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Synergistic interaction of endogenous platelet-activating factor and vasopressin in generating angina in rats

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Abstract

We examined the involvement of endogenous vasopressin and platelet-activating factor (PAF) in the pathogenesis of two types of experimental angina in urethane-anaesthetised male Wistar rats. In the first model, epinephrine (10 μg kg^{-1}) was injected into the tail vein, followed at the development of the maximum blood pressure response, i.e., 30 s later, by phentolamine (15 mg kg^{-1}). In the second model, the vasopressin V_1 receptor agonist ornithine-vasopressin (ornipressin; 0.5 IU kg^{-1}, i.v.) was administered. The heart rate, mean arterial blood pressure and surface electrocardiogram (ECG, standard lead II) were registered simultaneously. As a measure of myocardial ischaemia, at 1 min after phentolamine or ornipressin administration, we found significant ST-segment depression, lasting for more than 10 or 5 min, respectively. Pretreatment (15 min, s.c.) with the vasopressin V_1 receptor antagonist Mca^1,$Tyr(Me)^2$AVP (the Manning peptide; 0.02–0.2 μg kg^{-1}) or the platelet-activating factor receptor antagonist ginkgolide B (BN 52021; 0.25–2.5 mg kg^{-1}) alone caused a dose-dependent reduction of the ST-segment depression. Concurrent administration of the two antagonists in their threshold doses (0.02 μg kg^{-1} and 0.25 mg kg^{-1}) also attenuated the ST-segment depression in both models. Neither antagonist affected the blood pressure or heart rate changes throughout the studies. Our results suggest that endogenous vasopressin and platelet-activating factor interact synergistically in provoking myocardial ischaemia in vivo in experimental angina in the rat.

Keywords: Platelet-activating factor; Vasopressin; Experimental angina; Synergistic interaction; Pathogenesis of myocardial ischaemia

1. Introduction

The platelet-activating factor (PAF) is a potent phospholipid mediator released from inflammatory cells in response to different immunologic and nonimmunologic stimuli. In vitro animal studies have implicated PAF as a major mediator in coronary artery constriction, the modulation of myocardial contractility and the generation of arrhythmias, processes involved in such cardiac disorders as ischemic cardiac disease, myocardial infarction and sudden cardiac death (Feuerstein et al., 1997). However, in vivo animal studies have furnished conflicting results. For example, low doses of intracoronially administered PAF produced coronary vasodilatation in the dog (Jackson et al., 1986). In contrast, the systemic administration of PAF provoked coronary vasoconstriction in the same species (Sybertz et al., 1985). The effects of PAF and its analogues are thought to be mediated by specific cell surface receptors (Shimizu et al., 1992). At present, there is no conclusive evidence of PAF receptor heterogeneity on either molecular or pharmacological grounds. A number of natural products have been identified as PAF receptor antagonists, including ginkgolide B (BN 52021; Braquet, 1985). Unfortunately, there are only a limited number of animal models, in which PAF antagonists have demonstrated consistent efficacy (Feuerstein et al., 1997).

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Arginine-vasopressin (vasopressin) is a neurohypophyseal nonapeptide hormone that is released into the circulation by a variety of stimuli; it has vasopressor and antidiuretic effects (Vittet et al., 1986; Jard, 1988). It promotes the reabsorption of water in the renal tubular cells through the activation of its antidiuretic (V_2) receptors (Michell et al., 1979). Most of the remaining actions of vasopressin on vasoconstriction, platelet adhesion and coronary smooth muscle cell proliferation are mediated via its pressor (V_1) receptors. In the heart, vasopressin, at physiological concentrations causes dose-dependent coronary constriction, myocardial depression and coronary smooth muscle cell proliferation, actions reversed by vasopressin V_1 receptor antagonists (Boyle and Segel, 1986, 1990; Bax et al., 1995; Tahara et al., 2002). Of the peptide-type vasopressin V_1 receptor antagonists, Mca^1,Tyr $(Me)^2$AVP (the Manning peptide) has generally been used in both animal and human studies (László et al., 1991).

In the present study, we evaluated the actions and interactions of the local pro-inflammatory mediator PAF and the circulating hormone vasopressin on the development of cardiac ischaemia in vivo, by using specific receptor antagonists in two novel models of experimental angina in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 230–270 g, which were fed standard laboratory chow and which received water ad libitum, were used throughout the study. All manipulations were performed in accordance with the standards of the European Community guidelines on the care and use of laboratory animals and were approved by the institutional ethics committees.

