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Original Article

Effects of platelet-activating factor on nitrergic transmission in rabbit corpus cavernosum

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Abstract ***Aim***: The information currently available suggests that nonadrenergic noncholinergic (NANC) transmitters, particularly nitric oxide, are involved in the relaxation of penile erectile tissues. Platelet-activating factor (PAF) is a chemical mediator and is involved in many physiological and pathophysiological events. It is well known that several of the vascular actions of PAF are mediated by the generation of nitric oxide. We designed this study to test the hypothesis that PAF has an effect on NANC responses in rabbit corpus cavernosum strips.

Methods: Rabbit corpus cavernosum strips were precontracted with phenylephrine (10^{-5} mol/L). Isometric tension changes produced by carbachol (10^{-9}–10^{-5} mol/L), sodium nitroprusside (10^{-8}–10^{-5} mol/L) and electrical field stimulation (for 10 s at sequential frequencies of 2, 4, 8, 16, and 32 Hz as square-wave pulses of 50 mV) were recorded with a pressure transducer. These relaxations were compared to those obtained in the presence of PAF.

Results: PAF had no effect on endothelium-dependent, endothelium-independent or electrical field stimulation-induced NANC relaxation responses in isolated rabbit corpus cavernosum strips. There was no statistically significant difference between the pD_2 and E_{max} values for carbachol or sodium nitroprusside in the presence of PAF.

Conclusions: Our results suggest that PAF does not modify the endothelium-dependent, endothelium-independent or electrical field stimulation-induced NANC relaxation responses in isolated rabbit corpus cavernosum strips.

Key words nitric oxide, platelet activating factor, rabbit corpus cavernosum.

Introduction

In response to sexual stimulation in men, nonadrenergic noncholinergic (NANC) nerves, endothelial cells, and cavernosal smooth muscle cells release nitric oxide (NO), which promotes the production of cyclic guanosine monophosphate (cGMP) via the cytosolic enzyme guanylate cyclase.[1] The resulting molecular cascade decreases intracellular calcium levels, allowing the relaxation of smooth muscle cells in the cavernosal bodies. Penile blood flow increases and the sinusoidal spaces expand, preventing venous outflow of blood and resulting in erection.[2]

Platelet-activating factor (PAF) is a biologically active phospholipid mediator that was first derived from rabbit basophils upon IgE-mediated immunological challenge.[3] PAF is produced by many cell types, including endothelial cells, neutrophils, monocytes, macrophages, basophils, eosinophils, mastocytes and vascular smooth muscle.[4] Neurons, glial and microglial cells, monocyte cell populations, macrophages and the endothelial cells of blood vessels are the targets of PAF.[5] Although it was named for its ability to induce platelet aggregation, intense investigation has revealed that PAF is active in a broad range of cell types and tissues. Pharmacological studies have suggested that PAF functions in a variety of settings, including allergy,

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inflammation, neural functions, reproduction and atherscleros is.[6]

To our knowledge, there are no data demonstrating that PAF is present in the corpus cavernosum of any species. However, it has been shown that PAF stimulates the production of NO from L-arginine by acting on the PAF receptors in the endothelium, which in turn stimulates soluble guanylate cyclase in the smooth muscle cells, and so increases the production of cGMP, thus relaxing the arteries.[7] It has been suggested that PAF could constitute a paracrine/autocrine system for modulating smooth muscle cell contractility, in addition to the well-known vasoactive substances of endothelial origin such as NO and prostacyclin.[8] Based on this information, we aimed to investigate the effects of PAF on the nitrergic system in rabbit corpus cavernosum strips in order to evaluate the role of PAF in the physiology of penile erection.

