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Short communication

Neuropeptide Y Y_1 receptors are involved in the vasoconstriction caused by human sympathetic nerve stimulation

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Abstract

Neuropeptide Y, a novel neurotransmitter, interacts with selective membrane receptors to cause vasoconstriction. Frequency- and concentration-dependent isometric contractions were observed in human inferior mesenteric artery and vein mounted rings that were stimulated with either electrical pulses (70 V, 0.5 ms, 2.5–20 Hz) or noradrenaline. The antagonism elicited by 100 nM tetrodotoxin and 1 μM guanethidine confirmed the neuronal and sympathetic origins of the vasomotor response. Incubation with BIBP 3226 ((*R*)-N^2-(diphenacetyl)-*N*-(4-hydroxyphenyl)-methyl-D-arginineamide), a selective neuropeptide Y Y_1 receptor antagonist, significantly reduced the vasoconstriction. The incomplete antagonist activity of BIBP 3226 tends to support the hypothesis of sympathetic co-transmission involving neuropeptide Y, adenosine 5′-triphosphate and noradrenaline. These findings were confirmed in parallel studies using rat superior mesenteric artery and vein ring preparations. © 1997 Elsevier Science B.V.

Keywords: BIBP 3226; Neuropeptide Y receptor; Neuropeptide Y Y_1 receptor; Sympathetic stimulation; Mesenteric artery, human, inferior; Mesenteric vein, human, inferior

1. Introduction

Neuropeptide Y, a member of the avian pancreatic polypeptide family, is expressed in both the central and peripheral nervous systems. In both regions this neuropeptide is frequently, but not exclusively, co-localized with noradrenaline. Immunohistochemical studies of human perivascular sympathetic neurons have identified parallel staining distributions for neuropeptide Y and tyrosine hydroxylase, an enzyme involved in noradrenaline synthesis (Gulbenkian et al., 1993; Saetrum Opgaard et al., 1995). These findings of simultaneously expressed neuropeptide Y and adrenergic components lend support to the hypothesized complementarity of noradrenaline, neuropeptide Y, and adenosine 5′-triphosphate in co-transmission.

While neuropeptide Y has been positively identified in human coronary blood vessels (Gulbenkian et al., 1993; Saetrum Opgaard et al., 1995), relatively little experimental evidence has surfaced to explain its human physiological role. Nonetheless, several hypotheses have been developed on the basis of the results of animal and a limited number of human studies. Neuropeptide Y has been found to act both directly and indirectly to affect blood vessel tone (Potter, 1991). As a direct transmitter molecule, neuropeptide Y has been shown to promote vasoconstriction in the human and canine coronary circulatory systems (Mabe et al., 1985; Clarke et al., 1987; Macho et al., 1989) and to elevate systemic blood pressure following its i.v. administration (Mabe et al., 1985; Clarke et al., 1987). In contrast, neuropeptide Y can also function indirectly as an intermediate with the capacity to, in some circumstances, potentiate the vasomotor activity of pressor molecules, such as catecholamines, angiotensin II, and 5-hydroxytryptamine (Edvinsson et al., 1984; Wahlestedt et al., 1985; López et al., 1989), while in other instances it serves to attenuate the relaxant response to such molecules as acetylcholine and vasoactive intestinal peptide

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(Gulbenkian et al., 1992). In addition to its modulatory potential, neuropeptide Y can affect vascular tone by regulating the release of pressor molecules like catecholamines from sympathetic fibers, resulting in an absolute reduction of this system's physiological influence (Westfall et al., 1987).