2.2. Experimental protocol

The animals were anaesthetized with urethane (1.6 g kg^{-1}, i.p.). Cannulae were inserted into the tail vein and trachea for the administration of drugs and the maintenance of spontaneous respiration, respectively. The animals were placed on a heating blanket (Harvard Instruments, UK), and the core temperature was maintained at 37 °C. Via a cannula inserted into the left carotid artery, connected to a pressure transducer, the mean arterial blood pressure and heart rate were registered continuously by the HAEMOSYS computerized complex haemodynamic analysis system (Experimetria, London, UK). Simultaneously, the standard limb lead II of the surface electrocardiogram (ECG) was recorded by the HAEMOSYS system. The change in the ST segment was measured and used as the index of angina severity. The mean ECG voltage 13 ms after the peak of the S wave was defined as the value of the ST segment, as described previously (Mori et al., 1995). The difference in amplitude of the ST segment after and before the administration of angina-provoking agents was calculated and expressed as the depression of the ST segment in mV.

2.3. Experimental angina provoked by ornipressin

In the ornipressin model, a bolus injection of the selective vasopressin V_1 receptor agonist, ornithine-vasopressin (ornipressin, 0.5 IU kg^{-1}, dissolved in 0.2 ml saline) was administered into the tail vein over 2 s. The ECG, heart rate and blood pressure changes were recorded simultaneously.

2.4. Experimental angina provoked by epinephrine plus phentolamine

In the epinephrine plus phentolamine model, a single dose of epinephrine (10 μg kg^{-1}) was administered into the tail vein of rats, followed at the time of the maximum blood pressure response, i.e., 30 s later, by the α-adrenoceptor antagonist phentolamine (15 mg kg^{-1}). Each agent was dissolved in 0.2 ml of physiological saline and injected over 2 s. The ECG, heart rate and blood pressure changes were recorded simultaneously.

2.5. Administration of PAF and vasopressin antagonists alone or in combination

Different groups of animals received the PAF receptor antagonist BN 52021 (0.25–2.5 mg kg^{-1}, s.c.) or the vasopressin V_1 receptor antagonist Manning peptide (0.02–0.2 μg kg^{-1}, s.c.). The antagonists were diluted in saline, in a volume of 0.2 ml, and were injected 15 min before the provocation of angina. The dose ranges and the route and time of administration of the two antagonists were selected on the basis of previous studies (László and Whittle, 1994; László et al., 1994; Varga et al., 1998).

A separate group of animals were injected with the threshold doses of the two antagonists in separate syringes into different sites at the same time (15 min before angina induction), by the same route (s.c.) and in the same volume (0.2 ml). The threshold doses of the PAF antagonist (0.25 mg kg^{-1}) and the vasopressin antagonist (0.02 μg kg^{-1}) were established in the dose–response studies in the present work.

In the ornipressin model, we performed an autocontrol experiment: control angina was provoked by ornipressin; this was followed by antagonist administration 30–35 min later, when the ornipressin-provoked blood pressure, heart rate and ST-segment changes had returned to the baseline for a minimum of 10 min; finally, another bolus injection of ornipressin was administered 15 min after the antagonist.

In the epinephrine plus phentolamine model, all the animals were subjected to only one provocation, because of the long-term (more than 60 min) profound action of phentolamine on the blood pressure and heart rate.

2.6. Chemicals

Ornithine-vasopressin, epinephrine, phentolamine, BN 52021 and the Manning peptide were purchased from Sandoz Pharma (Switzerland), Gedeon Richter (Hungary), Novartis Pharma (Switzerland), Beaufour Ipsen (France) and Bachem (Switzerland), respectively. All nonspecified agents were from Sigma.

2.7. Statistics

The data are expressed as the means±S.E.M. of the results on n rats per experimental group. The data were analysed by the Tukey–Kramer Multiple Comparison test, a level of $P<0.05$ being taken as significant.

3. Results

3.1. Ornipressin-induced cardiac ischaemia

Within 30 s after the administration of ornipressin, the blood pressure increased significantly from the baseline 89±3 mm Hg to its maximum, 158±2 mm Hg (Fig. 1). In parallel, the heart rate decreased significantly, from 353±19 to 270±12 beats min^{-1}(Fig. 1). The blood pressure returned to the baseline after 20 min, and the heart rate after 10 min. The maximum depression of the ST segment was observed 3 min after ornipressin administration (Fig. 1), and the amplitude returned to the baseline after 7 min. No cardiac arrhythmia was detected following the ornipressin challenge.

