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Effects of a selective neuropeptide Y Y_1 receptor antagonist BIBP 3226 on double peaked vasoconstrictor responses to periarterial nerve stimulation in canine splenic arteries

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1 The periarterial electrical nerve stimulation (30 s trains of pulses at a frequency of 1, 4 or 10 Hz) induced a double peaked vasoconstriction consisting of an initial transient constriction (first peak) followed by a prolonged response (second peak) in the isolated, perfused canine splenic artery.

2 At low frequencies (1 and 4 Hz), a neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226 (0.1–1 μM) produced a dose-dependent inhibitory effect on the second peak, but did not modify the first peak. At a high frequency (10 Hz), 1 μM BIBP 3226 induced a slight, but significant inhibition on both the first and second peaked responses.

3 At a low frequency (1 Hz), the first peak was not influenced by blockade of α_1-adrenoceptors or NPY Y_1 receptors with prazosin (0.1 μM) or BIBP 3226 (1 μM), respectively, but abolished by P2X receptor desensitization with α,β-methylene ATP ($\alpha\beta$-m ATP, 1 μM). At a high frequency (10 Hz), the first peak was mostly inhibited by $\alpha\beta$-m ATP and partially by prazosin and BIBP 3226. On the other hand, the second peak at a low frequency was largely decreased by BIBP 3226 and partially by prazosin and $\alpha\beta$-m ATP, whereas at a high frequency, it was largely attenuated by prazosin and partially by $\alpha\beta$-m ATP and BIBP 3226.

4 The results suggest that at a low frequency, the firstly transient constriction of double peaked responses is mainly induced *via* an activation of P2X-receptors, whereas at a high frequency, it is mostly mediated by the P2X-receptors, and partially by α_1-receptors and NPY Y_1-receptors. The secondary prolonged vasoconstriction at frequencies used is predominantly mediated *via* both α_1-receptor and NPY Y_1 receptor activations, and in part by P2X-receptors. Furthermore, an activation of NPY Y_1 receptors may play an important role in evoking the prolonged vasoconstrictor response to longer pulse trains of stimulation at a low frequency, whereas an α_1-adrenoceptor activation exerts a main vasomotor effect for the prolonged response at a high frequency.

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Abbreviations: ATP, adenosine 5′-triphosphate; BIBP 3226, (R)-N^2-(diphenylacetyl)-N-[(4-hydroxyphenyl]methyl-argininamide; ES, electrical periarterial nerve stimulation; LP-NPY, Leu^{31} Pro^{34} neuropeptide Y; $\alpha\beta$-m ATP, α,β-methylene ATP; NA, noradrenaline; NPY, neuropeptide Y

Introduction

The evidence support the idea that neuropeptide Y (NPY) acts as a cotransmitter with noradrenaline (NA) and adenosine 5′-triphosphate (ATP) in the control of peripheral sympathetic vascular tone (Fried *et al*., 1986; Donoso *et al*., 1997a,b; Han *et al*., 1998; Phillips *et al*., 1998; Racchi *et al*., 1999). Further observations indicate that endogenous NPY accounts for the long-lasting component of the sympathetic vasoconstriction in response to nerve stimulation (Lundberg & Modin, 1995; Malmström *et al*., 1996; 1997; Racchi *et al*., 1997). Recently, a potent and selective non-peptide Y_1 receptor antagonist BIBP 3226 was developed (Rudolf *et al*., 1994). Using BIBP 3226 as a functional NPY Y_1 receptor antagonist, it is demonstrated that NPY Y_1 receptors dominantly mediated the direct and indirect vascular contractile effects of endogenous NPY (Lundberg & Modin, 1995; Malmström & Lundberg, 1995; Westfall *et al*., 1996; Donoso *et al*., 1997a,b; Han *et al*., 1998; Racchi *et al*., 1999). In the canine splenic artery, the time course of vasoconstrictor response to pulse trains of up to 30 s duration of stimulation appears to be double peaked vasoconstrictor responses consisting of an initial, transient constriction (first peak) followed by a prolonged response (second peak) (Yang & Chiba, 1998). It is reported that the first peaked constriction might have mainly a purinergic component, and the second peak response, mostly an adrenergic component, because the first peak was mostly inhibited by α,β-methylene ATP ($\alpha\beta$-m ATP), a P2X receptor desensitizing agent, whereas the second peak dominantly by prazosin, an α_1-adrenoceptor antagonist (Yang & Chiba, 1998). However, an α_1-adrenoceptor blockade with prazosin has been found to antagonize NA-induced potentiation of NPY vasomotor response of rat mesenteric arterial bed, suggesting that an α_1-adrenoceptor blockade is able to modify the postjunctional cooperation between NPY and NA (Cortés *et al*., 1999). It is therefore hypothesized that an activation of NPY Y_1 receptor may participate into the vasoconstrictor responses to periarterial nerve stimulation in the canine splenic artery. In this study, we tried to examine this hypothesis by determining the effects of blockade of NPY Y_1 receptors with BIBP 3226 on the double peaked vasoconstrictor responses to periarterial electrical nerve stimulation in the sympathetically innervated canine splenic artery.