Methods

Corpus cavernosum tissues were prepared from adult male New Zealand white rabbits weighing 2.5–3 kg. Total penectomy was performed in rabbits that were anesthetized with pentobarbital sodium (30–40 mg/kg intravenously) and killed by exsanguination through the carotid artery. Ventral incisions were made on the right and left corpora, the tunica was dissected and the corpus cavernosum tissue was exposed and immediately placed in organ chambers containing physiological saline maintained at 37°C. Strips of corpus cavernosum tissue measuring approximately 2 mm × 2 mm × 15 mm were placed in 15-mL water-jacketed tissue baths for isometric tension measurements. One end of each of the corporal strips was attached to the bottom of the organ bath and the other end was tied to a force transducer (FT 03, Grass Instruments, Quincy, MA, USA), which was connected to a pen polygraph (79 E, Grass Instruments). Each strip was allowed to equilibrate with a basal tension of 2 g for 1 h. Corporal tissues had an intact endothelium, as assessed by the capacity of acetylcholine (10^{-6} mol/L) to produce relaxation. The physiological saline was composed as follows (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11. The pH of the solution was 7.4, after being bubbled with a mixture of gaseous O_2 and CO_2.

Each preparation was precontracted submaximally with phenylephrine (10^{-5} mol/L). At the contraction plateau, relaxation responses to cumulative concentrations of carbachol and sodium nitroprusside (SNP), to test endothelium-dependent and independent relaxations, respectively, were assessed.

Endothelium-dependent responses

Relaxant responses to carbachol were elicited in endothelium-intact corpus cavernosum strips that were contracted with submaximal concentrations of phenylephrine (10^{-5} mol/L). These relaxations were compared with those obtained in the presence of PAF at concentrations of 10^{-10}–10^{-7} mol/L. PAF was applied to the preparations 30 min before precontraction with phenylephrine.

Endothelium-independent responses

Relaxant responses to SNP were produced after precontraction with phenylephrine (10^{-5} mol/L) and compared with responses in the presence of PAF (10^{-10}–10^{-7} mol/L). PAF was applied to the preparations 30 min before precontraction with phenylephrine.

Electrical field stimulation

For electrical field stimulation (EFS), strips of corpus cavernosum were stimulated for 10 s with two parallel platinum electrodes at sequential frequencies of 2, 4, 8, 16 and 32 Hz as square-wave pulses of 50 V (0.8 ms) delivered by a current amplifier and a stimulator (S 88, Grass Instruments). The strips were allowed to return to the baseline precontractile tension between tests at each frequency. Before EFS, the tissue was treated with an adrenergic nerve blocker, guanethidine (5×10^{-6} mol/L) and a muscarinic receptor blocker, atropine (10^{-6} mol/L) for 30 min to eliminate adrenergic and cholinergic responses and to study the relaxation responses induced by the stimulation of nonadrenergic, noncholinergic nerves.

Relaxation responses to EFS were determined after submaximal contraction with phenylephrine (10^{-5} mol/L). The technical procedure used for obtaining the responses to EFS has been described previously.[9] One strip from each rabbit was contracted with phenylephrine (10^{-5} mol/L) and the second strip remained unstimulated. Relaxant responses to EFS were compared with responses obtained in the presence of PAF (10^{-10}–10^{-7} mol/L).

The following drugs were used in the experiments: atropine sulfate, SNP, acetylcholine chloride, guanethidine sulfate, carbachol (carbamylcholine chloride), phenylephrine hydrochloride (Sigma, St Louis, MO) and PAF. All drugs except PAF were dissolved in distilled water and were freshly prepared on the day of the experiments. The PAF was dissolved in alcohol.

Analysis

The relaxant effects of the agonists were expressed as a percentage of precontraction in response to phenylephrine. Experimental values were expressed as mean ± SEM. To evaluate the effects of agonists, the maximum response (E_{max}) and pD_2 values (i.e. the negative logarithm of the concentration for the half-maximal response, ED_{50}) were calculated. The concentration–response data obtained in each experiment were plotted as the response/concentration against the response, producing a linear relationship in each experiment, as predicted from the Scatchard equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and were taken as a measure of the sensitivity of the tissues to each agonist. Groups were compared statistically using Student's *t*-test. Values of $P < 0.05$ were considered to be significant statistically.

