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Differences in the vascular selectivity and tolerance between the NO donor/β-blocker PF9404C and nitroglycerin

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Abstract

The vascular selectivity of PF9404C ((2′ *S*),(2*S*)-3-isopropylamine,1-[4-(2,3-dinitroxy)propoxymethyl]-phenoxy-2′ -propanol), a new β-blocker with nitric oxide (NO)-donor and vasodilator properties, was studied in different rabbit arteries and veins. Phenylephrine (10^{-6} M) or 35 mM K^+ were used to pre-contract the arteries and veins prior to study the relaxant effects of PF9404C and nitroglycerin. The potency of both drugs to depress the phenylephrine-induced contraction was greater than that shown in the blockade of the K^+-evoked contraction in most of the vessels studied, with the exception of the central ear artery. PF9404C exhibited about three-fold higher potency than nitroglycerin to relax the majority of the vessels studied, especially when they were contracted with K^+, and showed a certain selectivity of action for the renal artery. PF9404C produced autotolerance but this effect was about 20-fold less pronounced than that observed with nitroglycerin. Cross-tolerance in those preparations pre-exposed to PF9404C that were relaxed later on with nitroglycerin was much greater than autotolerance. The tolerance for nitroglycerin was practically abolished in the presence of *N*-acetylcysteine. However, this was not the case for PF9404C. These results indicate that, although sharing the property of being NO donors, PF9404C and nitroglycerin show a different profile in causing vasodilation; furthermore, the tolerance to this effect is lesser for PF9404C and seems to be mediated by a mechanism different to that of nitrates. This makes PF9404C a nice pharmacological tool to further develop novel NO-donor compounds with a lesser degree of vascular tolerance than those now available.

Keywords: PF9404C; NO donor; Vascular selectivity; Tolerance

1. Introduction

Classically, a strategy followed in the treatment of hypertension, which has been given promising results is the association of a vasodilator and a β-blocker (Strein and Sponer, 1990; Heidenreich et al., 1997; Witchitz et al., 2000; Cosentino et al., 2002). With the only administration of β-blockers, the peripheral vascular resistance rises initially to decrease gradually later but, even during chronic treatment, it remains higher than in normotensives, whereas the cardiac output is consistently reduced (Lund-Johansen, 1984). On the other hand, the administration of a vasodilator alone leads to a fall of peripheral resistance and arterial blood pressure which, in a counter-regulatory response, induces an increase in heart rate and catecholamine levels and the activation of the renin–angiotensin–aldosterone system. This counter-regulatory responses can be prevented by the simultaneous administration of a β-blocker. This was, for example, the idea in the case of carvedilol, a combined α–β-blocker that improves rest cardiac function and lessens symptoms in patients with heart failure (Mctavish et al., 1993; Olsen et al., 1995; Dunn et al., 1997).

In a previous paper (Villarroya et al., 1999), we described the characteristics of PF9404C ((2′ *S*),(2*S*)-3-

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isopropylamine,1-[4-(2,3-dinitroxy)propoxymethyl]-phenoxy-2′ -propanol), a new blocker of β-adrenergic receptors with vasodilator properties. We also presented some data clarifying the mechanism of that vasodilatory action, i.e. the slow liberation of nitric oxide (NO) by the molecule in the presence of vascular tissue. The potential benefits of this new drug would be that, as a vasodilator, it could correct the initial high peripheral resistance present in hypertensive patients treated with β-blockers. On the other hand, as a β-blocker, it would prevent the increase of heart rate and catecholamine levels that follows a fall of peripheral resistance and arterial blood pressure.

However, the beneficial effect of nitrates tend to diminish during long-term therapy. This phenomenon, termed tolerance, has been known and well documented since long time ago in congestive heart failure (Sharpe and Coxon, 1984; Elkayam et al., 1987; Packer et al., 1987) and gives place to a clinical condition in which, after continuous administration of the drug, the efficacy of the treatment is lost, leaving the patients vulnerable to ischemia. Many studies have been conducted on the origin and mechanisms underlying this phenomenon (Needleman et al., 1972; Chung and Fung, 1993; Bohyn et al., 1991; Munzel et al., 1995; Abrams et al., 1998; Parker and Parker, 1998; Chen et al., 2002) but they still remain controversial.