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Methods

Arterial preparations

Mongrel dogs of either sex, weighing 10–16 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1} i.v.). After treatment with sodium heparin (200 u kg^{-1} i.v), the dogs were killed by rapid exsanguination from the right femoral artery. The arterial main branches of the splenic artery were isolated, and side branches of the artery were tied with silk threads. Then, the artery (1–1.2 mm in an outer diameter) was cut into segments (15–20 mm in length), and only four segments were obtained from each splenic artery. Each segment was cannulated and set up for perfusion as described previously (Hongo & Chiba, 1983; Tsuji & Chiba, 1984). Briefly, a stainless steel cannula was inserted into the arterial segment from the distal to the proximal end. A proximal portion of the segment was fixed to the distal portion of a needle-type cannula with silk threads. The cannula was 3–4 cm long and 0.8–1.0 mm in an outer diameter and had small side holes 5 mm from the distal sealed end. The cannulated arterial segment was placed in a cup-shaped glass bath and was perfused by a roller pump (Tokyo Rikakikai, Tokyo, Japan) with Krebs-Henseleit solution gassed with 95% O_2 and 5% CO_2. The solution contained (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2 $NaHCO_3$ 25 and glucose 10. The flow rate was kept at approximately 2 ml min^{-1}. The perfusion pressure was continuously measured with an electric manometer (MPU-0.5A, Nihon Kohden, Tokyo, Japan) and recorded with a rectigraph (WT-685G, Nihon Kohden, Tokyo, Japan). After a stabilization period of 1 h, the preparation was removed from the bath solution and fixed in a horizontal position. The preparation was perfused at a constant flow rate during the experiment. The basal perfusion pressure was within 40–80 mmHg.

For electrical stimulation of the periarterial sympathetic nerve terminals, two platinum electrodes were placed on the extraluminal side of the arterial wall. Electrical stimulation was delivered by an electric stimulator (SEN-7203, Nihon Kohden) using 30 s trains of pulses at 10 V amplitude, 1 ms pulse duration, in a frequency range of 1, 4 and 10 Hz. The organ bath was sealed with the plastic film to maintain the preparation at 37°C. Ten minute intervals between electrical stimulation periods were needed to obtain the reproducible response. The intervals between frequency-response curves were over 1 h. The preparations were incubated for 1 h with all antagonists used before the next response curves were made for electrical stimulation. The drug solution for ATP, NA, Leu^{31} Pro^{34} NPY (LP-NPY) and NPY were administered into the rubber tubing close to the cannula in a volume of 0.01–0.03 ml, by use of microinjectors (Terumo, Tokyo, Japan).

Drugs

Drugs used were adenosine 5′-triphosphate disodium salt; α,β-methylene adenosine 5′-triphosphate lithium salt; prazosin hydrochloride; *dl*-noradrenaline hydrochloride (Sigma, St. Louis, U.S.A.); Leu^{31} Pro^{34} neuropeptide Y, human rat; neuropeptide Y, human, rat (Research Biochemicals International, Natick, MA, U.S.A.). BIBP 3226 was kindly provided by Boehringer Ingelheim Pharma KG, Biberach, Germany. Leu^{31} Pro^{34} neuropeptide Y and neuropeptide Y were dissolved in 0.5% (w v^{-1}) bovine serum albumin in distilled water before the start of the experiment. Other drugs were dissolved in distilled water. The stock solutions were kept at −20°C until used.

Statistical analysis

Vasoconstrictor responses to electrical stimulation are expressed as the maximal changes in perfusion pressure (mmHg) from their basal levels. The data are shown as mean ± s.e.mean. An analysis of variance with Bonferroni's test was used for the statistical analysis of multiple comparisons of data. *P* values less than 0.05 were considered statistically significant.