The recent discovery of BIBP 3226 ((*R*)-N^2-(diphenacetyl)-*N*-(4-hydroxyphenyl)-methyl-D-arginineamide), a selective neuropeptide Y Y_1 receptor antagonist, by Rudolf et al. (1994) has opened the way for a more complete understanding of its role in homeostasis by facilitating the elucidation of the physiological mechanism (s) through which neuropeptide Y is involved in the control of vascular tone. Despite the failure of BIBP 3226 to reduce systemic blood pressure in experimental animals, it was found to partially antagonize the stress-induced reduction in mesenteric blood flow observed in rats (Zukowska-Grojec et al., 1996). BIBP 3226's ability to antagonize vasoconstriction was similarly observed following electrical stimulation of the sympathetic nerve terminals of the guinea-pig vena cava (Malmström and Lundberg, 1995). The above results, based largely on non-human studies, have prompted us to assess, with the aid of BIBP 3226, the functional involvement of neuropeptide Y in human vasomotor activity. The present results suggest that neuropeptide Y is liberated as a result of electrical depolarization of the nerve terminals surrounding the human inferior mesenteric blood vessels and that it is at least partially responsible for the observed vasocontractile response.

2. Materials and methods

2.1. Human biopsies and the in vitro mounting of blood vessel rings for isometric recording of circular muscle layer contraction

Biopsy samples of the inferior mesenteric artery and vein were obtained from patients undergoing elective abdominal surgery (varying pathologies) at our School of Medicine's Clinical Hospital. Within minutes of intestinal resection, samples were transported in sterile saline solution from the nearby operating room to our laboratory. The samples were immediately transferred to a container with Krebs-Ringer solution bubbled with 95% O_2/5% CO_2 maintained at 37°C. Ring preparations, 3–4 mm in diameter, were mounted in a 6 ml bath chamber where circular muscle layer contractions were recorded isometrically. A basal tension of 1.5–2 g was maintained throughout the experiment.

2.2. Data analysis

All data are expressed as a percentage of the tension produced by 70 mM potassium chloride applied at the beginning of the experimental protocol. The tension generated by this standard did not differ significantly between the artery and the vein (1.76 ± 0.3 g vs. 1.9 ± 0.25 g, $n = 4$ per vessel group). Co-variance analysis was applied to the frequency-tension curves, with statistical significance being defined as a P value of less than 0.05. One vein ring and one artery ring were derived from each of the four patient biopsies. The noradrenaline concentration required for half-maximal contraction was interpolated from the concentration–response plots.

2.3. Experimental protocol: electrical stimulation and antagonist administration

The protocols performed with human tissues were carried out in accordance with the recommendations of the School of Medicine Ethics Committee, as regulated by the P. Catholic University. Mounted tissue samples, under conditions of constant buffer flux, were first equilibrated to 1.5–2 g of tension for 1 h. Then the analytical reference stimulation was carried out with 70 mM potassium chloride and tissue viability was assessed by cumulatively exposing samples to increasing concentrations of noradrenaline (0.1 nM to 10 μM noradrenaline). Rings that did not respond to the potassium challenge or rings that did not exhibit at least 2 g of tension with the maximal concentration of noradrenaline were discarded.

The rings were transmurally depolarized by electrical pulses (70 V, 0.5 ms duration) delivered through platinum electrodes located on opposite sides of the bath chamber. A series of stimuli (2.5, 5, 10, 20 Hz) were applied sequentially upon complete muscle relaxation, as indicated by a return to the basal tension. For each specimen, this stimulation protocol was performed twice under normal buffer conditions to define the control contraction response. Following a 10 min incubation with 1 μM BIBP 3226, the stimulation was repeated to identify the change in the frequency-tension relation caused by this agent. The drug was then thoroughly flushed from the system and another frequency-tension curve was generated to assess the reversibility of this neuropeptide Y receptor antagonism. Next, the biopsies were incubated either for 5 min with 100 nM tetrodotoxin or for 20 min with 1 μM guanethidine. A final stimulation protocol was completed in the presence of these drugs.

2.4. Parallel rat study

The experiments with human biopsies were paralleled by studies with adult Sprague-Dawley rats (250–300 g). Animals were anesthetized with sodium pentobarbital; a midline incision opened the abdominal wall and the superior mesenteric artery and vein were dissected from surrounding tissues and transferred to a Petri dish containing oxygenated Krebs-Ringer solution. The consequent procedure was as detailed for the human samples, except that

tension was adjusted to 1 g, and in most studies, guanethidine was used instead of tetrodotoxin.