In the frame of the potential cardiovascular use of PF9404C, in the present work, we intended to study a possible vascular selectivity for the different rabbit arteries and veins that were used (aorta, renal, femoral and central ear arteries and portal and saphena veins). In parallel experiments, we used nitroglycerin as a vasodilator of reference. Also, we assessed the development of tolerance by PF9404C and compared it to that to nitroglycerin.

2. Materials and methods

2.1. Experiments with rabbit vessels

Male New Zealand white rabbits (2.5 kg) were killed in a gas chamber with CO_2, and the following arteries and veins were dissected: thoracic aorta, femoral, renal and central ear arteries and portal and saphena veins.

All the vessels were placed in a Petri dish with Krebs–Henseleit solution of the following composition (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 1.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11; and $NaHCO_3$, 25; and cleaned of surrounding tissue; helical strips of aorta were cut and mounted in an organ bath containing 40 ml of Krebs–Henseleit solution at 37 °C, bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. The portal vein was divided into two segments that were mounted in the organ bath. Two-millimeter cylindrical segments of femoral artery, central ear artery, renal artery and saphena vein were cut and mounted in the bath through two wire hooks carefully introduced into the vessel's lumen. Thoracic aorta strips were loaded with 2.5 g; cylindrical segments of femoral artery, central ear artery, renal artery and saphena vein, and segments of portal vein were loaded with 0.5 g. All the vessels were allowed to equilibrate for 1 h with repeated washings. The responses were recorded isometrically by a force displacement transducer on a MacLab/4e acquisition system.

After 1 h equilibration of the vessels, the experiments were performed pre-contracting the preparations with the addition to the Krebs-bicarbonate solution of phenylephrine (1 μM) or a concentrated solution of KCl to get a final concentration of 35 mM K^+. Once the contraction reached a plateau, PF9404C was added to the bath in a cumulative manner.

2.2. Experiments with rat thoracic aorta

Male Sprague–Dawley rats weighing 225–250 g were used in all the experiments. The animals were killed by cervical dislocation. The thoracic aorta as close as possible to the heart was quickly removed and placed in a Petri dish containing Krebs bicarbonate solution and the excess fat and connective tissue were removed. The aorta was cut spirally into a strip approximately 2–3 mm wide. In each experiment, segments 1.5–2 cm long were used; the strips were kept in Krebs solution gassed with 95% oxygen and 5% CO_2 throughout the experiment.

The segments of aorta were mounted in an organ bath (muscle chamber with a capacity of 40 ml, kept at 37 °C), so that one end was fixed to an isometric transducer connected to an amplifier and recorder. In some experiments, 3-mm wide rings from the aorta were also used; they were mounted in the bath through two wire hooks carefully introduced into the vessel's lumen. In all experiments, the muscles were loaded with 1 g.

After 1 h equilibration of the aorta segments, experiments to study the development of tolerance by a molecule were performed. Preparations were incubated with the drugs during a total of 30 min, but the solution with the drug was replaced once after 15 min to avoid its consumption. Once the incubation period was terminated, the segments were pre-contracted by phenylephrine (1 μM) or KCl (final concentration 35 mM). Once the contraction reached a plateau, the compounds were added to the bath in a cumulative manner.

2.3. Materials and solutions

The following materials were used: PF9404C batch 130297 from Almirall Prodesfarma (Barcelona, Spain), nitroglycerin solution 1% (Merck) and phenylephrine (Sigma, Spain). All other chemicals used were reagent grade.

PF9404C and phenylephrine were dissolved in distilled water at 10^{-2} M and diluted in the saline solutions to be used.

2.4. Statistical analysis of the results

Data are expressed as means±S.E.M. IC_{50} for each drug was estimated through non-linear regression analysis using ISI software for a PC computer. Differences between non-paired groups were compared by an analysis of variance (ANOVA) with the statistical program StatView; a value of P equal or smaller than 0.05 ($P \leq 0.05$) was taken as the limit of statistical significance.

3. Results

3.1. Effects of PF9404C and nitroglycerin on rabbit aorta

Addition of 1 μM phenylephrine or 35 mM K^+ gave a comparable sustained contraction of aorta, 28.2±0.9 mN for phenylephrine (n=13) and 29.8±0.9 mN for K^+ (n=16). Usually, the contraction obtained was stable and exhibited little relaxation during the time of the experiment (about 1 h). In general, these concentrations of phenylephrine and K^+ produced similar contractile submaximal responses in all vessels studied (Table 1), as it was determined in previous experiments. This facilitated the comparison between the potencies and efficacies of PF9404C and nitroglycerin.