Results

Vascular responses to periarterial electrical nerve stimulation

The periarterial electrical nerve stimulation (30 s trains of pulses at a frequency of 1, 4 or 10 Hz) induced a double peaked vasoconstriction consisting of an initial transient constriction followed by a prolonged contractile response (Figures 1A and 3A), as reported previously (Yang & Chiba,

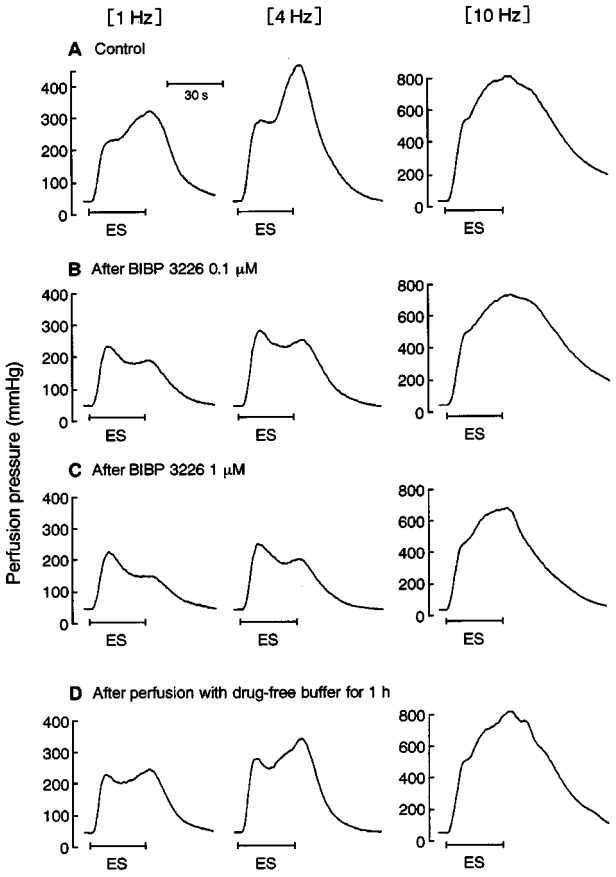

Figure 1 Double peaked vasoconstrictor responses to periarterial electrical nerve stimulation and the effects of BIBP 3226 in an isolated, perfused canine splenic artery. The double peaked vasoconstrictions were induced by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz (A). At low frequencies (1 and 4 Hz), the perfusion with 0.1 μM BIBP 3226 produced a clear inhibition on the second peaked constrictions (B), and a 10 fold increase of dose of BIBP 3226 (1 μM) exerted its greater inhibition (C), but BIBP 3226 at doses used did not affect the first peaked responses (B and C). At a high frequency (10 Hz), the first and second responses were not influenced by the treatment with a lower dose of BIBP 3226 (0.1 μM) (B), but obviously inhibited by a larger dose of BIBP 3226 (1 μM) (C). The BIBP 3226-induced inhibition was partially reversed by the perfusion with drug-free buffer for 1 h (D). (ES), Electrical nerve stimulation.

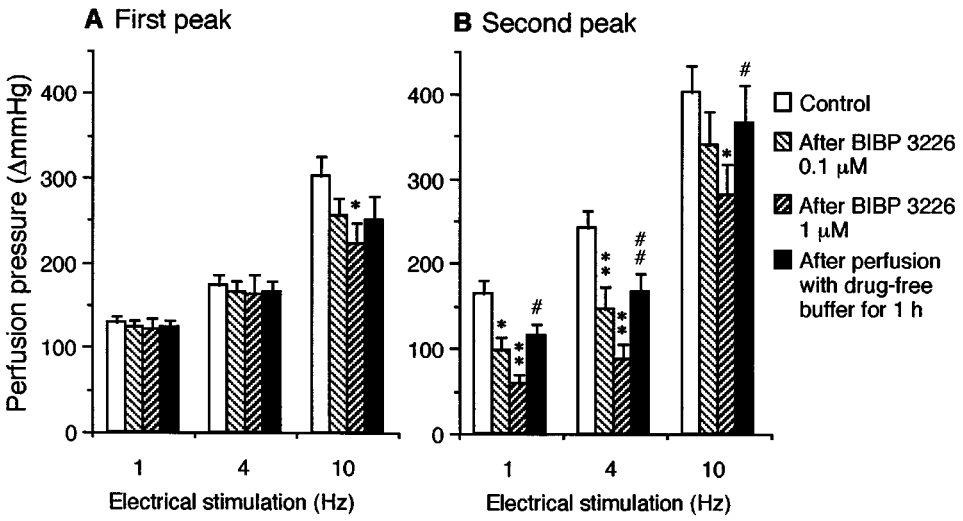

Figure 2 Effects of BIBP 3226 on the first (A) and the second peak (B) of the vasoconstrictor responses to periarterial electrical nerve stimulation, and reversed effect of perfusion with drug-free buffer for 1 h in the canine splenic arteries. The vessels were electrically stimulated by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. Data are presented as mean ± s.e.mean ($n=8$). *$P<0.05$; **$P<0.01$ as compared with the control group. #$P<0.05$; ##$P<0.01$ as compared with the preceding group.

1998). The first peak of vasoconstriction reached within 8–12 s, and the second peak within 30–35 s after the onset of electrical stimulation as shown in Figures 1A and 3A.

