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Apparent affinity and potency of BIBP3226, a non-peptide neuropeptide Y receptor antagonist, on purported neuropeptide Y Y_1, Y_2 and Y_3 receptors

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Abstract

The newly developed, purported non-peptide neuropeptide Y Y_1 receptor antagonist BIBP3226 was evaluated for its potential effect on the recently characterized Y_3 receptor subtype and for its apparent affinity in rat and human brain membrane binding assays using highly selective neuropeptide Y Y_1 and Y_2 radioligands. BIBP3226 potently blocked ($pA_2 = 7.36$) the contractile effect of neuropeptide Y in the rabbit saphenous vein, a Y_1 receptor bioassay and demonstrated nM affinity for Y_1/[^{125}I][Leu^{31},Pro^{34}]peptide YY binding sites. In contrast, it failed to antagonize the biological effects of neuropeptide Y in the rat vas deferens (Y_2) and rat colon (Y_3) and did not significantly competed for Y_2/[^{125}I]peptide YY-(3–36) binding sites in rat and human brain homogenates. Taken together, the results demonstrate further the high potency and selectivity of BIBP3226 for the neuropeptide Y Y_1 receptor by establishing its lack of antagonist activity on the Y_3 subtype.

Keywords: Neuropeptide Y; Neuropeptide Y_1, Y_2 and Y_3 receptor bioassay; Neuropeptide Y Y_1 receptor antagonist, non-peptide

Pharmacological data have suggested the existence of at least three (Y_1, Y_2 and Y_3) classes of neuropeptide Y (NPY) receptors (Gehlert, 1994; Wahlestedt and Reis, 1993). Only the neuropeptide Y Y_1 receptor subtype has been cloned so far (for example, Herzog et al., 1992). It is a typical member of the rhodopsin-like, G-protein coupled receptor super-family. In addition to its high affinity for neuropeptide Y and homologues such as peptide YY (PYY), the Y_1 receptor subtype demonstrates selectivity for agonists like [Leu^{31}, Pro^{34}]neuropeptide Y (Fuhlendorff et al., 1990) and [Leu^{31},Pro^{34}]peptide YY (Dumont et al., 1994) but not extended C-terminal fragments. In contrast, the neuropeptide Y Y_2 receptor subtype is preferentially recognized by neuropeptide Y and peptide YY fragments including peptide YY-(3–36), peptide YY-(13–36) and neuropeptide Y-(13–36) (Dumont et al., 1994; Wahlestedt and Reis, 1993). Much less is known about the purported neuropeptide Y Y_3 receptor subtype but it apparently only recognizes neuropeptide Y-, not peptide YY-, related molecules (Grundemar et al., 1991; Wahlestedt and Reis, 1993). However, progress as to the functional significance of each of these receptor classes has been hampered by the lack of selective antagonists.

Very recently, Rudolf and co-workers (Rudolf et al., 1994) reported on the development of BIBP3226 as the first highly potent non-peptide neuropeptide Y Y_1 receptor antagonist devoid of significant blocking activities on the Y_2 receptor subtype. However, its potential antagonism of the neuropeptide Y Y_3 receptor subtype was not investigated. The primary objective of this study was thus to evaluate the comparative antagonistic properties of BIBP3226 in prototypical Y_1 (rabbit saphenous vein), Y_2 (rat vas deferens) and Y_3 (rat colon) receptor in vitro bioassays (Dumont et al., 1993). Additionally, the apparent affinity of BIBP3226 for rat and human brain neuropeptide Y binding sites was investigated.

