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Short communication

Effects of the neuropeptide Y Y_2 receptor antagonist BIIE0246 on presynaptic inhibition by neuropeptide Y in rat hippocampal slices

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Abstract

We previously reported that (*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6*h*)-oxodibenz[*b*,*e*]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]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 a potent and highly selective neuropeptide Y Y_2 receptor antagonist. Neuropeptide Y Y_2 receptors have been proposed to mediate the inhibition by neuropeptide Y of excitatory synaptic transmission in rat hippocampus. Therefore, we investigated the effects of BIIE0246 on the electrophysiological properties of neuropeptide Y in rat hippocampal slices and determined the affinity of this novel antagonist for rat hippocampal neuropeptide Y Y_2 receptors. BIIE0246 displayed an affinity of $IC_{50} = 4.0 \pm 1.6$ ($n = 4$) for neuropeptide Y receptor binding sites labelled by ^{125}I-neuropeptide Y in rat hippocampal membranes. At a concentration of 1 μM, BIIE0246 completely antagonized the inhibitory effects of 300 nM neuropeptide Y on synaptic transmission in rat hippocampal slices. This is the first study showing that a selective neuropeptide Y Y_2 receptor antagonist is able to block neuropeptide Y mediated effects in the hippocampus and unambiguously characterizes the presynaptic receptor in the rat hippocampus as the neuropeptide Y Y_2 receptor.

Keywords: BIIE0246; Neuropeptide Y; Neuropeptide Y Y_2 receptor; Hippocampus

1. Introduction

The 36-amino acid peptide neuropeptide Y is widely distributed throughout the peripheral and central nervous systems, being especially abundant in limbic structures such as the hippocampus. For example, the CA1 region of the hippocampus is innervated by neuropeptide Y-immuno-reactive axons and terminals, which make numerous synaptic contacts with the dendrites of hippocampal neurons (Haas et al., 1987). The electrophysiological properties of neuropeptide Y have been investigated in several brain regions (for review, see Colmers and Bleakman, 1994) and demonstrate that effects of neuropeptide Y on central neurons are mainly inhibitory. Most extensively investigated in this respect has been the hippocampus. Neuropeptide Y is able to abolish excitatory neurotransmission in both hippocampal cultures as well as in slices (Bleakman et al., 1992; Colmers et al., 1988). These inhibitory effects are mediated by the blockade of glutamate release through stimulation of presynaptic pertussis toxin-sensitive receptors (Bleakman et al., 1992). Although neuropeptide Y can elicit its effects by at least five different receptor subtypes (Michel et al., 1998), it has been postulated that presynaptic inhibition by neuropeptide Y in rat hippocampus slice is mediated by a neuropeptide Y Y_2 receptor (Colmers et al., 1991; Greber et al., 1994). This pharmacological characterization is mainly based on the observation that C-terminal fragments of neuropeptide Y such as neuropeptide Y-(2-36) and neuropeptide Y-(13-36) potently inhibited excitatory postsynaptic potential amplitudes. Since these agonists are not only potent on the neuropeptide Y Y_2 receptor (Wieland et al., 1995) but also possess affinity for, e.g. the neuropeptide Y Y_5 receptor (Gerard et al., 1996), the final proof that the presynaptic inhibition is mediated by a neuropeptide Y Y_2 receptor can only be obtained by employing a selective neuropeptide Y

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Y_2 receptor antagonist. Therefore, to ascertain the neuropeptide Y receptor subtype responsible for the inhibition in hippocampus, we investigated the antagonistic properties of the novel and selective neuropeptide Y Y_2 receptor antagonist (*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6*h*)-oxodibenz[*b*,*e*]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]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, (Doods et al., 1999) and the neuropeptide Y Y_1 receptor antagonist (*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N^2-(diphenylacetyl)-argininamide trifluoroacetate, BIBO3304, (Wieland et al., 1998) on neuropeptide Y induced inhibition of synaptic transmission in rat hippocampal slices.

