlner et al., 1991). As well known as their therapeutic utility is the fact that tolerance can develop rapidly, sometimes in less than 48 h, to continued exposure to nitroglycerin (Ferratini, 1994; Fung, 1993; Harrison and Bates, 1993; Packer, 1990). The mechanism by which vascular tolerance develops is not completely understood. One line of investigation has focused on the depletion of sulfhydryl containing compounds in the vasculature which are postulated to participate in the biotransformation of nitroglycerin to the active nitrosothiol agent (Ignarro et al., 1981). However, equivocal results have been obtained in attempts to reverse nitroglycerin tolerance in the laboratory and clinic with such agents as captopril, *N*-acetylcysteine, and other sulfhydryl donating compounds (Boesgaard et al., 1991a, Boesgaard et al., 1991b; Shaffer et al., 1991, Van Gilst et al., 1991; Hogan et al., 1990; Horowitz et al., 1988). In addition to the biochemical tolerance, hemodynamic tolerance can also develop to the effects of nitroglycerin (Bauer and Fung, 1991).

Regardless of the mechanism of nitroglycerin tolerance, tolerance does appear to be associated with diminished elevations of cGMP in vascular smooth muscle cells in

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response to subsequent nitrate exposure (Pagani et al., 1993; Molina et al., 1987; Axelsson and Andersson, 1983). During nitroglycerin tolerance vasorelaxation to other guanylate cyclase activators remains unaltered (De Garavilla et al., 1993) and the response to nitrate vasodilators is not completely absent during nitrate tolerance (Pagani et al., 1993; Silver et al., 1991). These data suggest that the cGMP-mediated vasorelaxation pathway is intact, and functional, during nitroglycerin tolerance. Thus, elevating or preserving intracellular cGMP levels via alternative mechanism(s) may provide a means to reverse the effects of nitroglycerin tolerance. One viable approach would be to inhibit the cGMP-specific phosphodiesterase, also referred to as phosphodiesterase V (Pagani et al., 1992), in vascular smooth muscle cells and prevent the rapid breakdown of cGMP. It has already been shown that by inhibiting phosphodiesterase V one can potentiate the vasorelaxant effect of guanylate cyclase activators (Merkel et al., 1992; Harris et al., 1989). In previous studies, it has also been shown that the phosphodiesterase V-selective inhibitor zaprinast (also known as M&B 22948) can reverse nitroglycerin tolerance in vitro (Pagani et al., 1993; Merkel et al., 1992; Pagani et al., 1991). This effect appears to be related to the restoration of changes in vascular cGMP content in response to nitrate exposure (Pagani et al., 1993). Moreover, in limited studies in our laboratory, zaprinast was shown to reverse hemodynamic tolerance in vivo (Silver et al., 1991), although, only a single dose of zaprinast was tested and those studies did not include measurement of cGMP. In addition to zaprinast, other non-specific, less potent phosphodiesterase inhibitors like aminophylline and dipyridamole have been shown to potentiate the hypotensive effect of nitroprusside in dogs (Pearl et al., 1984) and reverse nitroglycerin tolerance in isolated strips of human veins (Bohyn et al., 1991), respectively; tissue cGMP was not measured in these studies either.

To date, there has been no confirmation that the reversal of tolerance by zaprinast or any other phosphodiesterase inhibitor in vivo is related to vascular tissue cGMP levels. Therefore, in the present study, zaprinast was evaluated in a model of nitroglycerin tolerance in spontaneously hypertensive rats (SHR) and tissue cGMP levels were quantified in situ. Zaprinast was also evaluated in an ex vivo paradigm using isolated vascular rings. For comparative purposes, dipyridamole was also evaluated since it had been shown to reverse tolerance in vitro (Bohyn et al., 1991).

2. Materials and methods

2.1. Animals and catheter implantation

All animal procedures were conducted in accordance with the recommendations of the Declaration of Helsinki and were approved by the Laboratory Animal Care and Use Committee at Sterling Winthrop Pharmaceuticals Research Division. All animal facilities and programs were accredited by the American Association for Accreditation of Laboratory Animal Care.

Male spontaneously hypertensive rats (17–25 weeks of age, 325–400 g body weight; Blue Spruce Farms, NY, USA) were used in these studies. Under sodium pentobarbital (50 mg/kg i.p.), anesthesia a femoral arterial and venous catheter were implanted as previously described (De Garavilla et al., 1993; Dundore et al., 1991). Following surgery, the rats were allowed at least 3 days recovery before testing.

