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Short communication

The involvement of neuropeptide Y Y_1 receptors in the blood pressure baroreflex: studies with BIBP 3226 and BIBO 3304

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Abstract

To ascertain the role of the neuropeptide Y Y_1 receptors in the vascular manifestations of the sympathetic baroreflex, 10-s bilateral carotid occlusions were performed in anesthetized cats; systemic blood pressure was monitored continually. This maneuver rose systolic blood pressure in 23 ± 2 mmHg. Following 100 μg/kg BIBP 3226 or BIBO 3304 i.v., the increase in blood pressure elicited by the occlusions was only 14 ± 1 and 15 mmHg, respectively. Both BIBP 3226 and BIBO 3304 displaced significantly 5.5 fold rightward the pressor dose–response curve elicited by exogenous neuropeptide Y, without altering the norepinephrine curve. Prazosin (10 μg/kg) reduced the pressor response elicited by the carotid occlusion to 12 ± 4 mmHg. The simultaneous administration of BIBP 3226 plus prazosin rose the systemic blood pressure following the occlusion only 9 ± 2 mmHg, supporting the involvement of neuropeptide Y in vascular sympathetic reflexes.

Keywords: Neuropeptide Y Y_1 receptor; BIBP 3226; BIBO 3304; Sympathetic reflex; Sympathetic vascular control; Blood pressure homeostasis

1. Introduction

Neuropeptide Y is commonly found stored together with catecholamines both in central and peripheral sympathetic neurons, particularly those innervating the cardiovascular system. Neuropeptide Y is the most abundant neuropeptide in the brain and heart (Zukowska-Grojec and Wahlestedt, 1993). Classic 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). Furthermore, chromatographic determinations coupled to radioimmunoanalysis evidenced the chemical identity of the peptide released following electrical stimulation of sympathetic perivascular nerve fibers (Donoso et al., 1997a). A variety of physiological and pharmacological evidence supports the notion that neuropeptide Y is involved in blood pressure homeostasis. The peptide is a potent vasoconstrictor (see review by Potter, 1991) and modulator of the vasomotor effect of norepinephrine (López et al., 1989) and adenosine 5′-triphosphate (ATP) (Westfall et al., 1995) in every animal species examined, including humans (Racchi et al., 1997).

Neuropeptide Y belongs to a family of peptides that includes peptide YY and the pancreatic polypeptide. At present, six receptors have been described for neuropeptide Y, only some of which have been cloned (Michel et al., 1998). Neuropeptide Y Y_1 receptors predominate in the vascular smooth muscles; the activation of this receptor mediates vasoconstriction, with little or no contribution of the neuropeptide Y Y_2 or other receptor subtypes (Zukowska-Grojec and Wahlestedt, 1993). The recent availability of selective neuropeptide Y receptor antagonists such as BIBP 3226 (Rudolf et al., 1994) and BIBO 3304 (Wieland et al., 1998) has opened the possibility to further study the involvement of neuropeptide Y in the regulation of blood pressure. While there is consensus that neuropeptide Y does not participate in the minute-to-minute regulation of blood pressure, the peptide is apparently involved in vascular responses following intense sympathetic nerve discharges (Donoso et al., 1997b; Racchi et al., 1997). Several evidence support this view, highlighting

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the notion that neuropeptide Y is a stress hormone (Zukowska-Grojec, 1995).

Based on the hypothesis that neuropeptide Y is released from sympathetic neurons following stressful stimuli, we were enticed to investigate the participation of neuropeptide Y in vascular sympathetic reflexes. To test this idea, we used the classic maneuver of the bilateral carotid artery occlusion (BCO) to elicit the baroceptor reflex, a stimuli known to evoke intense sympathetic vasoconstriction (Kaindl and Von Euler, 1951; Karasawa et al., 1982). To validate the participation of neuropeptide Y Y_1 receptors in this reflex response, selective neuropeptide Y Y_1 receptor antagonists were evaluated as blockers of the pressor response elicited by the bilateral carotid occlusion. This manuscript details the development of these protocols in cats.

2. Materials and methods

2.1. Neck surgery and the development of experimental protocols

Twenty adult cats (average 2.2 kg) were obtained from our Animal Reproduction Laboratories. The animals were anesthetized with 40 mg/kg sodium pentobarbital i.p.; a tracheotomy was performed to facilitate normal ventilation. Both carotid arteries were carefully dissected and cleaned from surrounding tissues avoiding damaging the sympathetic trunk of the neck. The left femoral artery was cannulated to insert a probe that was connected to a Stathan strain gauge for the continual recording of systemic blood pressure. The cannula was filled with heparinized saline. The contralateral femoral vein was cannulated to administer drugs i.v.