2. Material and methods

2.1. Electrophysiological studies

Transversal slices (400 μm thick) were prepared from the hippocampi of male Wistar rats (body weight: 150–200 g). Slices were transferred to the recording chamber which was perfused with artificial cerebrospinal fluid of the following composition (mM): NaCl 126, KCl 4, KH_2PO_4 1.4, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, glucose 4, pH 7.4. The solution was saturated with 95% O_2/5% CO_2 at 37°C.

Extracellular recordings from CA1 neurons were performed with borosilicate micropipettes (Hilgenberg, Malsfeld, Germany), which had resistances of 1–5 MΩ, when filled with 2 M NaCl. A bipolar electrode was used for orthodromic stimulation via the Schaffer collaterals. Stimuli (100 μs) were applied at a frequency of 0.033 Hz and an amplitude eliciting 70% of the maximum population spike amplitude. Signals were recorded with an Axoclamp 1-A amplifier (Axon Instruments, Foster City, USA) and stored and analyzed using a digital oszilloscope (1604, Gould Instruments, Madison, USA). Data are the means ± S.E.M. of 4–6 experiments for each treatment.

2.2. Receptor binding studies

Male rats (Chbb: Thom, 200–250 g) were decapitated, the brain removed and the hippocampi dissected. The tissue was homogenized in 50 volumes wet weight of ice-cold Tris–HCl buffer (mM: Tris, 50; NaCl, 100; and KCl, 5; pH 7.5) and centrifuged for 15 min, at 48,000 × *g* at 0°C. The resulting pellet was resuspended in 200 volumes of incubation buffer (50 mM Tris, 100 mM NaCl, 0.4 mM phenylmethyl sulfonylfluoride, 1% bovine serum albumin, 0.025% bacitracin, 1 mM EGTA, 5 mM $MnCl_2$ and pH 7.5) and homogenized again. The homogenate (0.1 ml) was incubated for 2 h at room temperature in the presence of 30-pM [^{125}I]neuropeptide Y and increasing concentrations of test compound in a final concentration of 0.25 ml. The incubation was stopped by centrifugation and (5 min, 1500 × *g*), the pellet was washed with 0.25 ml of incubation buffer, re-centrifuged and membrane-bound radioactivity was counted. Nonspecific binding was deter-