Effects of BIBP 3226 on the vasoconstrictor responses to electrical nerve stimulation

Figure 1 shows an original tracing of contractile force responses from typical experiments showing the effects of BIBP 3226 on the double peaked vasoconstrictor responses to nerve stimulation at 1, 4 and 10 Hz. Figure 2 shows the summarized data of effects of BIBP 3226 on the first peak (A) and the second peak (B). As shown in Figure 2, the treatment with BIBP 3226 (0.1–1 μM) produced a dose-dependent inhibition on the second peaked vasoconstrictor responses to nerve stimulation at low frequencies (1 and 4 Hz), but did not affect the first peaked responses. On the other hand, BIBP 3226 at a higher dose (1 μM) caused a slight but significant inhibition on both the first and second peaked responses to a high frequency of stimulation (10 Hz). At a low frequency (1 Hz), after treatment with 1 μM BIBP 3226 the second peak was inhibited by 63%, whereas at a high frequency (10 Hz) by 30% (Figure 2B). BIBP 3226-induced inhibition was partially reversed by the perfusion with drug-free buffer for 1 h (Figures 1 and 2).

Effects of αβ-m ATP and prazosin on the vasoconstrictor responses to electrical nerve stimulation in the presence of BIBP 3226

The treatment with 0.1 μM prazosin abolished the vasoconstrictor responses to exogenous NA (0.01–1 nmol), but did not influence the contractile responses to ATP (0.01–1 μmol). On the other hand, the perfusion with 1 μM $\alpha\beta$-m ATP blocked the contractile responses to ATP, but not those to NA, as reported previously (Yang & Chiba, 1998). Figure 3 shows an original tracing of contractile force responses from typical experiments showing the effects of $\alpha\beta$-m ATP and prazosin on the double peaked vasoconstrictor responses to nerve stimulation at 1, 4 and 10 Hz after treatment with 1 μM BIBP 3226. Figure 4 shows the summarized data of effects of $\alpha\beta$-m ATP and prazosin on the first peak (A) and the second peak (B) in the presence of 1 μM BIBP 3226. As shown in Figure 4, at a low frequency of 1 Hz, the application of $\alpha\beta$-m ATP (1 μM) totally abolished the first peak and the remaining second peaked responses after treatment with 1 μM BIBP 3226. At a high frequency of 10 Hz, the first peak in the presence of 1 μM BIBP 3226 was suppressed by $\alpha\beta$-m ATP (1 μM), whereas the second peak was not affected. A subsequent application of 0.1 μM prazosin blocked the remaining responses after treatment with BIBP 3226 and $\alpha\beta$-m ATP.

Effects of prazosin and αβ-m ATP on the vasoconstrictor responses to electrical nerve stimulation in the presence of BIBP 3226

As shown in Figure 5, at low frequencies (1 and 4 Hz), prazosin (0.1 μM) did not affect the first peaked responses after treatment with 1 μM BIBP 3226, but significantly inhibited the second peaked constrictions. On the other hand, at a high frequency of 10 Hz, prazosin strongly decreased both the first peak and the second peak responses in the presence of BIBP 3226. The remaining responses after treatment with BIBP 3226 and prazosin were abolished by a subsequent application of 1 μM $\alpha\beta$-m ATP (Figure 5).

Effects of BIBP 3226 and prazosin on the vasoconstrictor responses to electrical nerve stimulation in the presence of αβ-m ATP

The perfusion with 1 μm $\alpha\beta$-m ATP induced a transient increase of perfusion pressure and returned to its original level for 60 min. As shown in Figure 6, the nerve-stimulated first peaked response at a low frequency of 1 Hz was almost completely inhibited by treatment with 1 μM $\alpha\beta$-m ATP, whereas at a high frequency of 10 Hz, it was partially decreased. On the other hand, the second peaked responses at low frequencies (1 and 4 Hz) were slightly but significantly attenuated by $\alpha\beta$-m ATP, but the response at a high frequency of 10 Hz was rather unaffected by it (Figure 6). Furthermore, at low frequencies (1 and 4 Hz), BIBP 3226 (1 μM) strongly inhibited the second peaked responses after treatment with $\alpha\beta$-m ATP, but did not