2.2. Nitroglycerin tolerance and reversal in vivo

Tolerance to the hypotensive effect of nitroglycerin was induced by pretreatment with high doses of nitroglycerin (100 mg/kg s.c., 3 times/day) for 2 or 3 consecutive days. This regimen has been previously used to induce nitroglycerin tolerance in normotensive (Needleman, 1970) and hypertensive rats (De Garavilla et al., 1993; Silver et al., 1991). Nitroglycerin (ICI, Wilmington, DE, USA) was dissolved in a 50% polyethylene glycol 400 and 10% ethanol solution. Control animals were pretreated in the same manner with an equivalent volume (2 ml/kg s.c.) of diluent for 3 days. 18–22 h following the last pretreatment dose of nitroglycerin, hemodynamic tolerance was evaluated. Throughout this study, blood pressure was measured in conscious, freely moving SHR as described below.

Prior to the initiation of the 3-day pretreatment regimen, rats were placed individually in Plexiglas boxes and the baseline blood pressure of each animal was recorded (P53, Gould). Arterial pressure was continuously monitored using a polygraph (Model 7D, Grass Instruments) and mean arterial pressure (MAP) calculated as the sum of the diastolic pressure plus 1/3 the pulse pressure. Following a 30-min acclimation period, three consecutive MAP measurements were taken at 5-min intervals and averaged to give a baseline MAP. The arterial catheter was resealed, and the rats were returned to their cages.

The hypotensive effect of zaprinast and dipyridamole was evaluated in groups of tolerant (3-day pretreatment) and non-tolerant SHR. Zaprinast was administered at cumulative doses of 3, 6, 10, 18 and 30 mg/kg i.v. Dipyridamole was administered at cumulative doses of 10, 30, 60, 100 and 300 mg/kg i.v. These dose ranges were selected in order to achieve similar changes in MAP. The maximum hypotensive response was recorded for each dose. Zaprinast, synthesized at Sterling Winthrop Pharmaceuticals Research Division (Rensselaer, NY, USA), was dissolved in 10% (v/v) 0.5N NaOH and diluted to a final concentration of 10 mg/ml using sterile saline. Dipyridamole (Sigma, St. Louis, MO, USA) was dissolved in 30% (v/v) 0.5 N HCl to a concentration of 60 mg/ml in sterile saline.

In order to evaluate hemodynamic tolerance, bolus doses of nitroglycerin of 1, 10, 30, 100 and 300 μg/kg i.v. (hereafter referred to as challenge doses) were administered in a volume of 0.5 ml/kg. The maximum decrease in MAP was recorded for each dose. Administration of the vehicle (20% (v/v) PEG-400, 10% (v/v) ethanol in sterile water) at the same rate did not significantly affect MAP. Evaluation of tolerance with multiple doses of nitroglycerin took less than 30 min.

In separate groups of SHR that were made tolerant to nitroglycerin (3-day pretreatment), the ability of zaprinast and dipyridamole to reverse tolerance in vivo was evaluated. Rats were placed in the Plexiglas study boxes, and blood pressure was monitored. Following a stabilization period, rats were administered a subthreshold vasodepressor dose of either zaprinast (10 mg/kg i.v.), dipyridamole (60 mg/kg i.v.), or the corresponding vehicle. 5 min later, challenge doses of nitroglycerin (1–300 μg/kg i.v.) were administered and the maximum changes in MAP recorded. In additional groups of rats, zaprinast was tested at lower pretreatment doses ranging from 0.01 to 3 mg/kg, and dipyridamole at 30 mg/kg i.v.

2.3. Measurement of aortic cGMP content

cGMP content of abdominal aortic segments was measured in groups of tolerant and non-tolerant SHR using techniques previously described (Pagani et al., 1993; Dundore et al., 1991). Following 3 days of nitroglycerin pretreatment, rats were placed in the Plexiglas study boxes and administered a 100-μg/kg i.v. challenge dose of nitroglycerin. Within 30 s after administration and after the maximum hypotensive response was recorded, each rat was anesthetized with pentobarbital (25 mg/kg i.v.) and a laparotomy performed. The abdominal aorta was then isolated, ligated proximal to the tip of the femoral arterial catheter and flushed with ice-cold saline via the catheter to clear all the blood. A 2–5-mm segment of the aorta was then frozen in situ using a modified Wollenberger clamp, prechilled in liquid nitrogen. The aortic segment was excised and stored at −80°C. The time delay from the maximum hypotensive response to freezing of the aortic segment was no more than 90 s. cGMP was extracted from the tissue, and levels were measured using a commercially available radioimmunoassay (cGMP[^{125}I]; NEX-133, DuPont NEN Research Products).

