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Neuropeptide Y Y_1 receptor blockade does not alter adrenergic nerve responses of the rat tail artery

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Abstract

Using the selective neuropeptide Y Y_1 receptor antagonist, BIBP3226 [(N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-argininamide], the role of endogenous neuropeptide Y in mediating vasoconstrictor responses to adrenergic nerve stimulation was investigated by recording isometric force from isolated rat tail artery segments. BIBP3226 had no effect on contractile responses to adrenergic nerve stimulation (10 pulses; 0.5–2 Hz), but it completely blocked the enhancement of contraction produced by exogenous neuropeptide Y. When frequency and train length of the transmural nerve stimulation were increased (100 pulses; 1–16 Hz), contractile responses were still unaffected by BIBP3226. A peptidase inhibitor mixture known to increase responses to exogenous neuropeptide Y was added; however, BIBP3226 still did not influence contractile responses to adrenergic nerve stimulation. Thus, contractile responses to adrenergic nerve stimulation in the rat tail artery do not appear to involve the release and postjunctional action of endogenous neuropeptide Y; however, exogenous neuropeptide Y does potentiate these responses by acting on Y_1 receptors. © 1997 Elsevier Science B.V.

Keywords: Neuropeptide Y; Adrenergic neurotransmission; Neuropeptide Y receptor antagonist; BIBP3226

1. Introduction

Neuropeptide Y has been shown to have a variety of effects on blood vessels, depending on species and vessel type, including direct vasoconstriction, potentiation of contractile responses, and prejunctional inhibition of adrenergic nerve activity (Ekblad et al., 1984; Westfall et al., 1988; Vu et al., 1989). At least two peripheral neuropeptide Y receptors have been identified to account for these diverse responses: neuropeptide Y Y_1 receptors are thought to mediate primarily postjunctional contractile responses, including potentiation, and neuropeptide Y Y_2 receptors have been shown to mediate inhibition of neurotransmitter release (Gehlert, 1994; Bergdahl et al., 1996). However, there is some evidence that localization of receptor types may not always be this straightforward (Lundberg, 1996).

Neuropeptide Y is considered a co-transmitter in sympathetic adrenergic nerves and has been shown to be released during nerve stimulation (Kasakov et al., 1988; Warner et al., 1991). However, due to the lack of potent and selective neuropeptide Y receptor antagonists, the functional role of neuropeptide Y in neural transmission has not been clearly tested. Recently the development of a potent and selective non-peptide neuropeptide Y Y_1 receptor antagonist has been reported (Rudolf et al., 1994; Doods et al., 1995). BIBP3226, (N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-argininamide, was shown via radioligand binding and in vitro functional studies to have selectivity for Y_1 receptors and to block effects of neuropeptide Y on blood pressure in vivo.

In the isolated rat tail artery, neuropeptide Y has been shown to have no direct contractile effects, but to significantly enhance contractile responses to adrenergic nerve stimulation (Vu et al., 1989; Glenn et al., 1997). Endogenous neuropeptide Y is present in the tail artery (Glenn et al., 1997) which is exclusively innervated by adrenergic nerves (Sittiracha et al., 1987). Based on use of selective agonists, the postjunctional effects of neuropeptide Y in the tail artery have been shown to be consistent with the presence of an neuropeptide Y Y_1 receptor (Tschöpl et al., 1993). Therefore the goal of the present study was to

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determine if BIBP3226 would block contractile responses to neuropeptide Y in the rat tail artery and then, using this antagonist, to investigate to what extent endogenous neuropeptide Y participates in the contractile response to adrenergic nerve stimulation.

2. Materials and methods

Male Sprague Dawley rats (150–200 g) were decapitated. Tail arteries were carefully removed, placed in Krebs solution and cut into 3 mm segments. With the aid of a dissecting microscope, tail artery segments were mounted through the lumen on two platinum wires in a 50 ml tissue bath filled with oxygenated (95% O_2/5% CO_2) Krebs solution at 37°C. Composition of the Krebs solution was (in mM): NaCl, 122; KCl, 5.2; $CaCl_2$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 26; $MgSO_4$, 1.2; disodium EDTA, 0.027; and glucose, 11. Tissues were equilibrated for 1 h and then stretched to a resting tension of 1 *g*, which was previously determined to be optimal for force development. Tissues were equilibrated for an additional 1 h before starting the experiment.