2.5. Drug sources

Guanethidine sulfate, tetrodotoxin and noradrenaline hydrochloride were purchased from Sigma (St. Louis, MO, USA). BIBP 3226 was contributed by Dr. K. Rudolf (K. Thomae, Biberach, Germany).

3. Results

3.1. Frequency-tension curves in human inferior mesenteric arteries and veins

Electrical stimulation of artery and vein rings elicited frequency-dependent contractions that were abolished by tetrodotoxin, an indication of the response's neuronal origin. The inhibition was of about the same magnitude (75–85%) at all frequencies of nerve stimulation (see insets in Figs. 1 and 2) and was easily reversible upon removal of the toxin from the buffer. Guanethidine also blocked vasomotor activity, thus demonstrating the sympathetic nature of these contractions (see recordings in Figs. 1 and 2). The magnitude of the vasomotor response induced by electrical stimulation was larger in the vein than in the artery; however, the difference was not of statistical significance ($F(1,29) = 3.0$, n.s.). Parallel experiments demonstrated that the concentration of noradrenaline required to cause half-maximal contraction of the artery rings and the vein rings was essentially the same (0.50 vs. 0.45 μM, respectively).

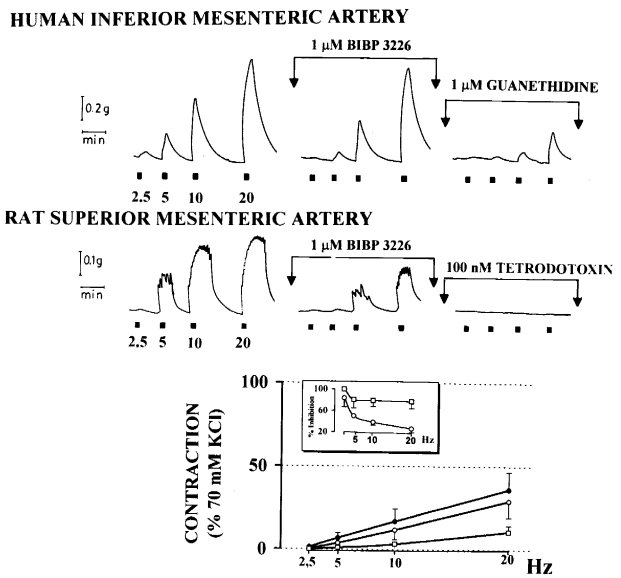

Fig. 1. Partial blockade of the electrically evoked sympathetic vasomotor responses of the human inferior mesenteric artery by BIBP 3226, a neuropeptide Y Y_1 receptor antagonist. Upper panel: Recording of vasomotor contractions evoked by transmural electrical depolarization of the perivascular nerve endings of the human inferior mesenteric artery ring preparation. Black squares indicate electrical pulses (70 V, 0.5 ms) of 2.5, 5, 10 and 20 Hz, delivered for 30 s either under control conditions, or in the presence of 1 μM BIBP 3226, or 1 μM guanethidine. Middle panel: Same protocol as performed in the upper panel, except that the ring was derived from the rat superior mesenteric artery and guanethidine was replaced by 100 nM tetrodotoxin. Lower panel: Frequency-tension curves derived from 4 human inferior mesenteric artery samples under control conditions (●), in the presence of 1 μM BIBP 3226 (○), and in the presence of 100 nM tetrodotoxin (□). Symbols indicate mean values, bars S.E.M. Inset shows the percentage of drug-induced inhibition of the control response (mean ± S.E.M. values) caused by BIBP 3226 and tetrodotoxin.