Results

In quiescent strips, no effect was observed with PAF (10^{-10}–10^{-7} mol/L). Contractions induced by phenylephrine (10^{-5} mol/L) in corpus cavernosum strips were also not altered significantly in the presence of PAF at the same concentrations (2120 ± 158 g for phenylephrine and 2086 ± 104 g for PAF [10^{-7} mol/L] + phenylephrine; $n = 6$).

Carbachol (10^{-9}–10^{-5} mol/L) and SNP (10^{-8}–10^{-5} mol/L) produced relaxations in the preparations that were precontracted with phenylephrine (10^{-5} mol/L; Fig. 1). PAF (10^{-10}–10^{-7} mol/L) had no significant effect on the carbachol- and SNP-induced relaxations, and there were no significant differences in E_{max} and pD_2 values. We have shown only the results obtained with a concentration of 10^{-7} mol/L PAF (Table 1, Fig. 1).

PAF produced no statistically significant effect on EFS-induced relaxation in corpus cavernosum (Table 1, Fig. 2).

The relaxation times for SNP (10^{-8}–10^{-5} mol/L) and carbachol (10^{-9}–10^{-5} mol/L) were not altered significantly (467.11 ± 26.81 s and 492.36 ± 23.19 s, respectively) in the presence of PAF (10^{-7} mol/L).

Discussion

To our knowledge, the expression of PAF receptors has not been investigated in the corpus cavernosum of

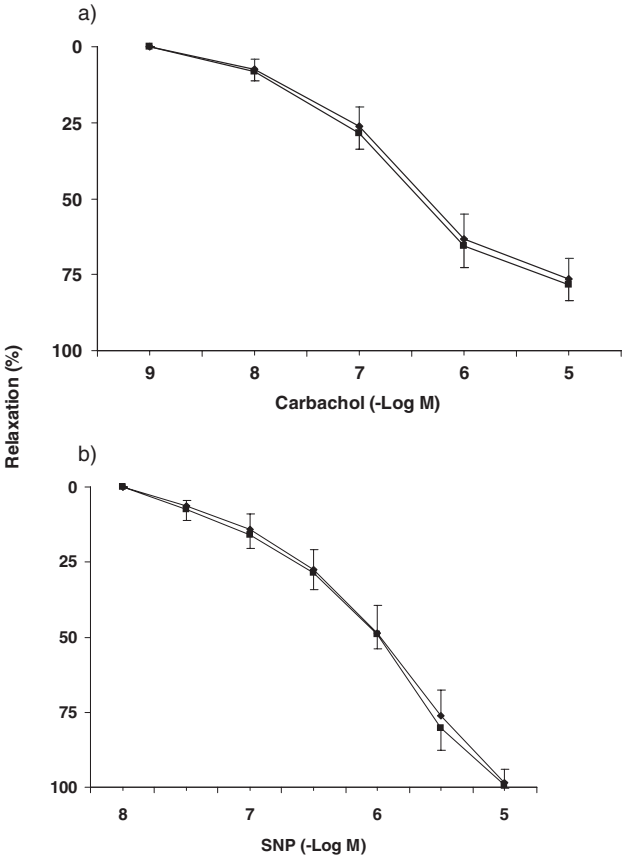


Fig. 1 Concentration–response curve for relaxation of isolated strips of rabbit corpus cavernosum (■) with and (◆) without platelet-activating factor in response to (a) carbachol and (b) sodium nitroprusside (SNP). Each point is expressed as a percentage of the contraction induced by 10^{-5} mol/L phenylephrine and is given as the mean ± SE. Six preparations were used for each group.