Fig. 1 shows that both PF9404C and nitroglycerin relaxed in a concentration-dependent manner the contraction of the aorta induced by phenylephrine, with an IC_{50} of 0.007 and 0.025 μM, respectively, PF9404C being 3.5-fold more potent than nitroglycerin. The vessel contracted by K^+ (Fig. 1B) was also relaxed in a concentration-dependent way, although the potency was lower (IC_{50} of 0.12 μM for PF9404C and not calculable for nitroglycerin). The maximum relaxation obtained with nitroglycerin was about 30% at the maximum concentration tested, i.e. 30 μM; thus, nitroglycerin was much less efficacious than PF9404C in relaxing the aorta contracted by K^+.

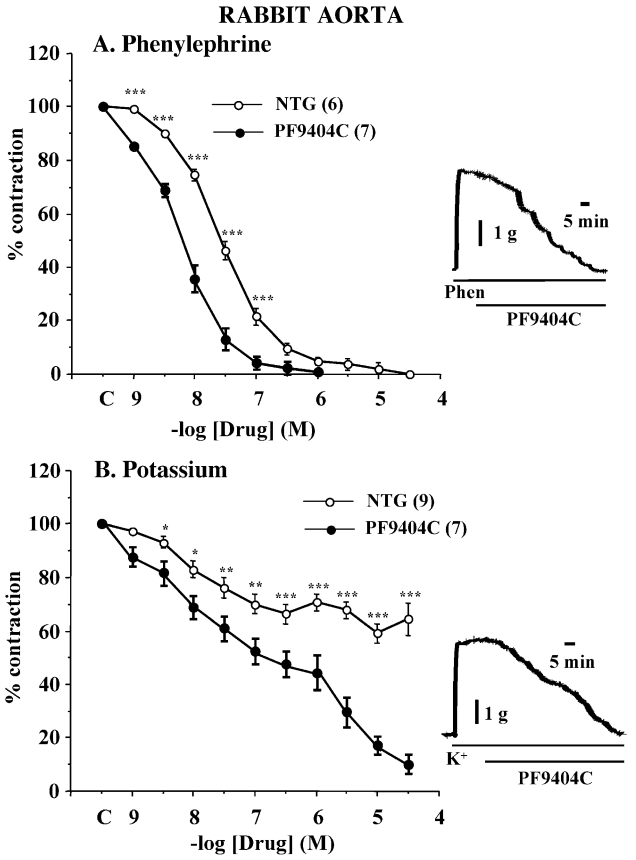

Fig. 1. Cumulative concentration–response curves in rabbit aorta helical strips pre-contracted with 1 μM phenylephrine (A) or 35 mM K^+ (B), for PF9404C (●) or nitroglycerin (○). Data are means±S.E.M. of six to nine strips from different animals. Insets show an example record of contractions elicited by phenylephrine (A) or potassium (B) and its relaxation by cumulative concentrations of PF9404C. *P<0.05, **P<0.01 and ***P<0.001 compared to concentration–response curves for PF9404C.

Table 1
Differences between PF9404C and nitroglycerin (NTG) in rabbit vessels pre-contracted with 1 μM phenylephrine or 35 mM K^+, expressed as IC_{50} to relax the vessels (μM)

	Vessel	n	Maximum contraction (mN)	PF9404C IC_{50} (μM)	NTG IC_{50} (μM)	Ratio NTG/PF9404C
Phenylephrine	Aorta	13	28.2±0.9	0.01	0.025	3.6
	Renal artery	17	36±4	0.015	0.04	2.5
	Femoral artery	22	40±5	0.1	0.3	3
	Central ear artery	17	26.5±1.8	3.70	4.5	1.2
	Portal vein	18	6.5±0.7	2.10	NC	NC
	Saphena vein	26	29±3	0.60	0.5	0.8
Potassium	Aorta	16	29.8±0.9	0.12	NC	NC
	Renal artery	13	48±6	1.34	4.1	3
	Femoral artery	15	41±5	2.86	NC	NC
	Central ear artery	21	20.4±2	1.95	2.2	1.1
	Portal vein	13	7.2±1.3	4.60	NC	NC
	Saphena vein	16	26±3	0.20	0.5	2.5

NC: not calculable, n: number of tissue strips.