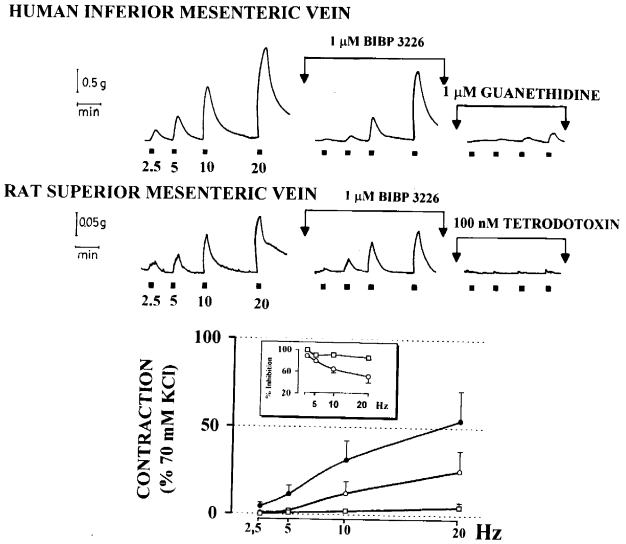

Fig. 2. BIBP 3226-induced blockade of the vasomotor responses induced in human inferior mesenteric vein biopsies. Upper panel: Frequency–tension curves generated from the biopsy complement of the tissue from Fig. 1. Black squares indicate the train of electrical pulses (70 V, 0.5 ms) delivered at frequencies of 2.5, 5, 10 and 20 Hz for 30 s, either under control conditions or in the presence of 1 μM BIBP 3226, or 1 μM guanethidine. Middle panel: As in the upper recording, a ring from the superior mesenteric vein of the same rat as shown in Fig. 1, was studied under the same conditions as detailed in the middle panel legend. Lower panel: Frequency–tension curves generated from human inferior mesenteric vein rings under control conditions (●), in the presence of 1 μM BIBP 3226 (○), and in the presence of 100 nM tetrodotoxin (□). Symbols indicate mean values, bars S.E.M. Inset shows the percent BIBP 3226 and tetrodotoxin induced inhibition of the control responses (mean ± S.E.M. values).

The inclusion of 1 μM BIBP 3226 in the bath solution reduced, but did not abolish the electrically induced vasomotor responses. Vasoconstriction was of smaller magnitude and slightly shorter duration as compared with the control response. Although this effect was more marked in the vein than in the artery, analysis of co-variance showed the significance of the blockade in the vein ($F(1,29) = 6.54$, $P < 0.025$) and a borderline significance in the case of the artery. The degree of contraction inhibition was notably greater at lower frequencies of nerve stimulation (see insets of Figs. 1 and 2). This result indicates the competitive pharmacology of the blockade. The persistence of inhibition 20 min after the removal of BIBP 3226

revealed the poor reversibility of the effect of this antagonist.

3.2. Blockade of the electrically induced vasomotor response by BIBP 3226 in rat superior mesenteric arteries and veins

Parallel experiments conducted with rings derived from the rat superior mesenteric artery and vein largely confirmed the results obtained for human biopsies. The neuronal and sympathetic origins of the response were confirmed and the BIBP 3226-induced reduction in contraction magnitude and duration was observed. Specifically, the inhibition elicited by 1 μM BIBP 3226 in 6 individual artery samples was significant (P value of less than 0.005; $F(1,37) = 11$), while in the corresponding vein rings the significance of the 1 μM BIBP 3226-induced antagonism was confirmed ($F(1,37) = 12.9,\ P < 0.005$). Interestingly, 1 μM BIBP 3226-induced blockade of the electrically evoked contractures in the artery rings was significantly greater ($P < 0.05$) than that attained in the veins (statistical data not shown). Furthermore, the tension recording from the rat vein (Fig. 2) clearly showed that BIBP 3226 substantially reduced the second component of the vasomotor response. Increasing the BIBP 3226 concentration from 1 μM to 3 μM did not elicit a parallel increase in the antagonist response.

4. Discussion

A plausible interpretation of the BIBP 3226 antagonism of the electrically evoked vasomotor response elicited in human and rat mesenteric vessels invokes the participation of neuropeptide Y Y_1 receptors. This interpretation relies on several premises: (i) the sympathetic perimesenteric nerves contain neuropeptide Y; (ii) electrical stimulation of the perimesenteric sympathetic nerves releases neuropeptide Y; (iii) the peptide directly, or indirectly, promotes vasoconstriction in this vascular territory; and (iv) BIBP 3226 selectively blocks neuropeptide Y Y_1 receptors.