2.4. Ex vivo evaluation of tolerance and reversal

In a separate group of naive SHR in which catheters were not surgically implanted, rats were pretreated with either nitroglycerin or vehicle for 3 consecutive days, as described above, to induce tolerance. 18–22 h following the last dose of nitroglycerin, the rats were anesthetized with pentobarbital (50 mg/kg i.p.) and euthanized by exsanguination. The thoracic and abdominal aortae were immediately removed from the animal, rinsed, and the endothelial cell layer gently removed. Carbachol (1 μM) was used to test for the presence of a functional endothelial layer and to confirm that the tissue had been denuded. Aortic rings (2–3 mm in length) were attached to stainless steel ring holders and immersed in 10 ml tissue baths containing a modified Krebs solution (in mM: NaCl, 118; KCl, 4.7; $MgCl_2$, 1.2; KH_2PO_4, 1.2; $CaCl_2$, 1.6; $NaHCO_3$, 21.4; dextrose, 11.1; EDTA, 0.026) pH 7.4 at 37°C. The rings were stretched with a preload of 2 gm, allowed to equilibrate for 75 min, and then precontracted with 1 μM phenylephrine. The force of contraction was similar for all tissues. In some of the aortic rings taken from nitroglycerin-pretreated SHR, either zaprinast (10 μM), dipyridamole (10 μM) or the corresponding vehicle was added to the bath. 10 min later a concentration-response curve was generated for nitroglycerin over a range of 0.0003–100 μM. Data were calculated as percentage relaxation relative to phenylephrine-induced contraction.

2.5. Data analysis

Data are expressed as mean ± S.E. Comparisons within groups were made using an analysis of variance followed by a Dunnett's test. Between group comparisons were made using an analysis of variance followed by a Newman-Keuls test. P values of ≤ 0.05 were considered to be significant.

3. Results

3.1. Inducing nitroglycerin tolerance in vivo

Baseline MAP values before and after a 2- or 3-day pretreatment regimen with nitroglycerin are listed in Table 1. No significant differences were detected between pre- and post-MAP values within each tolerant group. All subsequent data are presented as a change in MAP from the respective baseline value following nitroglycerin pretreatment.

Challenge doses of nitroglycerin caused a decrease in MAP which was rapid in onset and transient. Shown in Fig. 1 are the changes in MAP for groups of vehicle- and nitroglycerin-pretreated rats. In the vehicle-pretreated group, nitroglycerin caused dose-dependent reductions in

Table 1
Baseline mean arterial pressure (MAP, mean ± S.E.) before and after 2 or 3 days of nitroglycerin (100 mg/kg s.c., TID) pretreatment

Treatment group	n	MAP (mm Hg)	
		Pre-	Post-
Vehicle, 3 days (non-tolerant)	11	164 ± 4	168 ± 5
Nitroglycerin, 2 days (tolerant)	6	167 ± 3	158 ± 3
Nitroglycerin, 3 days (tolerant)	8	166 ± 4	166 ± 4

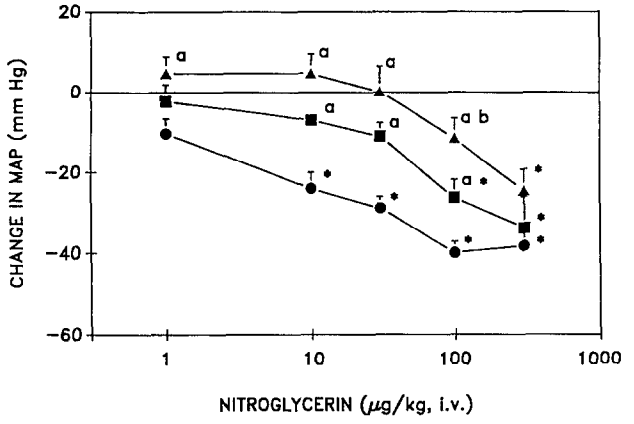

Fig. 1. Acute hypotensive effects of bolus i.v. doses of nitroglycerin in vehicle- (●) and nitroglycerin-pretreated SHR for either 2 (■) or 3 days (▲). Values reflect changes from baseline. * $P \leq 0.05$ vs. baseline value within each group. [a] $P \leq 0.05$ vs. vehicle-pretreated group and [b] $P \leq 0.05$ vs. 2-day pretreated group.

MAP between 10 and 40 mm Hg. In the nitroglycerin-pretreated groups, changes in MAP were significantly blunted at doses of 100 μg/kg and less, as compared to the vehicle-pretreated group. Significant changes in MAP were observed at a dose of 300 μg/kg nitroglycerin for all three groups; however, there was a tendency for the decrease to be less in the two nitroglycerin-pretreated groups.