3.2. Effects of PF9404C and nitroglycerin on rabbit renal, femoral and central ear arteries

In the renal artery, the contraction induced by phenylephrine was inhibited in a concentration-dependent manner by PF9404C and nitroglycerin with IC_{50} values of 0.015 μM for PF9404C and 0.038 μM for nitroglycerin (Table 1). The contraction evoked by K^+ was also blocked in a concentration-dependent manner by PF9404C and nitroglycerin (IC_{50} of 1.34 and 4.1 μM, respectively); again, the vasorelaxation caused by PF9404C was complete, while nitroglycerin (30 μM) still left a 20% of the initial contraction unblocked.

Once more, PF9404C was three-fold more potent in relaxing the femoral artery contracted by phenylephrine than nitroglycerin. In both cases, the relaxation was concentration-dependent. When the contraction was induced by 35 mM K^+, the blockade was also concentration-dependent; however, 30 μM nitroglycerin only produced a partial relaxation (50%) of the artery, while PF9404C completely relaxed the vessel.

In the central ear artery, both PF9404C and nitroglycerin relaxed the strips contracted by phenylephrine and K^+ in a concentration-dependent manner, with very similar potencies; thus, the phenylephrine-induced contraction was blocked by PF9404C with an IC_{50} of 3.7 μM and by nitroglycerin with an IC_{50} of 4.5 μM. The IC_{50} values for the relaxation of K^+-induced contractions were 1.95 μM for PF9404C and 2.2 μM for nitroglycerin. Again, neither phenylephrine- nor K^+-induced contractions of this vessel were completely relaxed by 30 μM nitroglycerin.

3.3. Effects of PF9404C and nitroglycerin on rabbit portal and saphena veins

Obviously, the magnitude of the contractile response attained in the rabbit portal vein after stimulation with phenylephrine or K^+ was much lower than in the arteries; thus, phenylephrine elicited a contraction of 6.5±0.7 mN (n=18) and K^+ of 7.2±1.3 mN (n=13). While PF9404C completely relaxed the tissue pre-contracted with phenylephrine or K^+ (IC_{50} of 2.1 and 4.6 μM), the maximum concentration of nitroglycerin tested (30 μM) only relaxed the initial contraction of the portal vein by 40% in both cases (Fig. 2).

The saphena vein contracted by phenylephrine was relaxed in a concentration-dependent manner by both PF9404C and nitroglycerin with IC_{50} values of 0.6 and 0.5 μM, respectively. The K^+-evoked contraction was also inhibited in a similar way by both drugs (IC_{50} of 0.2 and 0.5 μM), but neither PF9404C nor nitroglycerin did relax the tissue completely (Fig. 2B,D).

3.4. Development of autotolerance to PF9404C and nitroglycerin in rat aorta helical strips

Control rat aorta strips showed values of IC_{50} to relax the muscle pre-contracted with 1 μM phenylephrine of 0.0035