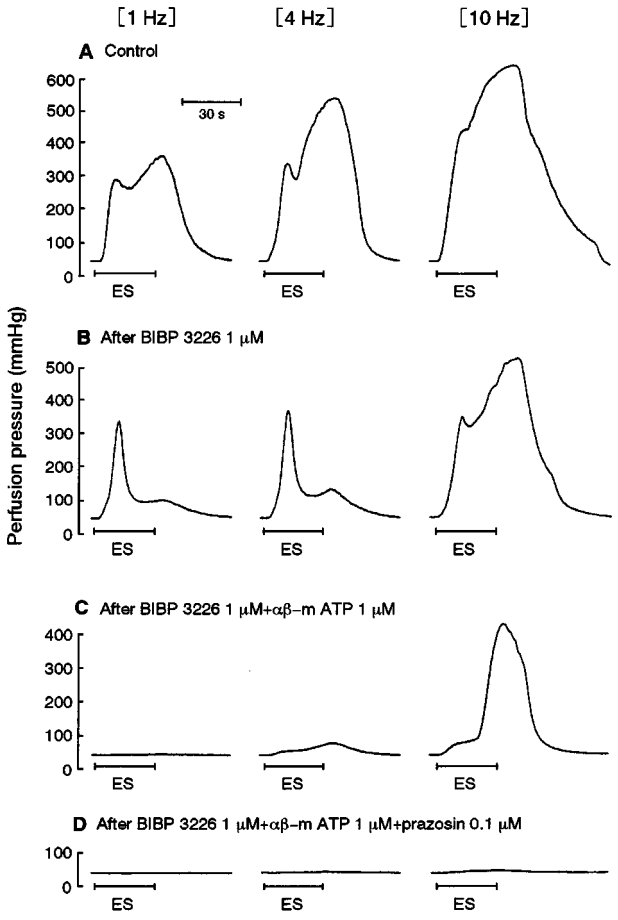

Figure 3 Double peaked vasoconstrictor responses to periarterial nerve stimulation and the effects of $\alpha\beta$-m ATP and prazosin on the BIBP 3226-resistant responses in an isolated, perfused canine splenic artery. The double peaked vasoconstrictions were induced by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz (A). After treatment with 1 μM BIBP 3226, the second peaked responses were strongly inhibited especially at low frequencies, and at 10 Hz, the second peaked response was slightly inhibited (B). At low frequency of 1 Hz, the perfusion with 1 μM $\alpha\beta$-m ATP completely inhibited the first and remaining second peaked constrictions after treatment with 1 μM BIBP 3226 (C). At a high frequency of 10 Hz, the first peaked response after treatment with 1 μM BIBP 3226 was suppressed by an application of 1 μM $\alpha\beta$-m ATP, whereas the second response was unaffected (C). The remaining responses after treatment with BIBP 3226 and $\alpha\beta$-m ATP were abolished by a subsequent administration of 0.1 μM prazosin (D). (ES), Electrical nerve stimulation.

modify the first peaked responses. At a high frequency of 10 Hz, both the first and second peaked responses were slightly but significantly decreased by BIBP 3226 in the presence of $\alpha\beta$-m ATP. A subsequent treatment with 0.1 μM prazosin completely inhibited the remaining responses after treatment with $\alpha\beta$-m ATP and BIBP 3226 (Figure 6).

Effects of BIBP 3226 on the vasoconstrictor responses to administered NPY, LP-NPY, ATP and NA

Intraluminally administered NPY (10 nmol) or LP-NPY (10 nmol) induced a moderate vascular contractile response in the isolated, perfused canine splenic arteries (Figure 7). The treatment with 1 μM BIBP 3226 abolished the vasoconstrictor responses to NPY and LP-NPY (Figure 7). On the other hand, the vasoconstrictor responses to 1 nmol NA and 0.3 μmol ATP were not modified by the treatment with 1 μM BIBP 3226, as shown in Figure 8.

Discussion

The present results strongly suggest that the nerve-stimulated vasoconstriction of this preparation also involves an activation of NPY Y_1 receptor, because blockade of NPY Y_1 receptors with BIBP 3226 inhibits the part of neuronally vascular contractile responses. Thus, it is proposed that NPY is a sympathetic cotransmitter and/or comodulator for the regulation of the canine splenic vessel tone.

It is generally accepted that the release of NPY from the sympathetic nerves is unaffected by BIBP 3226 (Lundberg & Modin, 1995; Donoso *et al*., 1997a). In addition, NPY Y_1 receptor-mediated vascular constrictions were readily blocked by BIBP 3226 in this study. Thus, the reduction of nerve-stimulated vasoconstrictions by BIBP 3226 is likely due to inhibition of postjunctional NPY Y_1 receptors.