3.2. Reversal of tolerance ex vivo

As shown in Fig. 2, aortic vascular rings isolated from SHR pretreated with nitroglycerin in vivo exhibited a lesser response to the vasorelaxant effect of nitroglycerin in vitro, as compared to rings isolated from vehicle-pretreated SHR. The biphasic nature of relaxant curve which is typical for tolerant tissues (Pagani et al., 1993; Silver et al., 1991; Malta, 1989) makes it difficult to compare responses. As estimated from the graph, the EC_{50} value to the vasorelaxant effect of nitroglycerin was approximately 3-fold greater for the first phase of the response and 20–50-fold greater for the second phase, as compared to non-tolerant tissues. Pre-incubation of tolerant rings with 10 μM zaprinast (Fig. 2, top) completely restored the vasorelaxant effect of nitroglycerin. Dipyridamole (Fig. 2, bottom), at 10 μM, was partially effective. Pre-incubation

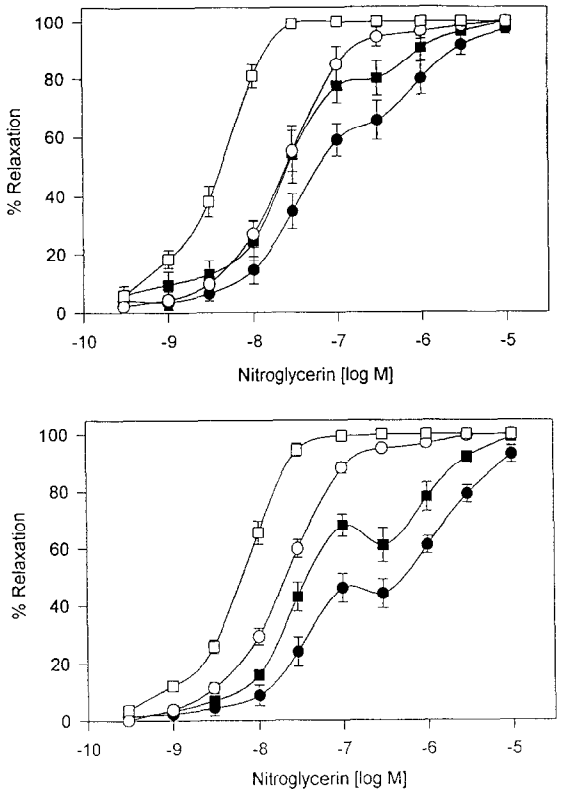

Fig. 2. Reversal of nitroglycerin tolerance ex vivo by zaprinast (top panel) or dipyridamole (bottom panel) in aortic vascular rings isolated from SHR pretreated with nitroglycerin or vehicle for 3 consecutive days. Percentage relaxation to ascending concentrations of nitroglycerin was calculated as a change in tension developed to 1 μM phenylephrine. The response to phenylephrine was similar for all tissues. ○, non-tolerant, vehicle pretreated; □, non-tolerant, zaprinast (10 μM) or dipyridamole (10 μM) pretreated; ●, tolerant, vehicle pretreated; ■, tolerant, zaprinast (10 μM) or dipyridamole (10 μM) pretreated. $n = 6–8$/group.

