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BIIE0246: A selective and high affinity neuropeptide Y Y_2 receptor antagonist

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Abstract

The in vitro biological characterisation of the first potent and selective non-peptide neuropeptide Y Y_2 receptor antagonist, (*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6h)-oxodibenz[*b*,*e*]azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cylopentyl] acetyl]-*N*-[2-[1,2-dihydro-3,5(4*H*)-dioxo-1,2-diphenyl-3*H*-1,2,4-triazol-4-yl]ethyl]-argininamid (BIIE0246) is reported. BIIE0246 displaced [^{125}I]neuropeptide Y with high affinity (IC_{50} = 3.3 nM) from the human neuropeptide Y Y_2 receptor and proved to be highly selective. BIIE0246 displayed antagonistic properties and thus represents the first selective non-peptide neuropeptide Y Y_2 receptor antagonist. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Neuropeptide Y; Neuropeptidc Y Y_2 receptor antagonist; BIIE0246

Five neuropeptide Y receptor subtypes have been pharmacologically characterised (Michel et al., 1998). However, only selective antagonists for the neuropeptide Y Y_1 receptor and the neuropeptide Y Y_5 receptor have been described so far. (*R*)-*N*-[[4-hydroxyphenyl]methyl]-N^2-(diphenylacetyl)-argininamide, BIBP3226, (Rudolf et al., 1994) and (*R*)-*N*-[[4-(aminocarbonylaminomethyl)phenyl]-methyl]-N^2-(diphenylacetyl)-argininamide, BIBO3304, (Wieland et al., 1998) are selective antagonists for the neuropeptide Y Y_1 receptor and 1-naphthalenesulfonamide, *N*-[[*trans*-4-[[(4-amino-2-quinazolinyl)amino]methyl]cyclohexyl]methyl]-, monohydrochloride, CGP71683A, is a selective neuropeptide Y Y_5 receptor antagonist (Criscione et al., 1998). The neuropeptide Y Y_2 receptor is pharmacologically characterised by its high affinity/agonistic potency for C-terminal neuropeptide Y or peptide YY fragments, e.g., neuropeptide Y (13–36) or peptide YY (3–36) (Gerald et al., 1995). However, these agonists also possess a moderate to high affinity for neuropeptide Y Y_5 receptors (Gerald et al., 1996). The 22-amino-acid peptide T4-[neuropeptide Y(33–36)]$_4$ has been suggested to possess neuropeptide Y Y_2 antagonistic properties (Grouzmann et al., 1997). However, this neuropeptide Y analogue has a rather low affinity for the neuropeptide Y Y_2 receptor (approx. 300 nM) and its receptor selectivity profile has not been reported. Because of this and its complex structure, T4-[neuropeptide Y (33–36)]$_4$ cannot be considered as a useful tool to examine the (patho) physiology of the neuropeptide Y Y_2 receptors in vitro and in vivo. In this study, we describe the receptor binding profile and the antagonistic properties of a novel and highly selective neuropeptide Y Y_2 antagonist: (*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6h)-oxodibenz[*b*,*e*]azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cylopentyl] acetyl]-*N*-[2-[1,2-dihydro-3,5(4*H*)-dioxo-1,2-diphenyl-3*H*-1,2,4-triazol-4-yl]ethyl]-argininamid (BIIE0246) (Fig. 1a).

Receptor-binding studies were performed employing SK-N-MC and SMS-KAN cells (neuroblastoma cell lines) to study Y_1 and Y_5 receptors. Chinese hamster ovary (CHO) and human embryonic kidney (HEK)293 cells were stably transfected with the Y_4 and the Y_5 receptor. Binding studies were performed according to Wieland et al., 1998, using [^{125}I]neuropeptide Y as the radioligand. BIIE0246 completely displaced the specific binding of radiolabelled neuropeptide Y from neuropeptide Y Y_2 sites on SMS-KAN cells expressing the human neuropeptide Y Y_2 recep-