Both common carotid arteries were simultaneously occluded using a home-made gadget. Routinely, 10-s occlusions were repeated at 2–3-min intervals several times prior to, and after drug administration. Occasionally, 30-s occlusions were performed. The pressor response elicited was remarkably reproducible.

The results are expressed as the rise in systolic or diastolic blood pressure (mmHg) elicited by the bilateral carotid occlusion procedure, prior to and following drug administration.

2.2. Comparative study on the influence of neuropeptide Y Y_1 and α_1-adrenoceptors antagonists on the pressor response elicited by bilateral carotid occlusion

Three separate sets of protocols were developed. In a first series of 13 cats, the effect of either 100 μg/kg BIBP 3226 or 100 μg/kg BIBO 3304 were separately examined on 10- and 30-s carotid occlusions-induced pressor responses. In a second separate series of four cats, the effect of 10 μg/kg prazosin on the pressor response elicited by 10-s occlusions were examined. In a third set of three experiments, the effect of the joint administration of 10 μg/kg prazosin plus 100 μg/kg BIBP 3226 on the 10-s occlusion-induced pressor response was evaluated.

2.3. Neuropeptide Y and norepinephrine dose–response protocols

To examine the specificity of BIBP 3226 and BIBO 3304 as neuropeptide Y Y_1 receptor antagonists, neuropeptide Y ($n = 13$) and norepinephrine ($n = 5$) pressor dose–response protocols were performed prior to and 10 min after the i.v. bolus administration of either 100 μg/kg BIBP 3226 ($n = 11$) or BIBO 3304 ($n = 1$).

2.4. Drugs and statistical analysis

Human neuropeptide Y was purchased from Peninsula Labs. and Bachem (CA, USA). Dr. K. Rudolf (Dr. Thomae, Biberach, Germany) generously supplied samples of BIBP 3226 ((*R*), N^2-(diphenylacetyl)-*N*-(4-hydroxyphenyl)-methyl-D-arginineamide) and BIBO 3304 ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N^2-(diphenylacetyl)-arginineamide trifluoroacetate). Prazosin hydrochloride and norepinephrine were purchased from Sigma (St. Louis, MO). Distilled water stock (10 μg/μl) aliquots of all drugs were defrosted daily and diluted in saline as necessary.

Results are expressed as the mean change in systolic or diastolic blood pressure ± S.E.M. Statistics were performed using the paired Student's *t*-test or the Dunnett's tables for multiple comparison with a single control.

3. Results

3.1. Increase in blood pressure following BCO; blockade by neuropeptide Y Y_1 receptor antagonists

Ten-second carotid occlusions caused a transient rise in systolic and diastolic blood pressure of 23 ± 2 and 24 ± 1 mmHg, respectively ($n = 19$, $P < 0.001$). Responses were consistently reproducible; series of six repeated 10-s occlusion maneuvers originated polygraphic tracings with identical raises in SBP and DBP. The rise in blood pressure following the 30-s procedure showed a plateau (Fig. 1).

Administration of 100 μg/kg BIBP 3226 or 100 μg/kg BIBO 3304, reduced significantly the increases in systemic blood pressure elicited by the carotid occlusions (Fig. 1). Both antagonists produced a maximal reduction of the pressor response between 6–8 min following the antagonist administration. Additionally, 100 μg/kg BIBP 3226 caused a significant but short-lived decrease in systolic and diastolic blood pressure of 4.4 ± 1.5 and 7.2 ± 2.0 mmHg, respectively ($n = 8$, $P < 0.05$ and 0.01). In contrast, 100 μg/kg BIBO 3304 did not reach a significant reduction in

systemic blood pressure (8.8 ± 5.5 and 7.5 ± 5.9 mmHg, respectively, $n = 4$). No significant variations in blood pressure were observed when saline injections were performed as the vehicle control.

3.2. Effect of prazosin and the co-administration of BIBP 3226 plus prazosin

Prazosin significantly reduced the rise in blood pressure elicited by the carotid artery occlusions. The joint administration of 100 μg/kg BIBP 3226 plus 10 μg/kg prazosin produced a significantly lower rise in systolic and diastolic blood pressure ($P < 0.05$) than that caused by BIBP 3226 alone (Fig. 1).

3.3. Selectivity of the antagonism

BIBP 3226 and BIBO 3304 caused a parallel displacement of the neuropeptide Y but not of the norepinephrine pressor dose–response curves (Fig. 2). Both antagonists significantly increased 5.5 the dose of neuropeptide Y required to rise systolic blood pressure by 20 mmHg ($P < 0.05$). No change in the dose of norepinephrine required to elicit a similar effect was observed, evidencing the selectivity of the antagonism.