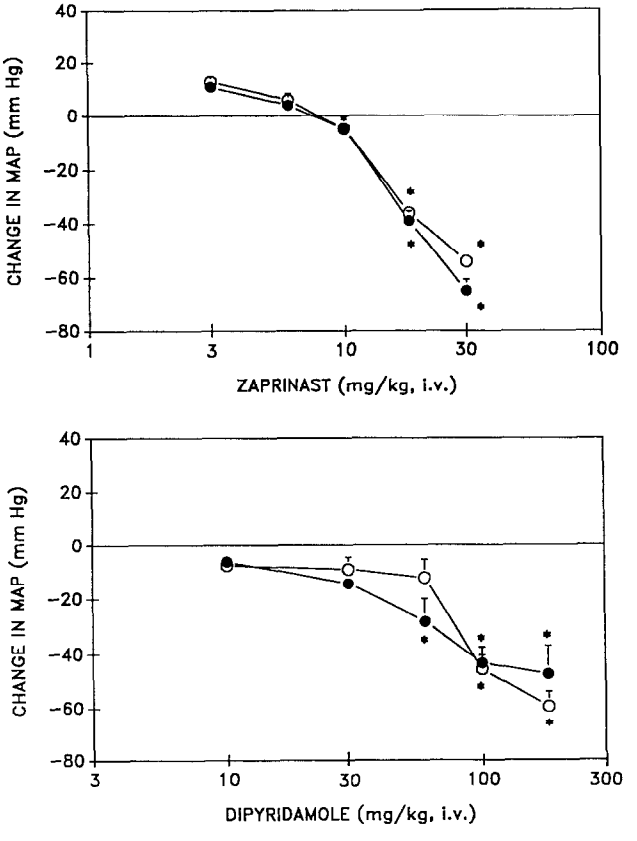

Fig. 3. Changes in mean arterial pressure (MAP) from baseline in response to cumulative doses of zaprinast and dipyridamole in groups of nitroglycerin-tolerant (3-day pretreatment, ○) and -non-tolerant (●) SHR. Maximum threshold doses of 10 mg/kg zaprinast and 60 mg/kg dipyridamole were selected from these data for use as a pretreatment doses in subsequent studies. * $P \leq 0.05$ vs. baseline value within each group. $n = 6–8$/group.

of non-tolerant tissues with zaprinast potentiated the response to nitroglycerin.

3.3. Hypotensive effects of zaprinast and dipyridamole

As shown in Fig. 3 (top), zaprinast caused a dose-dependent reduction in MAP in both vehicle- and nitroglycerin-pretreated SHR. Maximal changes of -65 ± 4 and -54 ± 3 mm Hg in vehicle- and nitroglycerin-pretreated SHR, respectively, were observed at a cumulative dose of 30 mg/kg zaprinast. Zaprinast caused similar changes in MAP in both tolerant and non-tolerant SHR. A maximum threshold hypotensive dose of 10 mg/kg i.v. of zaprinast was selected from these data for reversal studies in vivo.

As shown in Fig. 3 (bottom), dipyridamole caused similar dose-dependent reductions in MAP in both vehicle- and nitroglycerin-pretreated SHR. From these data, a maximum dose of dipyridamole of 60 mg/kg was selected for reversal studies in vivo. The hypotensive effects of zaprinast and dipyridamole persisted for at least 30 min.

3.4. Reversal of tolerance in vivo

Changes in MAP in response to i.v. challenge doses of 30 μg/kg nitroglycerin and higher were significantly greater in the non-tolerant (0-day) group of SHR acutely pretreated with 10 mg/kg zaprinast, as compared to the corresponding vehicle-pretreated SHR (Fig. 4, top). In tolerant (3-day) SHR acutely pretreated with vehicle, the changes in MAP were blunted (Fig. 4, bottom) demonstrat-

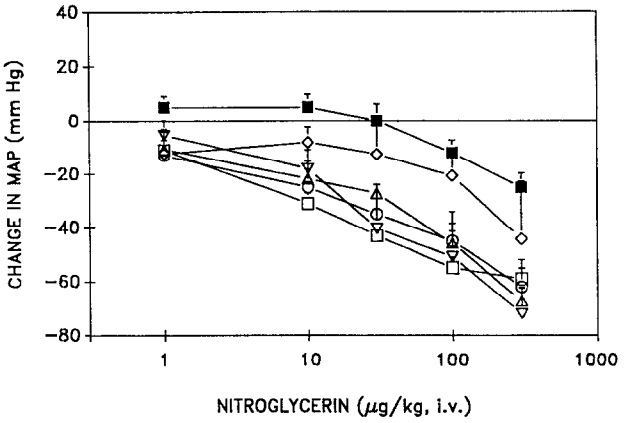

Fig. 5. Evaluation of the ability of lower pretreatment doses of zaprinast to reverse nitroglycerin tolerance in tolerant (3-day pretreatment) groups of SHR. Rats were acutely pretreated with zaprinast at doses of 3 (○), 1 (△), 0.1 (▽) or 0.01 (◇) mg/kg i.v. To facilitate comparison, the vehicle- (■) and 10-mg/kg zaprinast-pretreated (□) groups were included. $n = 4–8$/group.