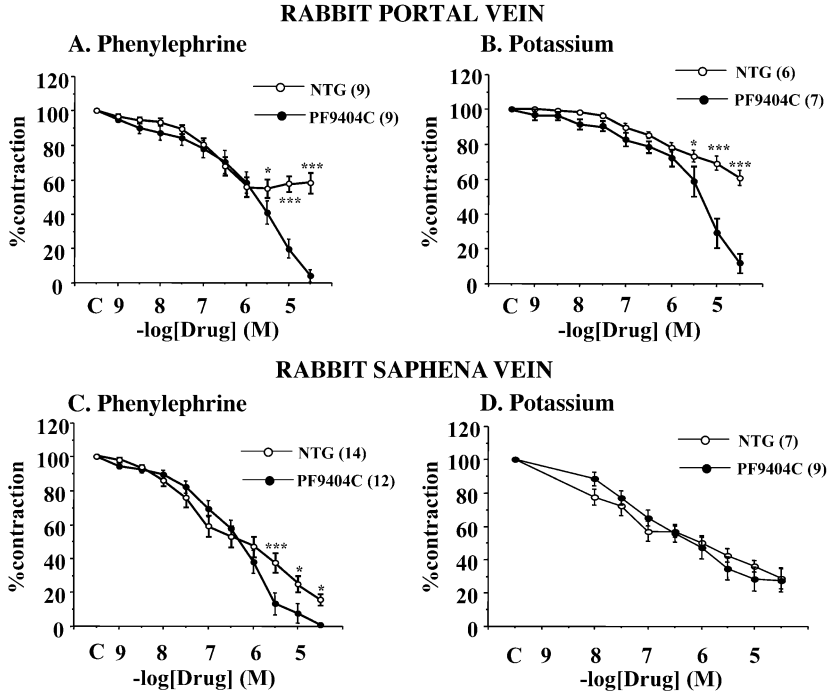

Fig. 2. Cumulative concentration–response curves in rabbit portal and saphena vein segments pre-contracted with 1 μM phenylephrine (A, C) or 35 mM K^+ (B, D), for PF9404C (●) or nitroglycerin (○). Data are means±S.E.M. of 6–14 vessels from different animals. *P<0.05 and ***P<0.001 compared to concentration–response curves for PF9404C.

μM (n=11) and 0.018 μM (n=14) for nitroglycerin and PF9404C, respectively, after an initial contraction of 3.7±0.3 mN. However, when the preparations were incubated with a high concentration of the compound (30 μM) for 30 min, before the performance of the concentration–response curve, much higher concentrations were required to obtain the same effect than in controls: IC_{50} of 2.9 μM for nitroglycerin (n=13) and 0.82 μM for PF9404C (n=22) (Fig. 3A,B), even though there was no statistically significant difference between contraction of controls before or after development of tolerance. Differences between the IC_{50} of control and pre-exposed preparations were 829-fold for nitroglycerin and 46-fold for PF9404C, which means that the effect of tolerance in the present experimental conditions is about 20-fold lower for PF9404C than for nitroglycerin (Table 2).

When the aortas were pre-contracted with 35 mM K^+ instead of phenylephrine, nitroglycerin relaxed the control preparations with an IC_{50} of 0.0043 μM (n=11), while in those pre-exposed to that compound, the IC_{50} could not be calculated, since the maximum relaxation attained at 0.1 mM was around 40% (n=11). For PF9404C, the IC_{50} for control strips was 0.011 μM (n=11) and for those pre-exposed during 30 min to the drug rose to 1.5 μM (n=11); however, the artery was fully relaxed at 10 μM PF9404C (Fig. 3C,D).

Table 2
Development of autotolerance to PF9404C and nitroglycerin (NTG) in rat aorta pre-contracted with 1 μM phenylephrine or 35 mM K^+, expressed as IC_{50} to relax the vessel (μM)

	Phenylephrine			Potassium		
	Control	Pre-exposed	Ratio	Control	Pre-exposed	Ratio
PF9404C	0.018	0.82	45	0.011	1.5	138
NTG	0.0035	2.90	829	0.0043	NC	–

3.5. Development of cross-tolerance to PF9404C and nitroglycerin in rat aorta helical strips

To study cross-tolerance, preparations were pre-exposed either to PF9404C or nitroglycerin (30 μM for 30 min) before contraction with 1 μM phenylephrine and concentration–response curves were performed with nitroglycerin or PF9404C, respectively (Fig. 4A,B). When the aortas were pre-exposed to PF9404C, the IC_{50} obtained for nitroglycerin was 5.9 μM (n=12) (cross IC_{50}/control IC_{50}=1.686). However, when the preincubation was carried out with nitroglycerin, the tolerance for PF9404C diminished with respect to autotolerance (IC_{50}=0.41 μM, n=14).