Isometric contractions of arterial segments were recorded using Fort 10 force transducers (World Precision Instruments, Sarasota, FL) and MacLab analog to digital converter system. Perivascular nerves were electrically stimulated via a Grass S48 stimulator and two electrodes placed on either side of the tissue (5 mm apart). Nerves were activated at various frequencies, 0.3 ms pulse duration, 15 V pulse height, with trains of either 10 or 100 pulses. The sodium channel blocker tetrodotoxin (1 μM) totally abolished contractile responses to electrical stimulation at 8 Hz, confirming dependence on nerve activation. The maximum contractile response of the tissue to norepinephrine was determined from cumulative concentration response curves (10^{-7} M–10^{-3} M).

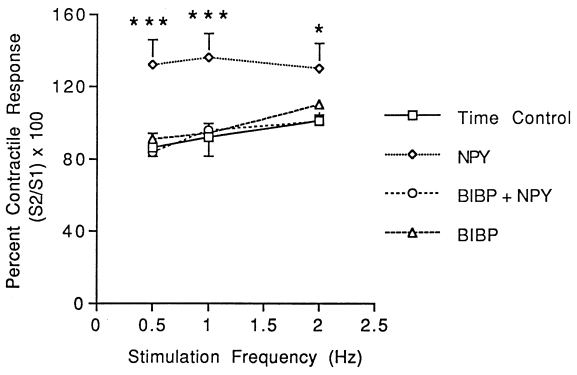

Fig. 1. Effect of BIBP3226 on neuropeptide Y-induced potentiation of contractile responses to adrenergic nerve stimulation. Contractile responses to adrenergic nerve stimulation (10 pulses) at various frequencies (0.5–2 Hz) were measured twice in each tail artery segment. A control stimulation (S1) was followed by a second stimulation (S2) in the presence of either neuropeptide Y (NPY; 10 nM), BIBP3226 (1 μM), or neuropeptide Y + BIBP3226 and compared with untreated tissues (time control). Contractile responses during S2 are calculated as a percent, in each tissue, of control responses in S1: (S2/S1)×100. Values are means ± S.E., $n = 7$. *** Different from three other groups by analysis of variance; * different from time control.

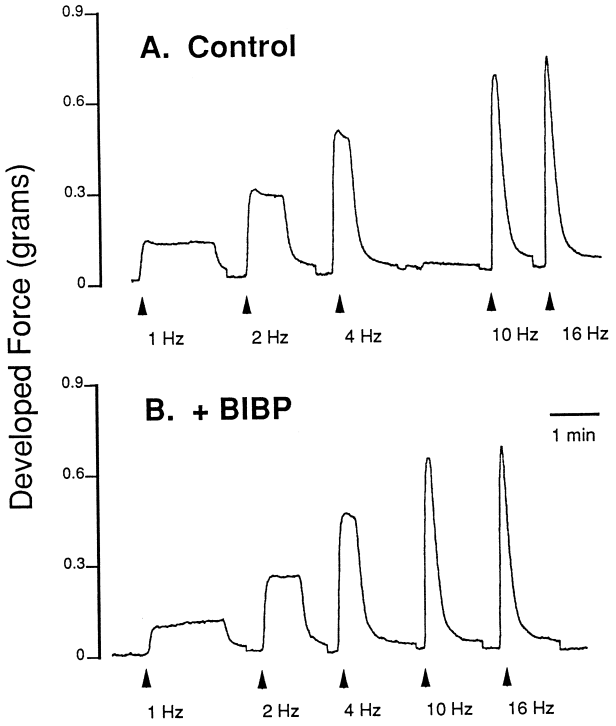

Fig. 2. Representative example of the effect of BIBP3226 on contractile responses to adrenergic nerve stimulation. Contractile responses to adrenergic nerve stimulation (100 pulses) in a single tissue are shown at various frequencies (1–16 Hz) (A) under control conditions and (B) in the presence of BIBP3226 (1 μM).