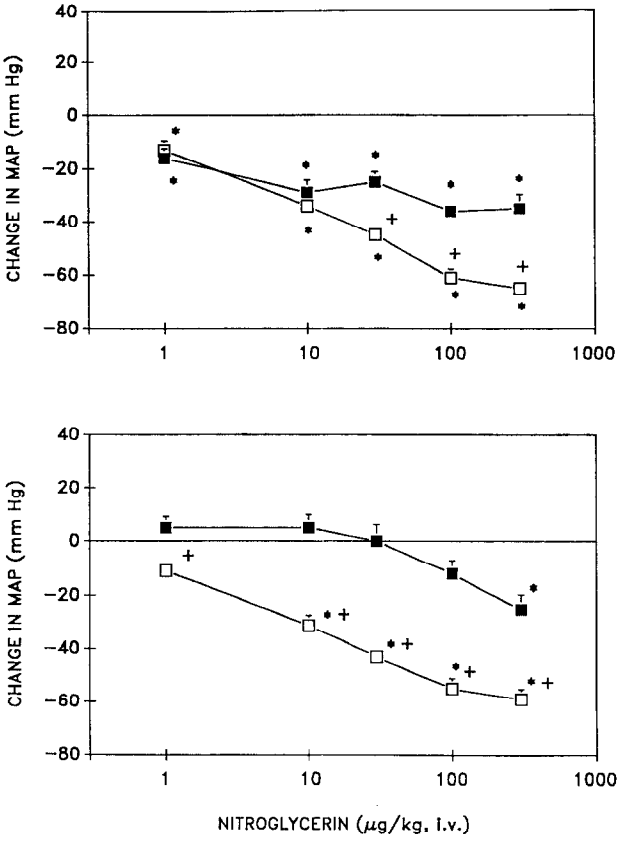

Fig. 4. Changes in mean arterial pressure (MAP) from baseline in response to bolus doses of nitroglycerin in non-tolerant (top) and tolerant (3-day pretreatment, bottom) groups of SHR. SHR were acutely pretreated with zaprinast (□) at 10 mg/kg or vehicle (■) i.v. * $P \leq 0.05$ vs. baseline value within each group. + $P \leq 0.05$ vs. corresponding vehicle-pretreated group. $n = 6–8$/group.

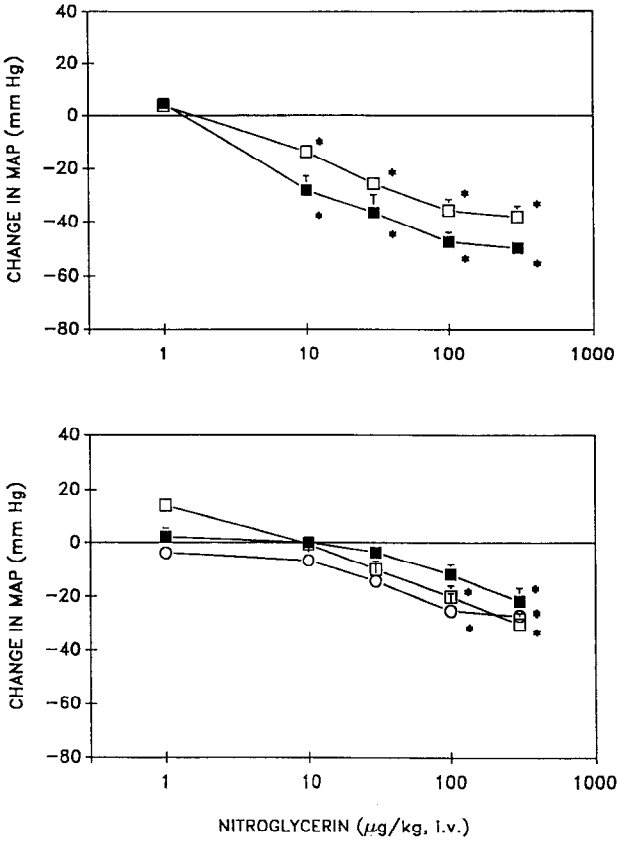

Fig. 6. Changes in mean arterial pressure (MAP) from baseline in response to bolus doses of nitroglycerin in non-tolerant (top) and tolerant (3-day pretreatment, bottom) groups of SHR. SHR were acutely pretreated with dipyridamole at doses of 30 (○) or 60 mg/kg (□), or vehicle (■) i.v. * $P \leq 0.05$ vs. baseline value within each group. No significant differences were detected between groups. $n = 6–8$/group.

ing that tolerance had been achieved. Conversely, in tolerant SHR pretreated with zaprinast a significant decrease in MAP was seen at 10 μg/kg of nitroglycerin and greater. In addition, the changes in MAP in this group were significantly different from that in the tolerant, vehicle-pretreated group at all doses of nitroglycerin tested.

The ability of zaprinast to reverse tolerance at doses less than 10 mg/kg was also evaluated. As shown in Fig. 5, acute pretreatment of tolerant SHR with doses of 0.1, 1 or 3 mg/kg zaprinast resulted in similar decreases in MAP in response to challenge doses of nitroglycerin as compared to the 10-mg/kg zaprinast-pretreated group. Acute pretreatment with a dose of 0.01 mg/kg zaprinast resulted in an intermediate decrease in MAP in response to nitroglycerin; thus suggesting partial reversal of tolerance at this dose.