Interestingly, the blockade of NPY Y_1 receptors with BIBP 3226 or other Y_1 receptor antagonists has been reported to inhibit the slow, long-lasting vasoconstriction evoked by nerve stimulation in several blood vessel preparations (Malmström & Lundberg, 1995; Racchi *et al*., 1997; Phillips *et al*., 1998), and also to attenuate the long-lasting blood pressure increase induced by the sympathetic nerve stimulation in the pig *in vivo* (Lundberg & Modin, 1995; Malmström *et al*., 1996; 1997). Our results confirm that neuronal NPY *via* an activation of Y_1 receptors may play an important role in eliciting the prolonged vasoconstriction of the canine splenic artery, since BIBP 3226 consistently inhibits the nerve-induced prolonged response. However, we found that BIBP 3226 exerted a remarkable inhibition on the prolonged vasoconstrictor responses to nerve stimulation at low frequencies (1 and 4 Hz), whereas at a high frequency (10 Hz), BIBP 3226 only produced a slight inhibition, although it is significant. Generally, the release of NPY is thought to be readily induced at high frequencies of electrical stimulation (Lundberg *et al*., 1989; Kennedy *et al*., 1997). Thus, our results may explain that a competitive Y_1 receptor antagonist BIBP 3226 produces a larger antagonistic effect on the response involving the release of a smaller amount of neurotransmitters when a low frequency stimulation is used. On the other hand, at a high frequency, for the prolonged vasoconstriction the release of NA might be increased, because the component of response not inhibited by BIBP 3226 and $\alpha\beta$-m ATP is markedly increased (Figures 4B and 6B), indicating an increased adrenergic component. The results indicate that neuronal NPY *via* the Y_1 receptor activation may mainly act as a vasomotor transmitter in response to a low frequency of stimulation, whereas neuronal NA acting at α_1-adrenoceptors dominantly exerts its vasoconstrictor effect in response to a high frequency. In addition, it has been suggested that a proper threshold of precontraction induced by NA suffices to trigger the physiological conditions that allow the NPY vasomotor effect to occur, whereas a strong precontraction by NA may limit the physiological capacity of the rat mesenteric vascular bed to contract with NPY (Cortés *et al*., 1999). Therefore, there might be a possibility that a strong vasoconstriction induced by an increased NA at a high frequency of stimulation partially attenuates the vascular contractile capacity of NPY, showing a smaller inhibition of BIBP 3226 at a high frequency. Thus, it is considered that NPY is readily released in the response to the longer pulse trains of stimulation at a high frequency in the canine splenic artery.

The present results showed that the transient vasoconstriction induced by nerve stimulation was completely suppressed by P2X receptor desensitization with $\alpha\beta$-m ATP at low frequencies, but not influenced by the blockade of NPY Y_1 receptors and α_1-adrenoceptors with BIBP 3226 and prazosin, respectively. At a high frequency, the transient vasoconstriction was inhibited

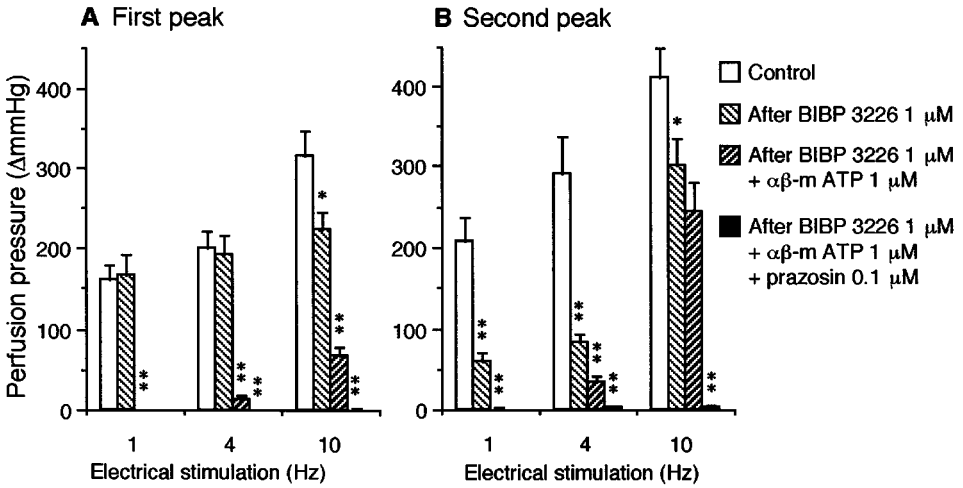

Figure 4 Effects of $\alpha\beta$-m ATP and prazosin on the BIBP 3226-resistant first peak (A) and the second peak (B) of the vasoconstrictor responses to periarterial electrical nerve stimulation in the canine splenic arteries. The vessels were electrically stimulated by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. Data are presented as mean ± s.e.mean ($n=6$). $^*P<0.05$; $^{**}P<0.01$ as compared with the preceding group.