3.2. Epinephrine plus phentolamine-induced cardiac ischaemia

In the epinephrine plus phentolamine model, the mean arterial blood pressure increased significantly from the baseline 88±4 mm Hg and reached its maximum of 162±4 mm Hg within 10 s after the intravenous bolus administration of epinephrine (Fig. 2). In parallel, the heart rate fell from the baseline 302±14 to 193±20 beats min^{-1} (Fig. 2). Administration of the antagonist phentolamine at the time of the maximum blood pressure response following epinephrine (30 s) caused a fall in the mean arterial blood pressure, which reached its maximum (50±2 mm Hg) after 60 s (Fig. 2). Simultaneously, the heart rate rose (381±10 beats min^{-1}), as demonstrated in Fig. 2. These haemodynamic changes lasted for more than 60 min following phentolamine injection. The administration of epinephrine alone raised the mean arterial blood pressure, decreased the heart rate and provoked atrial fibrillation and ventricular extrasystoles (these actions lasted for up to 10 min), but it did not cause a significant ST-segment depression (n=10; data are not shown). However, when phentolamine was administered 30 s after epinephrine, the cardiac arrhythmias disappeared and a significant ST-segment depression developed, which reached its maximum level after 1 min (Fig. 2), and the amplitude of the ST segment returned to the baseline after more than 10 min.

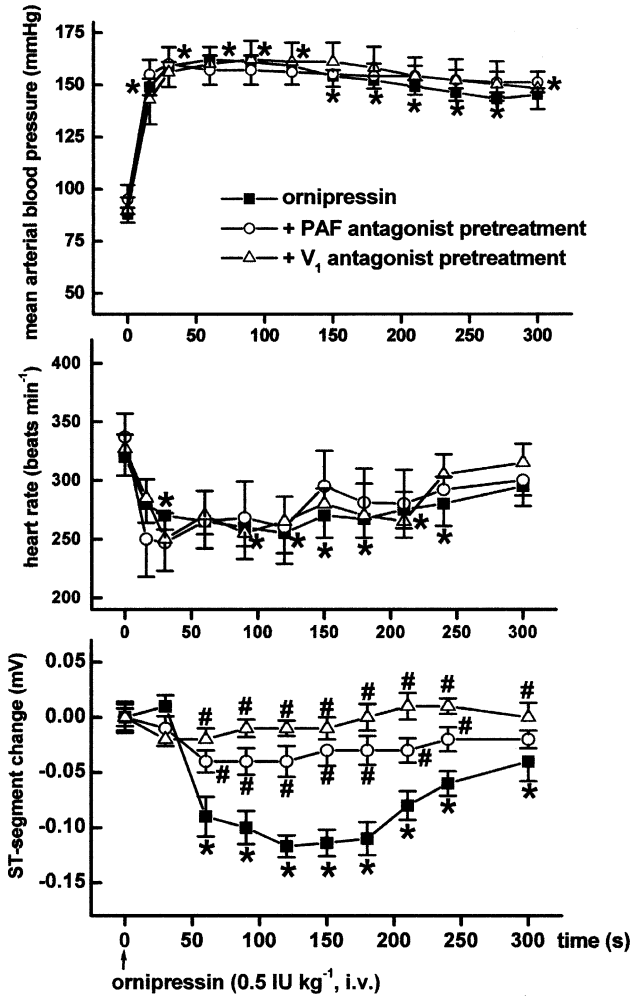

Fig. 1. Effects of an intravenous bolus injection of ornithine-vasopressin (ornipressin; 0.5 IU kg^{-1}; black squares) on mean arterial blood pressure (expressed in mm Hg; upper panel), heart rate (expressed in beats min^{-1}; middle panel) and ST-segment change on surface lead II ECG (expressed in mV; lower panel) in rats over a 5-min period. Actions of platelet-activating factor (PAF) antagonist BN52021 (2.5 mg kg^{-1}; open circles) or vasopressin V_1 receptor antagonist Manning peptide (0.2 μg kg^{-1}; open triangles) pretreatment (15 min, s.c.) on ornipressin-provoked changes in haemodynamics and ST segment. Data are means±S.E.M. of the results on 10–12 rats per experimental group. * indicates significant differences ($P<0.05$) between the baseline (0 min) values and the values following ornipressin injection, and # indicates significant differences ($P<0.05$) between the ornipressin values with or without pretreatment with the PAF or vasopressin V_1 receptor antagonist.