In non-tolerant (0-day) SHR acutely pretreated with 30 mg/kg dipyridamole, there was a tendency for the decrease in MAP to be slightly decreased, as compared to the corresponding vehicle-pretreated SHR (Fig. 6, top). However, this difference was not statistically significant. Unlike zaprinast, dipyridamole did not potentiate the effect of nitroglycerin. In tolerant (3-day) SHR acutely pretreated with dipyridamole at 30 or 60 mg/kg or the vehicle i.v., the response to nitroglycerin remained significantly blunted (Fig. 6, bottom); thus demonstrating an inability of dipyridamole to reverse tolerance in vivo. None of the pretreatment doses of zaprinast or dipyridamole significantly affected blood pressure.

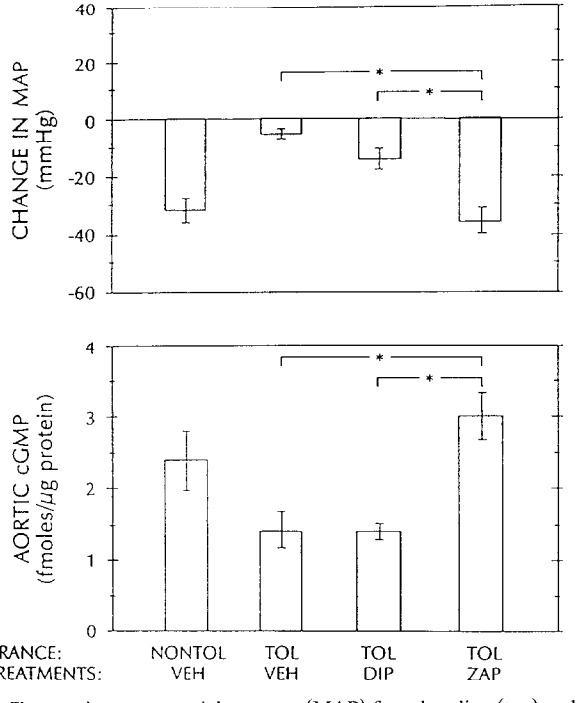

Fig. 7. Changes in mean arterial pressure (MAP) from baseline (top) and the corresponding amount of cGMP in the aorta (bottom) following an acute bolus dose of 100 μg/kg i.v., nitroglycerin in groups of non-tolerant (NONTOL) and tolerant (TOL, 3-day pretreatment) SHR. SHR were acutely pretreated with vehicle (VEH), dipyridamole (DIP, 60 mg/kg) or zaprinast (ZAP, 10 mg/kg). * $P \leq 0.05$. $n = 6–8$/group.

3.5. Aortic cGMP changes

Shown in Fig. 7 are the corresponding changes in MAP (top) and aortic cGMP (bottom) in response to a 100-μg/kg i.v. challenge dose of nitroglycerin in tolerant and non-tolerant SHR. The change in MAP and the level of aortic cGMP were less in the tolerant, vehicle-pretreated group, as compared to the non-tolerant, vehicle-pretreated group. In the tolerant, dipyridamole-pretreated group, the change in MAP and the amount of cGMP were not different from the tolerant, vehicle-pretreated group. Conversely, in the tolerant, zaprinast-pretreated group, the change in MAP and levels of aortic cGMP were completely restored, and shown to be significantly different from both tolerant, vehicle- and dipyridamole-pretreated groups.

4. Discussion

We have demonstrated that tolerance to the hemodynamic effect of nitroglycerin can be reliably achieved in SHR by administering high doses of nitroglycerin over a 2–3-day period. These results extend the previously reported findings of Needleman (1970) who showed that nitroglycerin tolerance could be developed in normotensive rats using a similar dosing regimen. The rationale for utilizing hypertensive rats in the present study was based on the need to achieve significant decreases in MAP to challenge doses and concentrations of nitroglycerin and to enhance the contrast between tolerant and non-tolerant responses. Tolerance is a relative phenomenon, thus, rather than relying on a single dose to confirm tolerance, we believe it is important to evaluate a full range of challenge doses of nitroglycerin as was done in this study. Moreover, since the degree of tolerance is relative it is imperative that the appropriate tolerant, vehicle-pretreated groups be concurrently examined when evaluating the ability of an agent to reverse tolerance.