To test effects of BIBP3226 on responses to exogenous neuropeptide Y, tissues were first stimulated at various frequencies (0.5–2 Hz) for trains of 10 pulses. Tissues were then divided into four treatment groups: time control; 10 nM neuropeptide Y; 1 μM BIBP3226; and 1 μM BIBP + 10 nM neuropeptide Y. After 10–15 min exposure to the appropriate treatments, contractile responses to the various frequencies of adrenergic nerve stimulation were again determined.

To test effects of BIBP3226 on responses to adrenergic nerve stimulation, tissues were first stimulated at various frequencies (1–16 Hz) for trains of 100 pulses. Tissues were then divided into two groups: time control and + BIBP3226 (1 μM). After appropriate treatment, contractile responses to each frequency were again determined. In another series of experiments a mixture of peptidase inhibitors was used: diprotinin A, 10^{-5} M; leupeptin, 10^{-6} M; aprotinin, 10^{-6} M; pepstatin, 10^{-6} M and bacitracin, 0.05% (Glenn et al., 1997). Tissues treated with peptidase inhibitors were exposed throughout the experiment; some were treated with BIBP3226 during the second stimulation train while others, studied in parallel, served as untreated time controls.

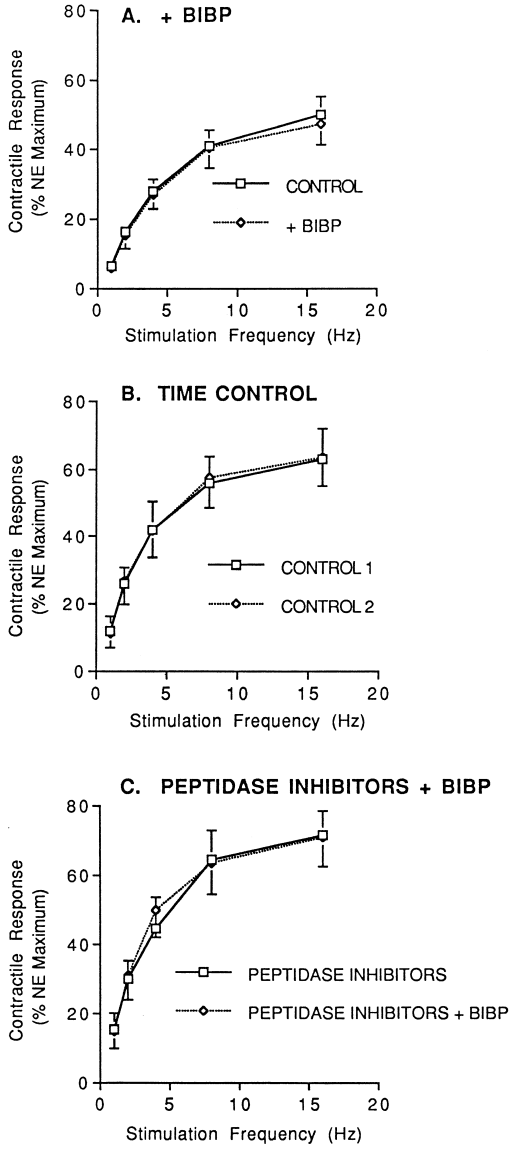

Fig. 3. Effect of BIBP3226 in the absence and presence of peptidase inhibitors on contractile responses to adrenergic nerve stimulation. Peak contractile responses to adrenergic nerve stimulation (100 pulses) are shown at various frequencies (1–16 Hz). (A) shows the effect of BIBP 3226 (1 μM); (B) shows results of time controls; and (C) shows the effect of BIBP3226 in the presence of peptidase inhibitors (see Section 2). Values are means ± S.E., $n = 5$.