3.3. Actions of PAF antagonist on ornipressin- or epinephrine plus phentolamine-induced cardiac ischaemia

Pretreatment (15 min, s.c.) with the PAF antagonist BN 52021 (2.5 mg kg^{-1}) did not affect the mean arterial blood pressure and heart rate changes either following ornipressin

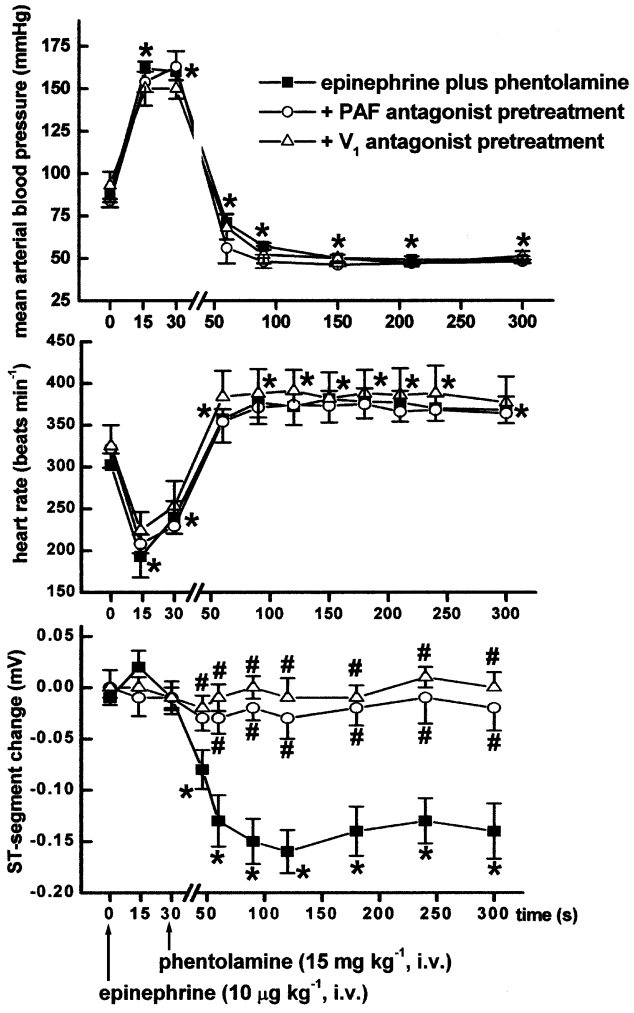

Fig. 2. Effects of intravenous bolus injections of epinephrine (10 μg kg^{-1}) plus (30 s later) phentolamine (15 mg kg^{-1}) on mean arterial blood pressure (expressed in mm Hg; upper panel), heart rate (expressed in beats min^{-1}; middle panel) and ST-segment change on surface lead II ECG (expressed in mV; lower panel) in rats over a 5-min period (black squares). Actions of platelet-activating factor (PAF) antagonist BN52021 (2.5 mg kg^{-1}; open circles) or vasopressin V_1 receptor antagonist Manning peptide (0.2 μg kg^{-1}; open triangles) pretreatment (15 min, s.c.) on epinephrine plus phentolamine-provoked changes in haemodynamics and ST segment. Data are means±S.E.M. of results on 10–12 rats per experimental group. * indicates significant differences (P<0.05) between the baseline (0 min) and following epinephrine plus phentolamine, and # indicates significant difference (P<0.05) between the epinephrine plus phentolamine values with or without pretreatment with the PAF or vasopressin V_1 receptor antagonist.

or after epinephrine plus phentolamine administration, but it decreased the ST-segment depression in each experimental angina model (Figs. 1 and 2). The ST-segment depression reduction following BN52021 pretreatment (0.25–2.5 mg kg^{-1}) was dose-dependent. The most effective doses of BN52021 in the ornipressin model or in the epinephrine plus phentolamine model were 1 mg kg^{-1} (maximum efficacy: 69±11%; n=11; P<0.005) or 2.5 mg kg^{-1} (maximum efficacy: 79±14%; n=10; P<0.01), respectively (Fig. 3).