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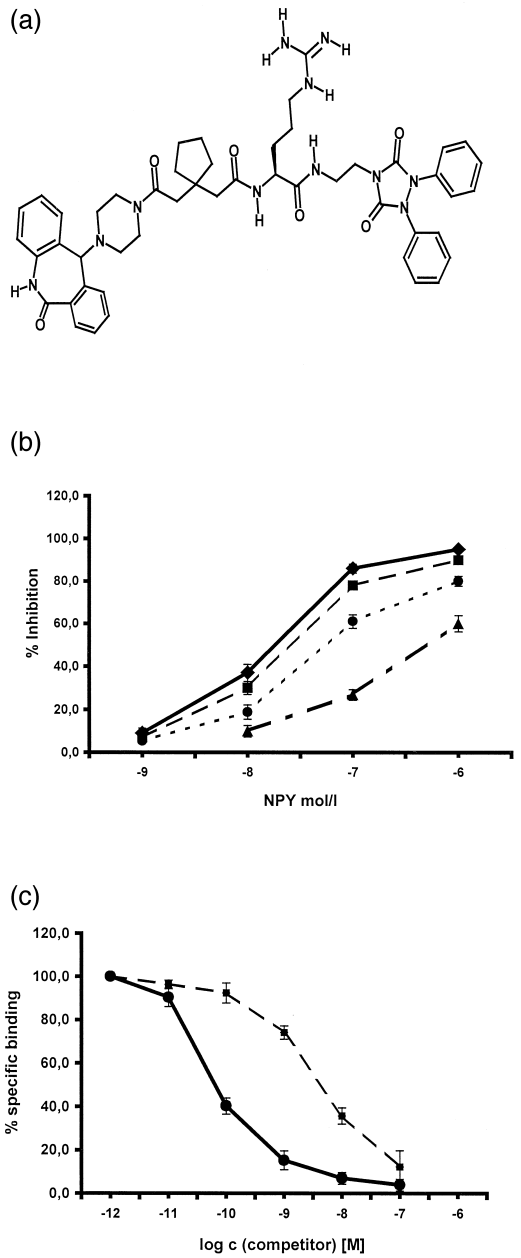

Fig. 1. (a) Structural formula of BIIE0246. (b) Competition of [^{125}I]neuropeptide Y with different concentrations of neuropeptide Y (· — ·) and BIIE0246 (■ — ■) in human neuropeptide Y Y_2 receptor expressing SMS-KAN cells. Data are presented as mean + S.E.M. (c) Inhibition of the twitch-response elicited by electrical field stimulation by neuropeptide Y in the rat vas deferens in the absence and presence of BIIE0246. Data are presented as mean ± S.E.M. (♦ control; ■ 0.01 μM; · 0.1 μM; ▲ 1.0 μM).

tor, with an inhibition constant of (IC_{50}) of 3.3 ± 1.5 nM ($n = 3$). Neuropeptide Y exhibits an IC_{50} value of 0.04 ± 0.01 nM ($n = 4$). The affinity of 3.3 nM for the human neuropeptide Y Y_2 receptor was comparable to the affinities observed for neuropeptide Y Y_2 receptors in other species such as rat and rabbit (data not shown). The Hill coefficients were not significantly different from unity. BIIE0246 binds selectively to the neuropeptide Y Y_2 receptor as in concentrations up to 1 μM no displacement was observed for the human neuropeptide Y Y_1, Y_4 and Y_5 receptor, nor did it cross-react with a wide variety of 60 other receptor types or enzyme systems. In order to assess whether BIIE0246 has antagonistic properties we examined the ability to inhibit the neuropeptide Y induced inhibition of the twitch response in the isolated electrically stimulated rat and rabbit vas deferens, bioassays for the Y_2 and Y_1 receptor, respectively (Doods and Krause, 1991). In those two in vitro preparations BIIE0246 displayed no agonistic properties at concentrations up to 3 μM. However, the concentration–response curve of neuropeptide Y in the rat vas deferens was shifted to the right in a concentration-dependent manner, indicating competitive antagonism (Fig. 1c). At a concentration of 1 μM a pK_b value of 7.63 ± 0.07 ($n = 4$) was calculated. A concentration of 3 μM did not influence the effects of neuropeptide Y on the neuropeptide Y Y_1 receptor in the rabbit vas deferens.

Accordingly we can conclude that BIIE0246 is the first potent and selective neuropeptide Y Y_2 receptor antagonist. The affinity (3.3 nM) of BIIE0246 is approximately 100-fold higher than that of previously reported peptidic Y_2 antagonist T4-[neuropeptide Y (33–36)]$_4$. In addition to its high affinity to the neuropeptide Y Y_2 receptor we could also show that BIIE0246 has virtually no affinity for neuropeptide Y Y_1, Y_4 and Y_5 receptors. Therefore, BIIE0246 is a useful tool to characterise neuropeptide Y Y_2 receptors and to elucidate the (patho) physiological role of the neuropeptide Y Y_2 receptor.

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