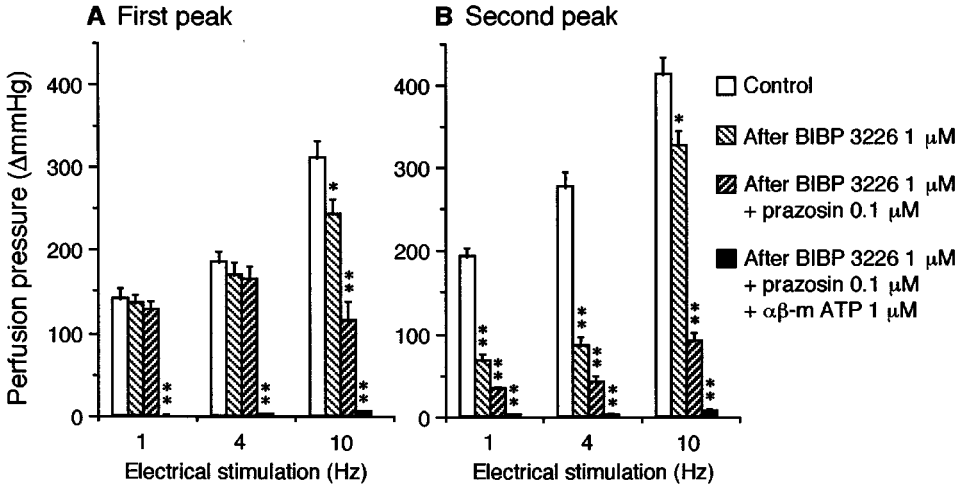

Figure 5 Effects of prazosin and $\alpha\beta$-m ATP on the BIBP 3226-resistant first peak (A) and the second peak (B) of the vasoconstrictor responses to periarterial electrical nerve stimulation in the canine splenic arteries. The vessels were electrically stimulated by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. Data are presented as mean ± s.e.mean ($n=6$). $^*P<0.05$; $^{**}P<0.01$ as compared with the preceding group.

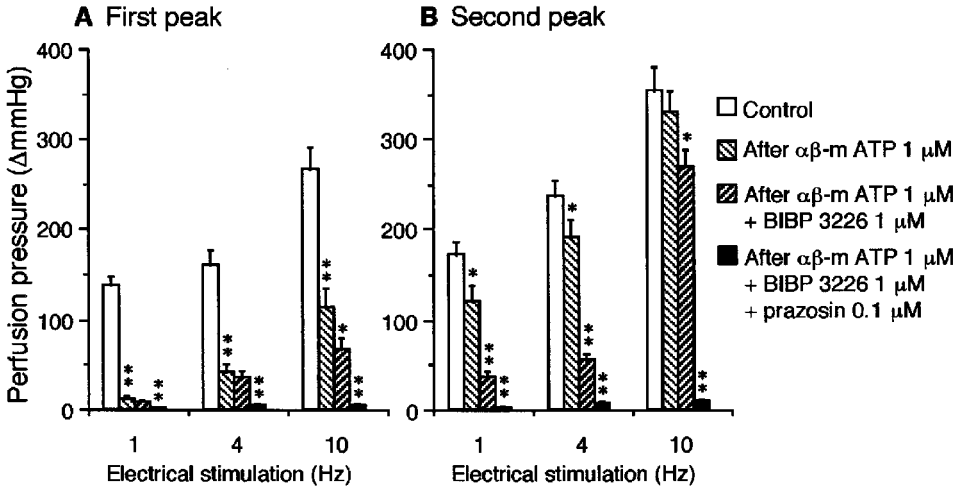

Figure 6 Effects of BIBP 3226 and prazosin on the $\alpha\beta$-m ATP-resistant first peak (A) and the second peak (B) of the vasoconstrictor responses to periarterial electrical nerve stimulation in the canine splenic arteries. The vessels were electrically stimulated by 30 s trains of pulses at 10 V amplitude and 1 ms pulse duration, with a frequency of 1, 4 or 10 Hz. Data are presented as mean ± s.e.mean ($n=7$). $^*P<0.05$; $^{**}P<0.01$ as compared with the preceding group.

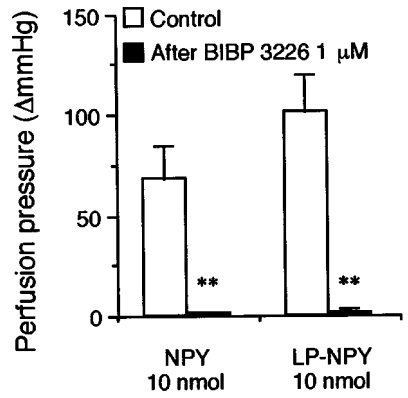

Figure 7 Effects of BIBP 3226 on the vascular contractile responses to NPY and LP-NPY in the isolated, perfused canine splenic arteries. Data are presented as mean ± s.e.mean ($n=6$). $^{**}P<0.01$ as compared with the control group.