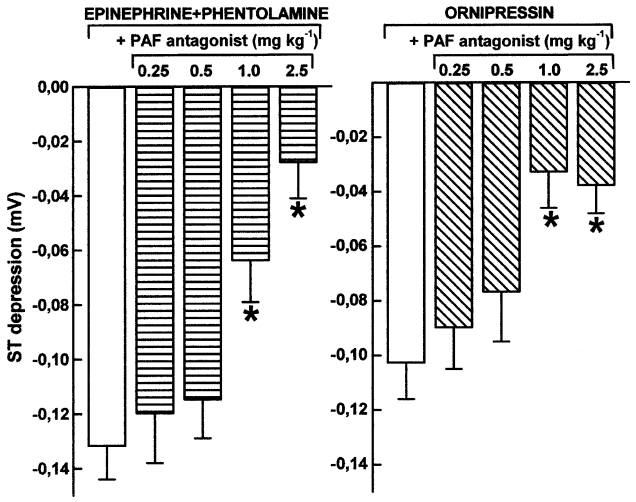

Fig. 3. Dose-dependent reductions of ST-segment depression (lead II surface ECG; expressed in mV) provoked by epinephrine (10 μg kg^{-1}, i.v.) plus (30 s later) phentolamine (15 mg kg^{-1}, i.v.; left panel) or ornithine-vasopressin (ornipressin; 0.5 IU kg^{-1}, i.v.; right panel) by the pretreatment (15 min) with the platelet-activating factor (PAF) receptor antagonist BN52021 (0.25–2.5 mg kg^{-1}). The ST-segment depression was measured 3 min after angina induction. Data are means±S.E.M. of the results on 7–12 rats per experimental group, where * indicates significant reductions (P<0.05) of the ST-segment depression.

3.4. Actions of vasopressin antagonist on ornipressin- or epinephrine plus phentolamine-induced cardiac ischaemia

Subcutaneous administration of the vasopressin V_1 receptor antagonist Manning peptide (0.2 μg kg^{-1}, s.c.) 15 min before the provocation of angina did not cause significant changes in the ornipressin- or epinephrine plus

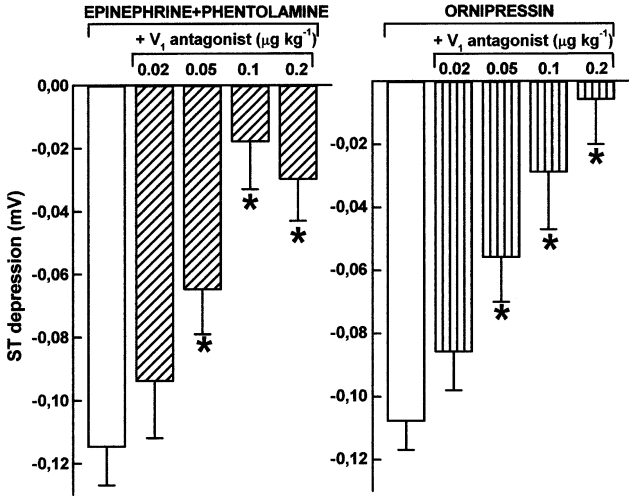

Fig. 4. Dose-dependent reductions of ST-segment depression (lead II surface ECG; expressed in mV) provoked by epinephrine (10 μg kg^{-1}, i.v.) plus (30 s later) phentolamine (15 mg kg^{-1}, i.v.; left panel) or ornithine-vasopressin (ornipressin; 0.5 IU kg^{-1}, i.v.; right panel) by the pretreatment (15 min) with the vasopressin V_1 receptor antagonist Manning peptide (0.02–0.2 μg kg^{-1}). The ST-segment depression was measured 3 min after angina induction. Data are means±S.E.M. of the results on 8–12 rats per experimental group, where * indicates significant reductions (P<0.05) of the ST-segment depression.

phentolamine-induced mean arterial blood pressure and heart rate responses (Figs. 1 and 2). However, it reduced the ST-segment depression significantly in both models (Figs. 1 and 2). The mitigation of the ST-segment depression by the vasopressin V_1 receptor antagonist was dose-dependent. The highest efficacies of the vasopressin antagonist in the ornipressin and in the epinephrine plus phentolamine model were 94±13% (at the 0.2 μg kg^{-1} dose; n=12; P<0.001) and 84±19% (at the 0.1 μg kg^{-1} dose; n=8; P<0.005), respectively (Fig. 4).