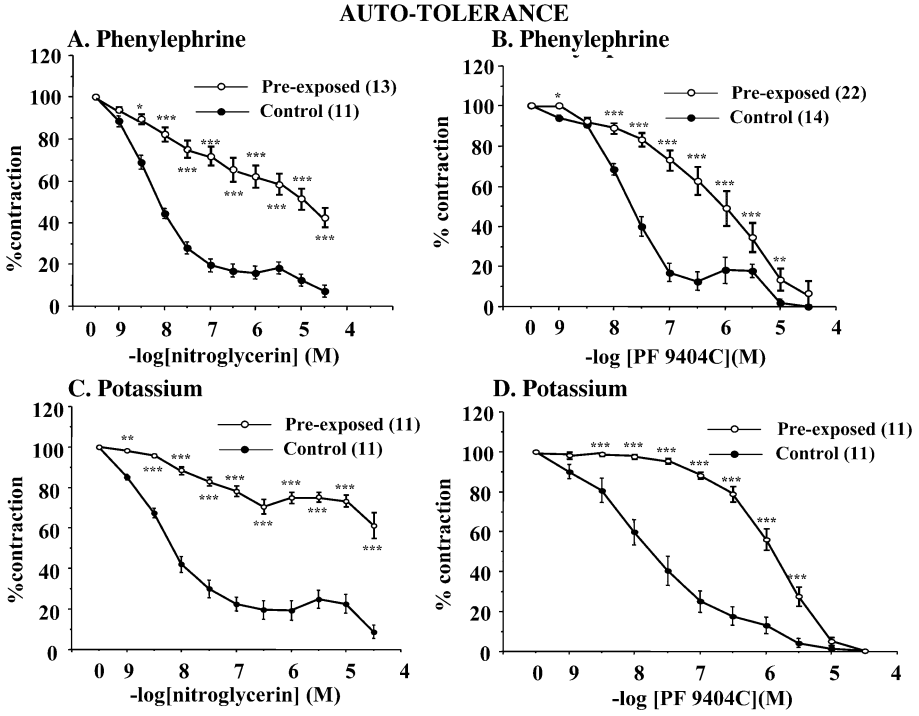

Fig. 3. Cumulative concentration–response curves for PF9404C and nitroglycerin in rat aorta helical strips pre-contracted with 1 μM phenylephrine (A, B) or 35 mM K^+ (C, D). Control curves (●) correspond to those performed without previous incubation of the drug and the pre-exposed (○) are concentration–response curves after 30 min of incubation with the drug at the concentration of 30 μM. Data are means±S.E.M. of 10–22 strips from different animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control curves.

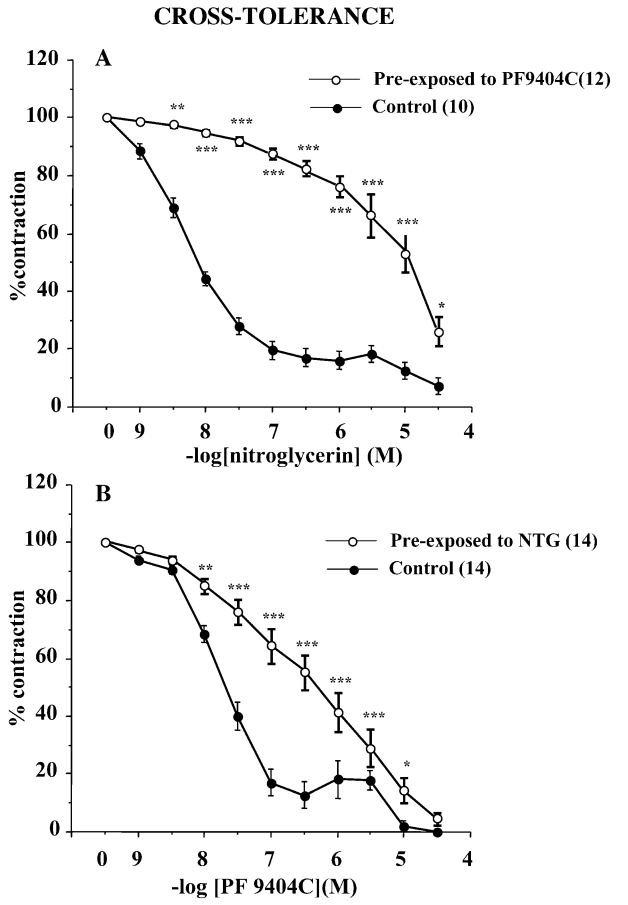

Fig. 4. Cumulative concentration–response curves for nitroglycerin (A) and PF9404C (B) in rat aorta helical strips pre-contracted with 1 μM phenylephrine. Control curves (●) correspond to those performed without previous incubation of the drug and the pre-exposed (○) are concentration–response curves after 30 min of incubation with the drug, contrary to that used to carry out the curves at the concentration of 30 μM. Data are means±S.E.M. of 10–14 strips from different animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control curves.