Drugs used included norepinephrine bitartrate, tetrodotoxin, and bacitracin (Sigma Chemical, St. Louis, MO); neuropeptide Y, diprotinin A, leupeptin, and pepstatin (Peninsula Laboratories, Belmont, CA); aprotinin (Calbiochem, San Diego, CA); and BIBP 3226 (N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-argininamide: courtesy of Dr. Karl Thomae, Biberach, Germany).

3. Results

The neuropeptide Y Y_1 receptor antagonist, BIBP3226, significantly blocked the potentiating effect of exogenous neuropeptide Y in the rat tail artery (Fig. 1). As we have previously reported (Vu et al., 1989), addition of neuropeptide Y to this tissue potentiates contractile responses to adrenergic nerve stimulation, an effect which is especially prominent at lower frequencies and shorter trains of stimulation. With short trains of adrenergic nerve stimulation (10 pulses), the potentiating effect of exogenously added neuropeptide Y was completely prevented when BIBP3226 was present in a concentration of 1 μM (Fig. 1). BIBP3226 by itself had no significant effect on contractile responses to adrenergic nerve stimulation with short trains of stimulation (10 pulses).

The effects of BIBP3226 against contractile responses to adrenergic nerve stimulation were then tested using longer trains and higher frequencies of stimulation. As shown in Figs. 2 and 3A and B, however, addition of BIBP3226 had no effect on responses to adrenergic nerve stimulation regardless of frequency from 1 to 16 Hz (100 pulses). Neither peak contractile responses to nerve stimulation nor the duration of the response were altered in the presence of BIBP3226.

We have previously demonstrated that addition of peptidase inhibitors to the tissue bath significantly increases responses to exogenous neuropeptide Y in the rat tail artery (Glenn et al., 1997). Therefore we tested whether inhibition of tissue peptidases would reveal a role for endogenous neuropeptide Y in modulating contractile responses to adrenergic nerve stimulation. However, even in the presence of peptidase inhibitors (diprotinin A, leupeptin, aprotinin, pepstatin and bacitracin), addition of BIBP3226 still did not alter contractile responses to adrenergic nerve stimulation, regardless of frequency from 1 to 16 Hz (Fig. 3C). Addition of peptidase inhibitors also had no significant effect on the magnitude of contractile responses to adrenergic nerve stimulation (Table 1), and repeated responses to adrenergic nerve stimulation in the

Table 1
Effect of peptidase inhibitors on contractile responses to adrenergic nerve stimulation (100 pulse trains) at various frequencies of stimulation

Frequency	Contractile response to adrenergic nerve stimulation (grams)				
	1 Hz	2 Hz	4 Hz	10 Hz	16 Hz
Control	0.20 ± 0.07	0.36 ± 0.09	0.56 ± 0.14	0.79 ± 0.16	0.92 ± 0.19
+ Peptidase inhibitors	0.16 ± 0.07	0.31 ± 0.14	0.48 ± 0.21	0.78 ± 0.30	0.86 ± 0.32

Values are means ± S.E., $n = 5$.

presence of peptidase inhibitors showed no variation over time (data not shown).

4. Discussion

This study demonstrates that BIBP3226 is, indeed, an effective antagonist of neuropeptide Y receptors in the rat tail artery and supports previous conclusions that smooth muscle neuropeptide Y receptors in this tissue are of the Y_1 subtype (Tschöpl et al., 1993; Gicquiaux et al., 1996). However, using BIBP3226 as a tool to explore the role of neuropeptide Y as an adrenergic co-transmitter, we found no evidence that neuropeptide Y contributes to the contractile response to stimulation of adrenergic nerves in this vascular tissue. Regardless of frequency, addition of a concentration of BIBP3226 that effectively blocked potentiation by exogenous neuropeptide Y had absolutely no effect on contractile responses to adrenergic nerve stimulation.