In our laboratory, it had been previously shown that zaprinast could reverse nitroglycerin tolerance in vitro (Pagani et al., 1993). In those studies, tolerance was developed and evaluated in vitro, whereas in the present study tolerance was developed in vivo and subsequently evaluated in aortic vascular rings ex vivo. Ex vivo, at equimolar concentrations (10 μM), both zaprinast and dipyridamole restored, to varying degrees, the vasorelaxant effect of nitroglycerin in tolerant aortae with zaprinast being more efficacious. Zaprinast has also been shown to reverse tolerance at a concentration as low as 300 nM (Pagani et al., 1993). These results suggest that, regardless of the method to induce tolerance, be it acutely in vitro or subchronically in vivo, the ability of an agent to reverse tolerance when tested in isolated vascular segments is

unaltered. The utility of this model is therefore extended to ex vivo evaluation of nitroglycerin tolerance.

To evaluate the ability of an agent to reverse tolerance in vivo, it was considered essential to pretreat with the maximum, minimally or subthreshold vasoactive dose. This ensured the separation of the direct effect of the phosphodiesterase inhibitor from the synergistic effect of reversing tolerance, i.e. there was no significant change in blood pressure after pretreatment with zaprinast or dipyridamole. Thus, to determine this dose the hypotensive effects of zaprinast and dipyridamole were evaluated in groups of tolerant and non-tolerant SHR to ascertain that the affect of each agent was not different during nitroglycerin tolerance. The dose-response to zaprinast and dipyridamole was similar in tolerant and non-tolerant SHR suggesting there was no cross-tolerance between nitroglycerin and either of these agents. In previous studies using this rat model, cross-tolerance was also shown not to develop between nitroglycerin and several endothelial derived relaxing factor-mediated vasoactive agents (De Garavilla et al., 1993). Doses of 10 and 60 mg/kg of zaprinast and dipyridamole, respectively, were selected as the maximum, threshold vasoactive doses. Despite the potential differences in potency, using equi-efficacious doses allowed for the valid comparison of zaprinast and dipyridamole to reverse hemodynamic tolerance to nitroglycerin. Moreover, the duration of the hypotensive effects of zaprinast and dipyridamole were similar and lasted for at least 30 min, the maximum time required to evaluate tolerance with i.v. challenge doses of nitroglycerin. Thus, neither pharmacokinetic nor pharmacodynamic differences between zaprinast and dipyridamole appear to account for the ability to reverse tolerance.

In contrast to zaprinast, dipyridamole did not reverse tolerance in vivo even at a dose as high as 60 mg/kg. As previously stated, equi-efficacious doses of zaprinast and dipyridamole were selected for pretreatment, which should have accounted for any relative difference in hypotensive potency. The apparent difference in these two agents to reverse tolerance in vivo appears to be explained by less of a change in aortic cGMP in response to an acute nitroglycerin challenge. Correlation of hemodynamic tolerance to tissue cGMP levels in the same animal demonstrates that zaprinast restores cGMP levels and changes in MAP in response to acute nitroglycerin challenges, whereas dipyridamole does not. Since cGMP was not measured in the animals receiving lower doses of zaprinast it is not known for certain whether the effect on aortic cGMP accounts for the efficacy of zaprinast at these doses. However, a positive correlation has been established between vascular wall cGMP content and vasorelaxation in response to various guanylate cyclase activators (Fukuda et al., 1992; Popescu et al., 1985) and phosphodiesterase inhibitors (Dundore et al., 1991). Although aortic cGMP was not measured following pretreatment with zaprinast or dipyridamole and before nitroglycerin challenge, the results of Dundore et al. (1991) do suggest that non-vasoactive doses of zaprinast do not change aortic cGMP.

Dipyridamole has been shown in the present study and in another (Bohyn et al., 1991) to reverse tolerance in vitro. The results of a most recent study showed that pretreatment of human subjects made tolerant to nitroglycerin with dipyridamole was also ineffective in reversing tolerance (Torfgård and Ahlner, 1993). Neither the findings in the present study nor that of Torfgård and Ahlner (1993) provide an explanation as to why dipyridamole was effective in reversing tolerance in vitro but not in vivo. These findings corroborate the results of the present study and perhaps provide supportive evidence for the predictive nature of the nitroglycerin tolerance model in rats. Understandably, full corroboration must await the testing of an agent that effectively reverses tolerance in both the rat model and in humans. To date, a potent, selective cGMP phosphodiesterase inhibitor has not been evaluated in humans for reversal of vascular tolerance to organic nitrates.

In summary, the selective cGMP phosphodiesterase inhibitor zaprinast has now been shown to reverse nitroglycerin tolerance in several preclinical studies, under in vitro, ex vivo and in vivo conditions. In addition, doses of zaprinast as low as 0.1 mg/kg i.v. have now been shown to effectively reverse hemodynamic tolerance. The ability of zaprinast to reverse tolerance in vivo is related to vascular cGMP preservation.

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