3.6. Reversion of the tolerance effect

It is widely admitted that the mechanism underlying the effect of nitroglycerin and other organic nitrates is linked to the liberation of NO, which activates guanylate cyclase and then relaxes the vascular smooth muscle (Murad et al., 1978; Ignarro et al., 1981). One of the mechanisms proposed to explain the phenomenon of tolerance is the impaired bioconversion of nitrates (Brien et al., 1986; Axelsson and Ahlner, 1987), which would have diminished their capacity to activate guanylate cyclase. In vitro, this activation by nitroglycerin is sulfhydryl-dependent (Ignarro and Gruetter, 1980) and certain sulfhydryl agents like *N*-acetylcysteine potentiate the effect of organic nitrates (Horowitz et al., 1983; Loscalzo, 1985). For this reason, we decided to study the effect of co-incubation with 0.5 mM *N*-acetylcysteine in the development of autotolerance by nitroglycerin and PF9404C. Experiments were carried out following the same protocol as for the studies of autotolerance, except that some aortic strips were incubated simultaneously with the drug (nitroglycerin or PF9404C) and *N*-acetylcysteine, before the concentration–response curves were performed. The effect of autotolerance for nitroglycerin was almost completely abolished in the presence of *N*-acetylcysteine (Fig. 5A), with an IC_{50} to relax the aortic strips of 0.010 μM (n=11). On the contrary, the effect of autotolerance for PF9404C was potentiated in the presence of *N*-acetylcysteine (Fig. 5B), with an IC_{50} to relax the aorta of 3 μM (n=11).

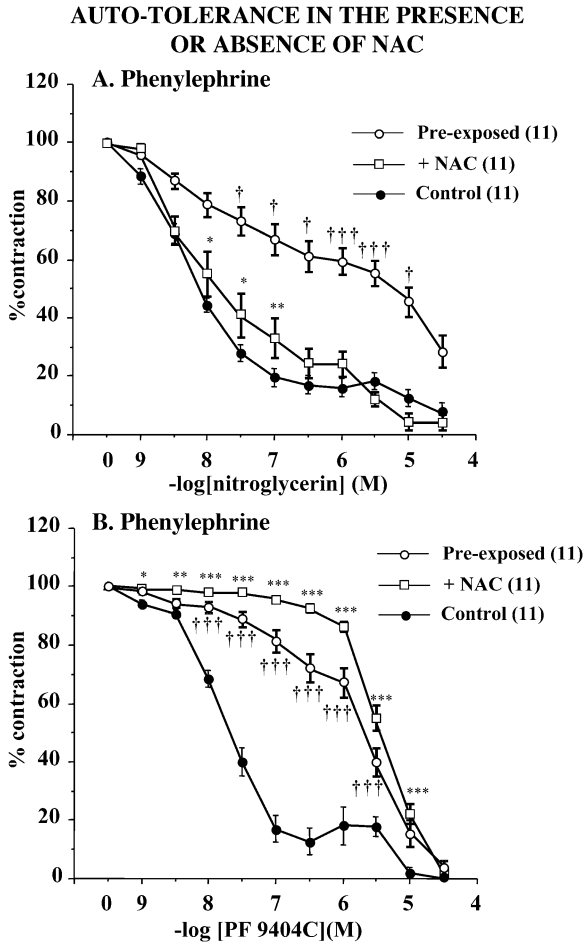

Fig. 5. Cumulative concentration–response curves for nitroglycerin (A) and PF9404C (B) in rat aorta helical strips pre-contracted with 1 μM phenylephrine. Control curves (●) correspond to those performed without previous incubation of any drug, the pre-exposed (○) are concentration–response curves after 30 min of incubation with the drug at the concentration of 30 μM and the +NAC (□) are concentration–response curves after 30 min of incubation with the drug at the concentration of 30 μM plus *N*-acetylcysteine at the concentration of 0.5 mM. Data are means±S.E.M. of 10–14 strips from different animals. *P<0.05, **P<0.01 and ***P<0.001 compared to control curves. †P<0.05, ††P<0.01, †††P<0.001 compared to NAC curves.

4. Discussion

Two different types of stimuli were used to pre-contract the arteries and veins prior to study the relaxant effects of PF9404C and nitroglycerin: (i) phenylephrine, which induces the α-adrenergic-mediated mobilization of calcium from the intracellular stores with the consequent contraction of the vessels (Saida and Van Breemen, 1983); and (ii) high K^+, which recruits the L-type calcium channels through membrane depolarization, permitting the entrance of calcium and the subsequent contraction of the vessel (Van Breemen et al., 1978; Bolton, 1979).