It has been generally hypothesized that peptide co-transmitters, including neuropeptide Y, are more likely to be involved in mediating contractile responses to nerve stimulation at higher stimulation frequencies or with longer stimulation trains (Lundberg, 1996). Therefore, for the purpose of investigating the possible role of endogenous neuropeptide Y, we selected trains of 100 pulses and stimulated with frequencies up to 16 Hz which produces the maximum contractile response obtained with nerve stimulation. However, regardless of frequency, BIBP3226 had no effect on contractile responses to adrenergic nerve stimulation. It is an interesting paradox that neuropeptide Y is thought to be released primarily during intense nerve stimulation (Lundberg, 1996); however, the contractile effects of exogenous neuropeptide Y are most evident when using low levels of stimulation in the tail artery (Vu et al., 1989; Glenn et al., 1997). We found that BIBP3226 also had no effect on contractile responses to shorter trains of adrenergic nerve stimulation (10 pulses). These findings support the conclusion that endogenous neuropeptide Y does not play an important role in mediating contraction to adrenergic nerve stimulation in the rat tail artery, regardless of stimulation frequency and train length.

We have previously demonstrated that addition of a mixture of peptidase inhibitors significantly augments contractile responses to exogenous neuropeptide Y in the rat tail artery in vitro (Glenn et al., 1997). Therefore, we tested the hypothesis that inhibition of endogenous peptidases might reveal a contractile effect of endogenously released neuropeptide Y. However, even in the presence of peptidase inhibitors, addition of the neuropeptide Y antagonist BIBP3226 still did not alter contractile responses to adrenergic nerve stimulation. These findings with peptidase inhibitors reinforce the conclusion that, in the rat tail artery, endogenous neuropeptide Y does not participate to any significant extent in the contractile response to adrenergic nerve stimulation. This conclusion is further supported by our observation that addition of peptidase inhibitors also had no significant influence on the magnitude of contractile responses to adrenergic nerve stimulation (Table 1).

Using other tissues, there are reports in the literature of significant effects of neuropeptide Y antagonists on contractile responses to adrenergic nerve stimulation. In the guinea pig vena cava, BIBP3226, but not its less potent enantiomer BIBP3435, significantly reduced contractile responses to nerve stimulation at frequencies of 10 or 40 Hz (Malmström and Lundberg, 1995). Furthermore, it was recently reported that, in the perfused rat mesentery, contractile responses to adrenergic nerve stimulation were significantly attenuated by addition of BIBP3226 (Han et al., 1996). Furthermore, in the reserpinized pig in vivo, vasoconstrictor responses to adrenergic nerve stimulation in kidney and hind limb are substantially reduced in the presence of BIBP3226 (Malmström et al., 1997). One difference between responses to adrenergic nerve stimulation in the tail artery and some of these other tissues is that, in the tail artery, upon cessation of stimulation there is very rapid smooth muscle relaxation (Fig. 2). In contrast, the guinea pig vena cava and reserpinized pig hindlimb vascular beds show prolonged responses after cessation of adrenergic nerve stimulation, and these prolonged responses are preferentially influenced by blockade of neuropeptide Y receptors. However, in the reserpinized pig renal circulation, treatment with BIBP3226 has significant effects even though no long-lasting vasoconstriction is seen (Malmström et al., 1997). These findings suggest that the role of neuropeptide Y in adrenergic transmission may vary depending on species, vascular bed, and, possibly, even within a vascular bed.

What, then, might be the physiological role of the endogenous neuropeptide Y and Y_1 receptors present in the tail artery? If neuropeptide Y is released by the nerve it could have access to prejunctional Y_2 receptors and possibly play a role in regulating neurotransmission (Gehlert, 1994; Glenn et al., 1997). While Y_1 receptors do not appear to contribute to nerve-evoked constriction in the tail artery, extrajunctional Y_1 receptors would respond to exogenous, circulating neuropeptide Y, which is elevated during certain conditions including stress (Zukowska-Grojec et al., 1996). It is also possible that the release of neuropeptide Y in the adventitia is not sufficient to alter contractility; however it may serve a trophic function by acting on smooth muscle neuropeptide Y receptors to stimulate growth (Erlinge et al., 1994). These possibilities require further investigation. However, our findings in the rat tail artery underscore that demonstration of the presence of neuropeptide Y as well as functional neuropeptide Y receptors is insufficient evidence to conclude that endogenous neuropeptide Y has a significant role in mediating contractile responses to nerve stimulation.

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