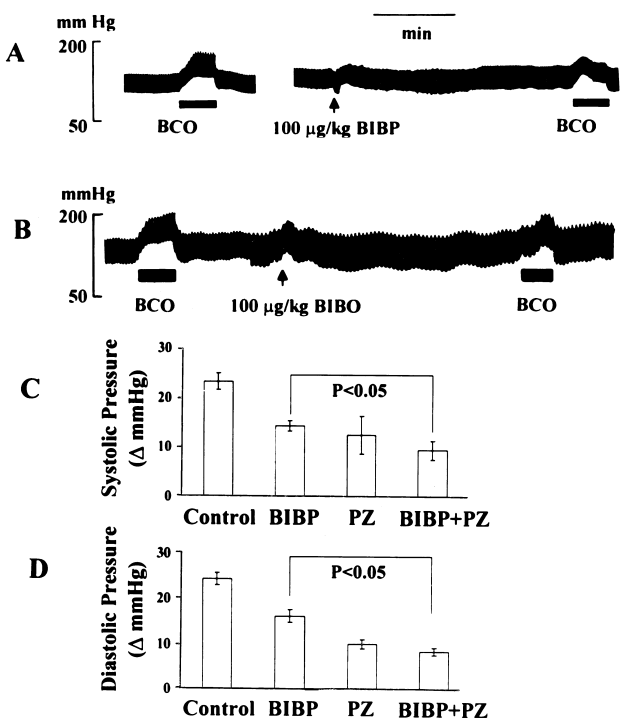

Fig. 1. BIBP 3226 and BIBO 3304 antagonize the pressor response elicited by bilateral common carotid occlusion (BCO). Representative polygraphic tracings illustrate that the administration of 100 μg/kg BIBP 3226 (A) and BIBO 3304 (B) reduce the pressor effect evoked by 30-s occlusions of the carotid arteries. Panels C and D show the reduction in the rise in systolic blood pressure and diastolic blood pressure elicited by 10-s occlusions. The maximal inhibition of the pressor response was attained 6–8 min following the administration of the antagonists. 100 μg/kg BIBP 3226 ($n = 11$), 10 μg/kg prazosin ($n = 4$) or the simultaneous administration of 100 μg/kg BIBP 3226 and 10 μg/kg prazosin ($n = 3$) significantly reduced the rise in systemic blood pressure evoked by the occlusion procedure prior to drug administration (control). The combination of BIBP 3226 plus prazosin further reduced the pressor effect elicited by the occlusion maneuver.

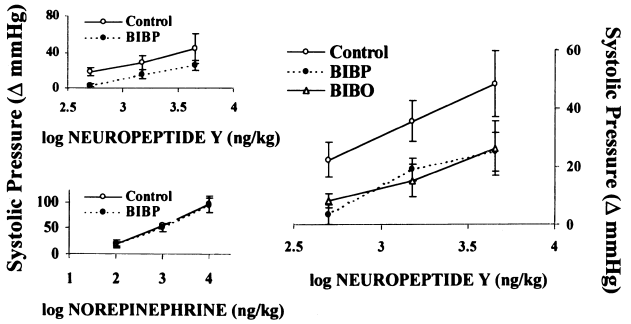

Fig. 2. Specific blockade of the neuropeptide Y Y_1 receptor by BIBP 3226 and BIBO 3304. The administration of 100 μg/kg BIBP 3226 i.v. causes a significant 5.5-fold rightward displacement of the neuropeptide Y dose–response curve, effect which is not observed with the norepinephrine. BIBP 3226 and BIBO 3304 are equally potent antagonists of the pressor response induced by exogenous neuropeptide Y.

4. Discussion

Bilateral occlusions of the common carotid artery are known to elicit an intense sympathetic reflex pressor response, which involves the systemic release of noradrenaline and adrenaline derived not only from the perivascular sympathetic nerves, but also from the adrenal medulla (Kaindl and Von Euler, 1951). In cats, this response is known to be partially attenuated by α_1-adrenoceptor blocking drugs such as prazosin and pharmacologically related compounds (Karasawa et al., 1982). To perform this study we chose cats since preliminary experiments in rats showed erratic pressor responses to carotid occlusion procedures. It is likely that we elicited vagal stimulations due to the difficulties in the proper dissection of the sympathetic nerve trunks running parallel to the carotid artery.