3.5. Interactions of PAF and vasopressin antagonists in ornipressin- or epinephrine plus phentolamine-induced cardiac ischaemia

To study the possible interaction of endogenous PAF and vasopressin, we administered the PAF antagonist concurrently with the vasopressin antagonist 15 min before angina provocation, in their threshold doses of 0.25 mg kg^{-1} and 0.02 μg kg^{-1}, respectively. We found no significant changes in the blood pressure and heart rate responses either following ornipressin or after epinephrine plus phentolamine administration (data not shown). However, the degree of the ST-segment depression was reduced in both models, with efficacies of 70±9% (n=10; P<0.01) and 56±10% (n=8; P<0.05) in the ornipressin model and epinephrine plus phentolamine model, respectively, as demonstrated in Fig. 5.

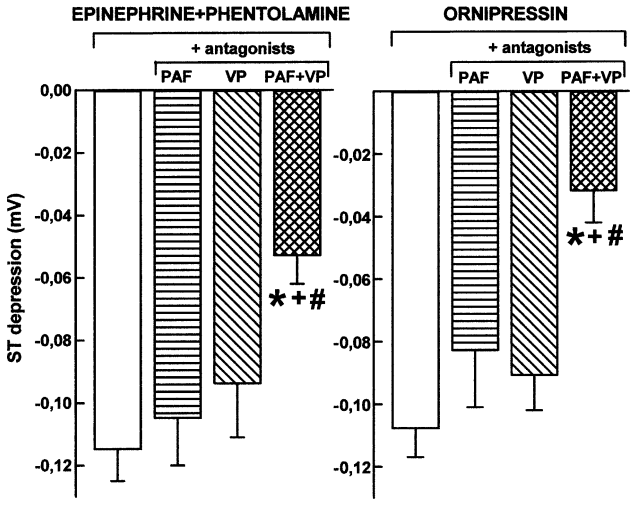

Fig. 5. Synergistic interaction of threshold doses of platelet-activating factor (PAF) receptor antagonist BN52021 (0.25 mg kg^{-1}, s.c.) and vasopressin (VP) V_1 receptor antagonist Manning peptide (0.02 μg kg^{-1}, s.c.) pretreatment (15 min) in the reductions of ST-segment depression (lead II surface ECG; expressed in mV). The ST-segment depression was provoked by epinephrine (10 μg kg^{-1}, i.v.) plus (30 s later) phentolamine (15 mg kg^{-1}, i.v.; left panel) or ornithine-vasopressin (ornipressin; 0.5 IU kg^{-1}, i.v.; right panel), and measured 3 min after angina induction. Data are means±S.E.M. of the results on 8–12 rats per experimental group, where * indicates significant differences (P<0.05) between PAF+VP antagonist and angina-provoking agent(s) alone, $^{+}$ indicates significant differences (P<0.05) between PAF+VP antagonist and angina-provoking agent (s)+PAF antagonist alone, and $^{\#}$ indicates significant differences (P<0.05) between PAF+VP antagonist and angina-provoking agent(s)+VP antagonist alone.

3.6. Control experiments

In both experimental angina models, administration of the solvent (physiological saline) of the angina-provoking agents, at the same time, by the same route and in the same volume as described above, did not affect the mean arterial blood pressure, heart rate or ST segment (n=4; data not shown).

An intravenous bolus injection of the vasopressin V_1 receptor antagonist (0.2 μg kg^{-1}) immediately before ornipressin (0.5 IU kg kg^{-1}, i.v.) administration reduced the ornipressin-induced increase in mean arterial blood pressure by 50% (n=4; data not shown).

Subcutaneous administration of the solvent (physiological saline) of the PAF or the vasopressin antagonist did not affect the ornipressin or epinephrine plus phentolamine-provoked ST-segment depression in the control animals (n=4; data not shown).

Subcutaneous administration of the PAF or the vasopressin receptor antagonists alone did not affect the baseline mean arterial blood pressure, the heart rate or the amplitude of the ST segment during the 15-min pretreatment period (n=7–12; data not shown).

4. Discussion

In the present work, we studied the actions and interactions of the PAF receptor antagonist BN 52021 and the vasopressin V_1 receptor antagonist Manning peptide in vivo in rats in two novel models of experimental angina, provoked by ornipressin and by epinephrine plus phentolamine.