In general terms, we can say that the potency of both drugs to inhibit the phenylephrine-induced contraction was greater than that shown in the inhibition of the K^+-evoked contraction in most of the vessels studied, with the exception of the central ear artery. PF9404C exhibited a slightly higher potency (about three-fold) than nitroglycerin. In addition, it is worth noting that PF9404C always exerted complete relaxation of all vessels, either contracted by phenylephrine or K^+; in contrast, nitroglycerin did not fully relax the vessels contracted with K^+. It is plausible that PF9404C has some calcium-antagonist property, thus explaining the greater blockade of K^+-evoked vessel contraction (Fleckenstein, 1983).

A second aspect to consider was the possible selectivity of the two drugs for the different arteries and veins. Nitroglycerin showed a certain selectivity for the aorta and renal artery with respect to the femoral artery (IC_{50} to inhibit the phenylephrine-induced contraction around 0.03 and 0.3 μM, respectively) and greater selectivity for these three vessels with respect to the central ear artery (IC_{50}=4.6 μM).

We found a clearer vasoselectivity for PF9404C. The order of potencies to inhibit the phenylephrine-induced contraction of the arteries were (Table 1): aorta (IC_{50}=0.007 μM)>renal artery (IC_{50}=0.015 μM)>femoral artery (IC_{50}=0.098 μM)>central ear artery (IC_{50}=3.7 μM). The differences were even greater when the contraction of the arteries was evoked with high K^+. While nitroglycerin never inhibited 100% of the contraction, PF9404C was a very efficacious inhibitor, being capable of relaxing completely all arteries, with the following order of potencies: aorta (IC_{50}=0.12 μM)>renal artery (IC_{50}=1.3 μM)>central ear artery (IC_{50}=1.9 μM)>femoral artery (IC_{50}=2.8 μM).

It is widely admitted that nitroglycerin, and most of the organic nitrates, are preferentially venoselective (Mackenzie and Parratt, 1977; Rosen et al., 1987; Bauer and Fung, 1996) but, surprisingly, we did not find a clear venoselectivity for either of the two drugs since, as mentioned in Results section, the IC_{50} to relax the portal vein or the saphena are higher than those for the aorta, the renal artery or the femoral artery (Table 1).

The fact that PF9404C shows a certain selectivity for the renal artery in relation to the femoral and the central ear arteries, give us basis to predict a possible beneficial effect improving renal blood flow in situations with a deficit in renal perfusion, like in congestive cardiac failure or in certain types of hypertension. Additionally, it could have a nephroprotective effect in different nephropathies.

As mentioned before, tolerance to organic nitrates develops after chronic treatment of patients. Intermittent dosage could be a way to overcome this problem, but patients in nitrate monotherapy would be unprotected against congestive heart failure or angina pectoris. In this context, it is interesting that PF9404C produces much lesser tolerance than nitroglycerin. Two major groups of mechanisms have been proposed to explain organic nitrate tolerance: impaired enzymatic release of NO ("impaired bioconversion") and increased endothelial generation of superoxide (Loscalzo, 2001; Gori and Parker, 2002a,b), the first one being more widely accepted at present. In the case of nitroglycerin, its activation of guanylate cyclase is sulfhydryl-dependent in vitro (Ignarro and Gruetter, 1980) and *N*-acetylcysteine is known to potentiate the effect of organic nitrates (Horowitz et al., 1983; Loscalzo, 1985). This is consistent with our observation in experiments in the presence of *N*-acetylcysteine, in which the nitroglycerin tolerance was practically abolished. However, this did not happen in the case of PF9404C; after co-incubation with *N*-acetylcysteine autotolerance was not abolished but slightly increased, which suggests that the mechanism of action of PF9404C relaxing smooth muscle vessels must be different from that of nitroglycerin. One possible cause would be the slower release of NO by PF9404C as compared with nitroglycerin (Villarroya et al., 1999).

In conclusion, we have found that in most vessels studied PF9404C was more potent than nitroglycerin in causing relaxation. Furthermore, PF9404C exhibited a selectivity for vasodilation of kidney vessels, suggesting a possible therapeutic relevance to increase kidney perfusion and in cardiovascular diseases; in vivo experiments will tell about these possibilities. Finally, it is interesting that the cross-tolerance and autotolerance to the vasorelaxing effects of PF9404C was 20-fold lower and apparently involves a different mechanism to that of nitroglycerin. Given the importance of the development of tolerance in the treatment with nitrates, ischemic heart disease or heart failure, PF9404C could serve as a basis of new model of NO-donor compounds with much less capability of developing tolerance.

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