The first three premises have been confirmed in a recent publication by Donoso et al. (1997). This study demonstrated that immunoreactive neuropeptide Y is abundant in the rat mesenteric arterial territory. Chromatographic evidence showed the release of neuropeptide Y from the nerve terminals surrounding these vessels into the venous perfusate. Finally, the investigation demonstrated the ability of neuropeptide Y to sensitize the contractile activity of endogeneously released noradrenaline. The similarities between human and rat vascular neuropeptide Y physiology, as seen in the present study, allows one to infer that the above results for the rat mesentery are applicable to its human counterpart. The observation that 1 μM BIBP 3226 attenuated the vasomotor response elicited by all frequencies of electrical stimulation both in human and rat preparations of either arteries or veins is puzzling. A large number of studies, including ours in the mesentery (Donoso et al., 1997), show that neuropeptide Y is primarily released with a high stimulation frequency. From a pharmacodynamical standpoint, it could be anticipated that a competitive antagonist would reduce to a larger extent the responses involving the release of a smaller amount of a neurotransmitter. However, this reasoning is at variance with the physiological observations documenting that neuropeptide Y, as well as other neuropeptides, are detected following high-frequency electrical nerve stimulation. The possibility that some degree of tachyphylaxis develops to the repeated electrically evoked contractions can be largely discarded on the basis of our experience of the high reproducibility of these responses. The possibility that this blood vessel bioassay detects minute amounts of neuropeptide Y cannot be ignored.

With regard to the pharmacodynamics of BIBP 3226, there is convincing evidence that this compound is a selective, potent and competitive antagonist of the neuropeptide Y Y_1 receptor. The initial characterization of BIBP 3226 as a competitive and selective neuropeptide Y Y_1 receptor antagonist has been largely confirmed by several laboratories and in a battery of pharmacological assays ranging from membrane binding experiments to in vivo pharmacological testing (Lundberg and Modin, 1995; Doods et al., 1995). In the vascular system, these receptors are likely localized in the smooth muscle membrane. Variable concentrations of receptors in the inferior mesenteric artery versus the vein may explain the relatively greater efficiency of BIBP 3226 antagonism in human veins. Recent results of Lundberg and Modin (1995) demonstrate that BIBP 3226 reduces the contractions evoked by perivascular nerve stimulation of the guinea-pig vena cava. The present results expand upon this observation by linking this neuropeptide Y activity not only to animal (rat) vasomotor responses, but also to parallel human responses in middle-sized arteries and veins. Although at present at least 6 subtypes of neuropeptide Y receptors have been characterized, only the Y_1 subtype has been clearly identified in blood vessels. We cannot rule out that neuropeptide Y receptors distinct from BIBP 3226-sensitive sites could be involved in the electrically evoked vasomotor responses, although the current literature emphasizes the preponderance of neuropeptide Y Y_1 receptors in blood vessels.

On the basis of the previously stated premises, one can infer that neuropeptide Y is an integral participant in the response to sympathetic nerve stimulation of the human inferior mesenteric artery and vein. As such, the findings suggest that the neuropeptide Y Y_1 receptor could be an important component in human vascular homeostasis. The findings of Zukowska-Grojec et al. (1996) that BIBP 3226 has the potential, when administered intravenously, to partially restore normal blood pressure in cold-stressed rats underlines the relevance of the present results to sympa-

thetically driven homeostasis. Furthermore, the tissues' resistance to complete BIBP 3226 inhibition indicates that neuropeptide Y participates as a complementary member in a coordinated co-transmission response involving noradrenaline and adenosine 5′-triphosphate. Further evidence of this phenomenon is demonstrated by the disappearance of the second component of the tension-response curve in the rat superior mesenteric vein, indicating that neuropeptide Y may be involved in the prolongation of the contractile event.

The clinical implications of these findings are of paramount interest as they relate to conditions such as the fight or flight response, stress, hemorrhage, pheochromocytoma and other pathological conditions characterized by intense sympathetic reflex discharges. The biomedical evaluation of these ideas, particularly the concept of sympathetic co-transmission, merits close attention.

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