The effective anti-anginal dose ranges of BN 52021 (0.25–2.5 mg kg^{-1}) and the Manning peptide (0.02–0.2 μg kg^{-1}) relate closely to those concentrations of these antagonists that had been used in previous studies. They were shown to prevent the vascular endothelial dysfunction provoked by high or low doses of endotoxin in vivo in rats (László and Whittle, 1994; László et al., 1994; Varga et al., 1998). Surprisingly, we found that subcutaneous administration of the vasopressin V_1 receptor antagonist 15 min before ornipressin did not affect the increase in mean arterial blood pressure. However, our control study demonstrated that an intravenous bolus injection of the highest dose (0.2 μg kg^{-1}) of the Manning peptide immediately before the administration of ornipressin reduced the mean arterial blood pressure elevation by 50%. It is in agreement with the previous observation of Kruszynski et al (1980) that the effective anti-vasopressor dose of the Manning peptide was 0.184 μg kg^{-1}. For the characterization of the relative potencies of novel vasopressin V_1 receptor antagonists, a standard in vivo bioassay method has been developed and

generally used, where the antagonist is given intravenously immediately before the agonist. The effective dose of a vasopressin antagonist is defined as the dose that reduces the response to the agonist to one-half of that given to the same dose of agonist administered in the absence of the antagonist (for a review, see László et al., 1991). In our present study, subcutaneous administration of the Manning peptide 15 min before ornipressin did not affect the increase in mean arterial blood pressure, probably because of its delayed absorption and widespread organ distribution.

In the ornipressin-induced angina model, we administered the specific vasopressin V_1 receptor agonist ornithine-vasopressin in order to induce an ST-segment depression in the surface ECG. Earlier studies had demonstrated that the coronary vasospasm provoked by the full vasopressin V_1/V_2 receptor agonist arginine-vasopressin in anaesthetized rats is useful in allowing an evaluation of the efficacy and potency of proposed antianginal drugs in vivo (Hiramatsu et al., 1970; Karasawa et al., 1988). A depression of the ST segment following a bolus injection of a high dose of vasopressin is considered to indicate subendocardiac ischaemia (Hiramatsu et al., 1970; Hatano et al., 1980; Kita et al., 1994). Moreover, vasopressin induces acute platelet retention and activation, e.g., it reverses the aspirin-provoked platelet dysfunction (Lethagen et al., 2000). In the coronary circulation, vasopressin evokes most of its effects through the vasopressin V_1-type receptors. Indeed, in our study, the selective vasopressin V_1 receptor agonist ornipressin led to an ST-segment depression in the surface ECG. The ornipressin-induced ischaemia exhibited similarities with the well-characterized full-agonist arginine-vasopressin model. Since, we registered similar degrees of ST-segment depression, blood pressure increase and heart rate decrease to those reported by other investigators in the arginine-vasopressin model (Uchida et al., 1993; Mori et al., 1995; Yamamoto et al., 2000). Moreover, the times of the ST-segment changes (the maximum ST-segment depression developed at 3 min and disappeared within 10 min following ornipressin administration) were closely similar to those in previous studies (Uchida et al., 1993; Mori et al., 1995; Yamamoto et al., 2000). Finally, in the present study, the administration of the vasopressin V_1 receptor antagonist caused a dose-dependent reduction of the ornipressin-provoked ST-segment depression, which appears to emphasize the pathogenic importance of vasopressin V_1 receptor activation in the development of angina.

In the epinephrine plus phentolamine-provoked angina model, we administered high doses of the physiological adrenoceptor agonist epinephrine, and at the time of the resulting maximum blood pressure increase we injected the α-adrenoceptor antagonist phentolamine. In response to phentolamine, we found that the blood pressure dropped and the heart rate increased virtually immediately, and within 1 min, an ST-segment depression appeared in the surface ECG. From certain aspects, our epinephrine plus phentolamine model closely relates to isoproterenol-induced experimental angina. Isoproterenol provokes an ST-segment depression because of both β_1- and β_2-adrenoceptor stimulation (Harada et al., 1993; Yamamoto et al., 2000). In our epinephrine plus phentolamine model, an important role of β-receptor activation is strongly suspected in the development of the ST-segment depression. Following stimulation of both the α- and β-receptors by high doses of epinephrine, we initiated the sudden and complete blockade of the α-receptors by the bolus injection of phentolamine, however, the haemodynamic changes were much more profound in the epinephrine plus phentolamine model as compared with the isoproterenol model. To some extent, the phentolamine plus epinephrine model may be associated with the well-known clinical situation in which angina or myocardial infarction develops, even with morphologically intact coronaries, because of the fall in blood pressure (Sobel, 1996). Finally, epinephrine acutely stimulates the platelets, causing rapid increments in arterial platelet count and volume (Lande et al., 1985), an additional process that may be involved in the cardiac ischaemia provoked by epinephrine plus phentolamine.