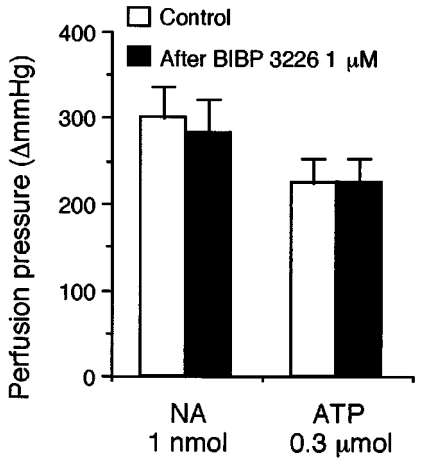

Figure 8 Effects of BIBP 3226 on the vascular contractile responses to ATP and NA in the isolated, perfused canine splenic arteries. Data are presented as mean ± s.e.mean ($n=6$).

mostly by $\alpha\beta$-m ATP and partially by blockade of NPY Y_1 receptors and α_1-adrenoceptors with BIBP 3226 and prazosin, respectively. It indicates that the transient response is probably induced *via* an activation of P2X receptors at low frequencies, whereas at a high frequency it probably involves mostly P2X receptors, and partially NPY Y_1 receptors and α_1-adrenoceptors. On the other hand, the results presented that the prolonged vasoconstrictor response was mostly decreased by blockade of NPY Y_1 receptors with BIBP 3226 and partially by α_1-adrenoceptor blockade with prazosin and P2X receptor desensitization with $\alpha\beta$-m ATP at low frequencies. At a high frequency, the prolonged response was mainly inhibited by prazosin and partially by $\alpha\beta$-m ATP and BIBP 3226. In addition, the treatment with $\alpha\beta$-m ATP inhibited the prolonged vasoconstrictor response to a high frequency of stimulation in the presence of α_1-adrenoceptor blockade with prazosin, but did not affect the response in the absence of α_1-adrenoceptor blockade, as reported previously (Yang & Chiba, 1998; 1999). It has been suggested that $\alpha\beta$-m ATP may also cause a desensitization of prejunctional P2X receptors, in addition to its postjunctional effects, which in turn causes a blockade of prejunctional inhibitory mechanisms of NA release, i.e. at a high frequency, the adrenergic component of prolonged response after $\alpha\beta$-m ATP might be enhanced (Yang & Chiba, 1999). Thus, it is considered that the total vasoconstriction of prolonged response at a high frequency may be unaffected by $\alpha\beta$-m ATP in the absence of α_1-adrenoceptor blockade. The present results indicate that the prolonged vasoconstriction is possibly mediated largely *via* an activation of NPY Y_1 receptors and partially *via* an activation of α_1-adrenoceptors and P2X receptors at low frequencies, whereas at a high frequency, it possibly involves mainly α_1-adrenoceptors and partially NPY Y_1 receptors and P2X receptors.

The previous results obtained in the canine splenic artery showed that nerve-stimulated prolonged vasoconstriction was mostly inhibited by α_1-adrenoceptor blockade with prasozin at the same experimental conditions (Yang & Chiba, 1998). In this study, the blockade of NPY Y_1 receptors with BIBP 3226 also exerted its stronger inhibition on the prolonged responses at low frequencies. The question is raised that of which α_1-adrenoceptor- or NPY Y_1 receptor-mediated mechanisms mainly contributes to the prolonged vasoconstrictor response to nerve stimulation at low frequencies. Interestingly, Cortés *et al.* (1999) documented that NPY requires a precontraction with NA or other receptor agonists linked to phospholipase C to elicit its characteristic vasomotor effect in the rat mesenteric bed. Furthermore, blockade of α_1-adrenoceptors with prazosin not only abolishes the precontraction tone induced by NA but also abolishes NPY-induced vasoconstrictions (Cortés *et al.*, 1999). One the other hand, NPY released from rat mesenteric periarterial sympathetic nerves can facilitate the NA-induced vasoconstriction, and the facilitation of NPY was blocked by BIBP 3226 (Donoso *et al.*, 1997a). These findings emphasize the physiological importance of synergism between NPY Y_1 receptors and α_1-adrenoceptors in the regulation of the peripheral sympathetic vascular tone. It remains to be clarified in future studies whether the cooperation between postjunctional α_1-adrenoceptors and Y_1 receptors accounts for the prolonged vasoconstrictor response to a low frequency of stimulation in the canine splenic artery.

It is concluded that the double peaked vasoconstrictor responses to nerve stimulation in the canine splenic artery involve an activation of postjunctional P2X receptors, α_1-adrenoceptors and NPY Y_1 receptors. Furthermore, the ATP released from the sympathetic nerves acting at P2X receptors may mainly mediate a transient vasoconstrictor response to nerve stimulation in the canine splenic artery. On the other hand, the neuronal NPY *via* an activation of NPY Y_1 receptors may dominantly act as a vasomotor transmitter in evoking the prolonged vasoconstrictor response to longer pulse trains of stimulation at a low frequency, whereas at a high frequency, neuronal NA *via* an α_1-adrenoceptor activation exerts its main vasoconstrictor effect for the prolonged response.

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