In parallel with the observation that the acute administration of the neuropeptide Y Y_1 receptor antagonists transiently lowered the systemic blood pressure of the cat, these drugs consistently blocked the pressor response elicited by occlusions of the carotid arteries. A parsimonious interpretation of these findings suggests that neuropeptide Y is released from sympathetic nerves only upon intense sympathetic nerve stimulation. There is general agreement that the pressor action of neuropeptide Y is almost exclusively related to stimulation of neuropeptide Y Y_1 receptors (Zukowska-Grojec and Wahlestedt, 1993), demonstrating its abundant presence in the cat vascular tree. At the light of the concept of sympathetic co-trans-

mission (Donoso et al., 1997a,b), it is likely that high frequency discharges of the sympathetic terminals, as observed in the present results with the baroceptor reflex, co-releases neuropeptide Y, and ATP in addition to norepinephrine. Consonant with the short half-life of BIBP 3226 (Doods et al., 1995), the time course of the blockade of the pressor response induced by carotid occlusion likely mirrors the concentration of the antagonists in the vascular neuroeffector junction.

In full agreement with the report of Karasawa et al. (1982), the present results demonstrate that a small dose of 10 μg/kg prazosin reduced the rise systemic blood pressure elicited by carotid artery occlusion. Furthermore, the co-application of prazosin, a well characterized competitive α_1-adrenoceptor antagonist plus BIBP 3226, causes significantly more attenuation of the rise in blood pressure elicited by the occlusion than that attained by prazosin alone. This supports the notion that the pressor response elicited following the baroreflex likely encompasses the simultaneous activation of neuropeptide Y plus α_1-adrenoceptors receptors located in the vascular smooth muscles. This observation is consonant with the interpretation that both neuropeptide Y_1 and α_1-adrenoceptors are involved in the physiology of the sympathetic baroceptor reflex control of blood pressure. Since prazosin is also known to antagonize α_{2B}-adrenoceptors, we cannot discard that post-junctional α_{2B}-adrenoceptors receptors might also be involved. Further support for this interpretation derives from other studies by Racchi et al. (1997) who reported that BIBP 3226 reduces the magnitude of the vasomotor response elicited by electrical nerve stimulation of sympathetic perivascular fibers from isolated human mesenteric vessels.

The present results demonstrate the specificity of BIBP 3226 and BIBO 3304 to block the pressor responses elicited by neuropeptide Y. Both compounds exhibit in the cat similar in-vivo potencies to displace the pressor dose–response curve elicited by exogenous neuropeptide Y; none altered the pressor activity elicited by norepinephrine. The displacement of the neuropeptide Y dose–response curve was parallel suggesting a competitive interaction, in agreement with the in-vivo findings reported by Mezzano et al. (1998) in the rat. Little literature is yet available for BIBO 3304, a newer non-peptide Y_1 receptor antagonist (Wieland et al., 1998) compound which apparently does not have a significantly different pharmacological profile compared to BIBP 3226 (Rudolf et al., 1994; Doods et al., 1995; Mezzano et al., 1998).

On the basis of the previously stated premises, it is possible to infer that neuropeptide Y is an integral participant of sympathetic reflexes. A strong sympathetic stimulation such as the one observed following carotid artery occlusion, must release neuropeptide Y, which cooperates, together with norepinephrine and ATP, in the manifestation of the physiological response. Along this vein, Zhang et al. (1997) recently reported that the stress evoked tachycardia in conscious spontaneously hypertensive rats is attenuated by BIBP 3226 administration, supporting the concept elaborated by Zukowska-Grojec (1995) that neuropeptide Y is a stress hormone, as illustrated by these results. A more detailed analysis of this data allows the suggestion that neuropeptide Y is not primarily involved in the minute-to-minute regulation of blood pressure homeostasis. However, it is evident that stressful stimuli such as observed in the present experimental conditions releases neuropeptide Y from sympathetic varicosities to the vascular neuroeffector junction. Consonant with this conclusion, it is notorious that while BIBP 3226 lowered modestly systemic blood pressure, the attenuation of the pressor response elicited by the occlusion of the carotid arteries was significant. This finding adds further evidence in favor of the notion that neuropeptide Y is a sympathetic co-transmitter involved in stressful responses.

The conclusion raised by Han et al. (1998) who suggested that in the arterial mesenteric bed, 30% of the vasoconstriction produced by sympathetic nerve stimulation depends on neuropeptide Y receptor activity, is fully supported by the present findings. The clinical implications of our findings showing that neuropeptide Y is an integrand component of cardiovascular sympathetic reflexes open new opportunities for biomedical research and therapeutic strategies for medical intervention. The involvement of neuropeptide Y in the clinical manifestations of cardiovascular sympathetic reflexes in different types of shock and in cardiac failure remains to be evaluated. In sum, the present findings document that neuropeptide Y is an active participant of sympathetic vascular reflexes. The notion that neuropeptide Y and norepinephrine are sympathetic co-transmitters offers new insights of the possible clinical relevance of neuropeptide Y in vascular homeostasis. The availability of Y_1 receptor antagonists offers opportunities to test this working hypothesis in the clinic.

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