In in vitro experiments on Langendorf preparations of the rat heart, PAF has shown to induce coronary vasoconstriction (as measured by the decrease in coronary flow), which is attenuated by the PAF-receptor antagonist BN 52021 (Piper and Stewart, 1987). Montrucchio et al (1986) studied patients with coronary artery disease subjected to myocardial ischaemia, induced by atrial pacing. During pacing, PAF release was detected in blood samples taken from the coronary sinus and aorta, indicating the production and release of PAF from the heart during myocardial ischaemia in humans. Moreover, animal studies suggest that PAF antagonists may be useful in reducing myocardial infarct size, ischaemia-induced arrhythmias (Wainwright et al., 1988), post-ischaemic hypo-perfusion and depression of the myocardial function (Loucks et al., 1997). Our findings are in agreement with these results, since we found that administration of the PAF antagonist BN 52021 dose-dependently decreased the severity of cardiac ischaemia in two different models of experimental angina, which supports the aggressive role of PAF in the development of angina.

With an intact baroreceptor reflex, the infusion of arginine-vasopressin does not increase the blood pressure, except when it is given in very high concentration (100 ng ml^{-1} min^{-1}), although vasopressin is known to be an extremely potent arterial vasoconstrictor (Padfield et al., 1976). It is apparent from studies on both humans and dogs that, in the absence of autonomic reflex control, small changes in plasma arginine-vasopressin levels within the daily physiological range can exert significant vaso-

constrictor actions, these being associated with substantial elevations in arterial blood pressure (Cowley et al., 1983). It is interesting in this context that, at one time in clinical practice arginine-vasopressin was given as a provocative test for angina and coronary artery spasm in humans (Ruskin, 1947). Furthermore, other early investigators reported that the administration of high doses of arginine-vasopressin may lead to acute myocardial infarction, arrhythmias and sudden cardiac death in humans (Mills et al., 1949; Slotnik and Teigland, 1951). More recently, in prospective randomized clinical trials, it became clear that in the course of acute myocardial infarction, the degree of the early increase in the endogenous plasma arginine-vasopressin level is an independent predictor of such combined end-points of the clinical outcome as mortality, and the development of severe heart failure or myocardial re-infarction (Rouleau et al., 1994). In patients with advanced heart failure, where the circulating vasopressin level is high, administration of the vasopressin V_1/V_2 receptor antagonist conivaptan improves the clinical picture through its beneficial effects on the haemodynamics and urine output without acting on the blood pressure or heart rate (Udelson et al., 2001). In our study, the vasopressin V_1 receptor antagonist likewise displayed a protective effect against the development of cardiac ischaemia, which lends to support the aggressive role of vasopressin in the processes leading to angina.

Our experiments revealed that the concurrent administration of PAF and vasopressin V_1 receptor antagonists in low threshold doses could evoke a significant attenuation of cardiac ischaemia. This synergistic interaction of the local mediator PAF and the circulating hormone vasopressin in the development of myocardial ischaemia may involve a number of mechanisms. The liberation of PAF provokes platelet aggregation, leukocyte adhesion, the release of different vasoactive phospholipid mediators and coronary vasoconstriction (Braquet, 1985; Feuerstein et al., 1997). On the other hand, vasopressin stimulates the aggregation and secretion of human platelets in vitro and causes coronary vasoconstriction in vivo and in vitro. All of these effects of vasopressin develop via the activation of its vasopressin V_1 receptors (Thomas et al., 1983; Thibonnier and Woloschak, 1988), even at physiological vasopressin concentrations, and can be reversed by vasopressin V_1 receptor antagonists (Bax et al., 1995; Wun et al., 1996). It seems that through the activation of this complex system, endogenous PAF and vasopressin mutually intensify the aggressive effects of the other towards the generation of cardiac ischaemia.

In conclusion, our results suggest that endogenous vasopressin and PAF play aggressive roles in the ornipressin- or epinephrine plus phentolamine-induced angina models in vivo in rats. At lower release rates, they are capable of interacting synergistically in the course of the myocardial ischemic processes.

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