Table 1 Maximum relaxation response (E_{max}) and pD_2 values for carbachol and sodium nitroprusside (SNP) obtained on exposure to platelet-activating factor (PAF; 10^{-7} mol/L) in rabbit corpus cavernosum strips precontracted with phenylephrine. E_{max} values for electrical-field stimulation (EFS)-induced relaxations are also indicated in the presence of PAF (10^{-7} mol/L). Agonists produced no statistically significant effect on relaxation responses. Data are mean ± SE

	pD_2	E_{max}
Carbachol	6.74 ± 0.07	76.4 ± 5.2
Carbachol + PAF	6.82 ± 0.05	77.1 ± 6.2
SNP	5.62 ± 0.08	97.6 ± 2.3
SNP + PAF	5.71 ± 0.03	98.1 ± 1.8
EFS		73.2 ± 4.2
EFS + PAF		72.4 ± 2.1

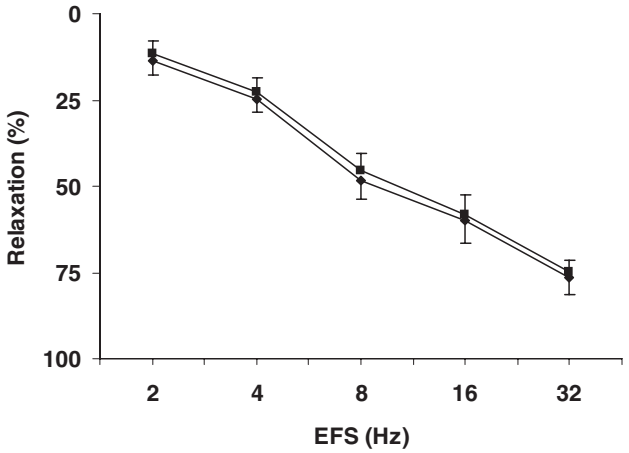


Fig. 2 Relaxation responses evoked by electrical field stimulation (EFS) on strips precontracted with phenylephrine (10^{-5} mol/L) (■) with and (◆) without platelet-activating factor. Each point is expressed as a percentage of the contraction induced by phenylephrine and is given as the mean ± SE. Six preparations were used for each group.

laboratory animals or humans. There are also no data in the literature indicating the existence of PAF in corporeal tissue. This is the first study examining the role of PAF in the modulation of the nitrergic system in corporeal tissue.

In the present study, PAF had no effect on relaxation amplitude or relaxation time in rabbit corpus cavernosum strips that were precontracted with phenylephrine. Endothelium-dependent, endothelium-independent and EFS-induced NANC relaxation responses were unchanged in the presence of PAF. These results suggest that PAF receptors are not involved in the modulation of the nitrergic system in rabbit corpus cavernosum.

It has been shown that PAF elicits endothelium-dependent relaxation in several vasculatures.[7,9] Evidence for the contribution of NO to the action of PAF is that PAF antagonists have been shown to attenuate or abolish PAF-induced relaxation and cGMP formation.[7] In this context, it has been reported that PAF receptors exist in endothelial cells.[8] With regard to the effects of PAF on smooth muscle (except for vascular smooth muscle), it has been found that PAF induces relaxation in guinea pig trachea. However, these relaxations have been attributed to the products of cyclooxygenase.[11] As has been mentioned above, the NO-mediated effects of PAF are prominent in vascular smooth muscle. When these studies are taken together, it is feasible to speculate that since the effects of PAF on the nitrergic system can vary between species and tissues, PAF receptors may not be available in rabbit corpus cavernosum. In order to clarify whether PAF has a modulatory role in the nitrergic system in corpus cavernosum, the effects of PAF should be investigated in other species.

According to various studies, the relaxant effect of PAF is exerted over a broad range of doses.[7,9,11] Therefore, it is possible that in the present study the dose used was not adequate to induce a response in rabbit corpus cavernosum strips. This may be another explanation for the lack of effect of PAF.

In conclusion, these results suggest that PAF does not take part in modulation of the nitrergic system in rabbit corpus cavernosum in *in vitro* conditions.

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