Tissues used as neuropeptide Y Y_1, Y_2 and Y_3 receptor bioassays were prepared as described in detail

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Table 1
Comparative antagonistic potencies of BIBN3226 in purported neuropeptide Y Y_1, Y_2 and Y_3 in vitro receptor bioassays

	Rabbit saphenous vein Y_1	Rat vas deferens Y_2	Rat colon Y_3
	ED_{50} (nM)		
Neuropeptide Y	20 ± 5	25 ± 4	86 ± 19
+ BIBP3226 (nM)			
10	26 ± 17	ND	ND
1000	295 ± 80 *	30 ± 6	95 ± 30
	* ($P < 0.05$)	(NS)	(NS)
	pA_2 7.36	Ineffective	Ineffective

Mean ± S.E.M. of 3–6 experiments. The pA_2 value represents the negative logarithm of the molar concentration of BIBP3226 required to produce a 2-fold increase in the ED_{50} of neuropeptide Y. This value was calculated from the Schild regression of log (concentration ratio − 1) vs. log of antagonist concentration (10^{-8}–10^{-6} M; 6 concentrations tested). ND, not determined; NS, non-significant vs. neuropeptide Y alone.

elsewhere (Dumont et al., 1993). In the neuropeptide Y Y_1 receptor preparation, neuropeptide Y related molecules induce a concentration-dependent contraction of the rabbit saphenous vein (Dumont et al., 1993) while these peptides inhibit the electrically evoked contractions of the rat vas deferens, a well established neuropeptide Y_2 receptor bioassay (Dumont et al., 1993; Wahlestedt and Reis, 1993; Rudolf et al., 1994). In the neuropeptide Y Y_3 rat colon in vitro assay, neuropeptide Y and related analogues and fragments act as contractile agents while peptide YY-related derivatives are inactive (Dumont et al., 1993). BIBP3226 (up to 5 μM; a generous gift from Thomae, Germany) failed to induce any agonist-like activities in these three preparations and was then tested for antagonistic properties against various concentrations of neuropeptide Y. For binding assays, rat and postmortem normal control human brain (Douglas Hospital Brain Bank) membranes were prepared as described in detail elsewhere (Dumont et al., 1993,1994) using 25–35 pM of high pressure liquid chromatography purified [^{125}I][Leu^{31},Pro^{34}]peptide YY and [^{125}I]peptide YY-(3–36) as highly selective Y_1 and Y_2 receptor radioligand (2000 Ci/mmol), respectively. Neuropeptide Y (1 μM) was used to determine specific binding (over 75–80% for both ligands) and concentrations of BIBP3226 ranging between 10^{-12} and 10^{-5} M were evaluated in competition binding assays.

As shown in Table 1, BIBP3226, potently and in a competitive manner, antagonized the contractile effect of neuropeptide Y in the rabbit saphenous vein while being inactive in the rat vas deferens. The apparent pA_2 value in the neuropeptide Y Y_1 receptor bioassay was calculated to be 7.36 with a Schild plot of 0.83. These results confirm and extend those most recently reported by Rudolf et al. (1994) using the rat kidney and the rat vas deferens as neuropeptide Y Y_1 and Y_2 receptor bioassays, respectively. Thus, BIBP3226 demonstrated high antagonistic potency and selectivity for neuropeptide Y Y_1 vs. Y_2 receptors. Binding data support these results as BIBP3226 possessed an affinity in the low nM range in neuropeptide Y Y_1- (rat frontoparietal cortex; K_i of 0.7 ± 0.2 nM; $n = 4$) but not Y_2- (rat hippocampus, $n = 4$ and human brain frontal cortex, $n = 4$, both $K_i > 10\,000$ nM) enriched receptor preparations (Dumont et al., 1994).

To our knowledge, BIBP3226 had not been evaluated for its potential activity on the purported neuropeptide Y_3 receptor subtype. As shown in Table 1, BIBP3226 failed to alter the contractile effect of neuropeptide Y in the rat colon, a neuropeptide Y Y_3 receptor bioassay (Dumont et al., 1993,1994). It would thus appear that in addition to its lack of antagonistic effects on the neuropeptide Y Y_2 receptor subtype, BIBP3226 is not active on the Y_3 receptor establishing further its exquisite selectivity as a tool to investigate the physiological role of the neuropeptide Y Y_1 receptor subtype. It also suggests the existence of significantly different pharmacophores for the neuropeptide Y Y_1 and Y_3 receptors, in addition to their known differential ability to recognize neuropeptide Y- and peptide YY-related molecules (Grundemar et al., 1991, Wahlestedt and Reis, 1993).

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