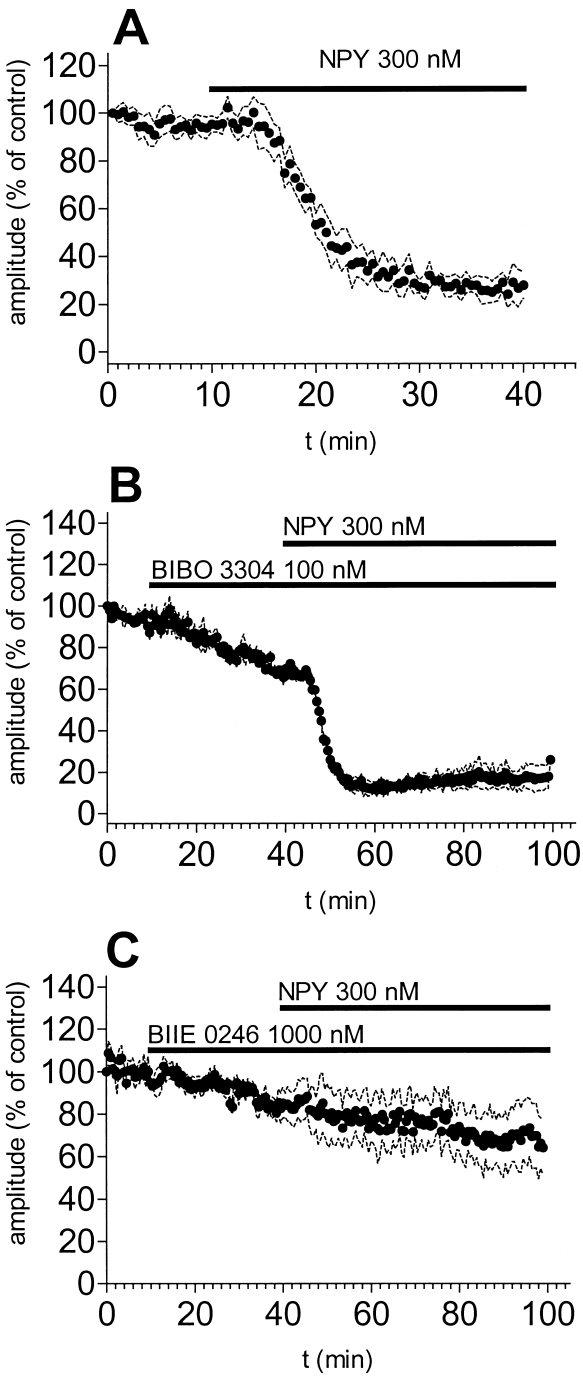

Fig. 1. Effects of neuropeptide Y and neuropeptide Y receptor antagonist on population spike amplitudes in hippocampal slices. (A) Neuropeptide Y at a concentration of 300 nM reduced population spike amplitudes to about 25% of the controls. (B) Bath application of the neuropeptide Y Y_1-selective receptor antagonist, BIBO3304 (100 nM), did not affect the neuropeptide Y-induced reduction of spike amplitudes. (C) BIIE0204 (1000 nM), a neuropeptide Y Y_2-selective receptor antagonist, almost completely suppressed the effects of neuropeptide Y.

mined in the presence of 100 nM neuropeptide Y. Competition binding experiments were analyzed by using an iterative nonlinear least-square regression analysis (RS/1 software package, BBN Research Systems, Cambridge, MA, USA). All data are expressed as mean ± S.E.M.

[^{125}I]human neuropeptide Y-Tyr^{36} (81 Tbq/mmol specific activity) was purchased from Anawa Trading (Wangen/Zürich, Switzerland), neuropeptide Y from Bissendorf Biochemical (Hannover, Germany). BIIE0246 and BIBO3304 were provided by the Department of Chemistry of Boehringer Ingelheim Pharma, Biberach, Germany.

3. Results

3.1. Electrophysiological studies

The inhibition of excitatory synaptic transmission in the CA1 region by neuropeptide Y is concentration-dependent (0.03–1.0 μM). A concentration of 300 nM caused a marked inhibition of the population spike amplitude of approximately 75% and was used to investigate the effect of the antagonists (Fig. 1A). BIBO3304 (50 or 100 nM) was not able to antagonize the effect elicited by 300 nM neuropeptide Y (Fig. 1B). In contrast, at a concentration of 500 nM, BIIE0246 antagonized neuropeptide Y-mediated effects by approximately 50%, whereas a concentration of 1000 nM caused an almost complete suppression of the neuropeptide Y effect (Fig. 1C).

3.2. Receptor binding studies

[^{125}I]neuropeptide Y is specifically bound to neuropeptide Y receptors in rat hippocampal membranes. BIIE0246 completely displaced the radioligand and the Hill coefficient was not significantly different from unity (Fig. 2).

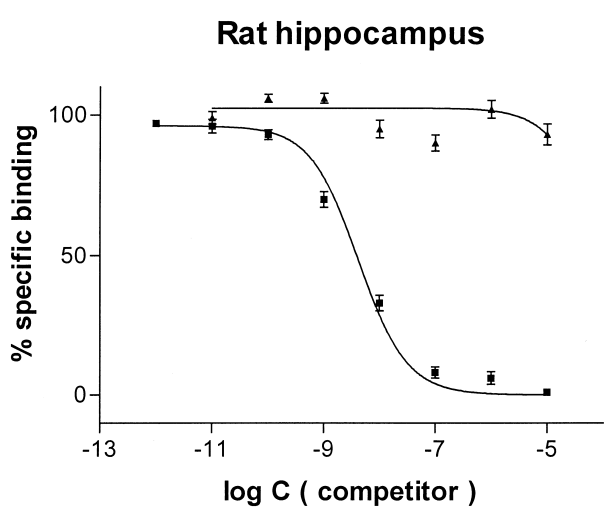

Fig. 2. (a) Displacement of specifically bound [^{125}I]human neuropeptide Y by (■) BIIE0246 and (▲) BIBO3304 using rat hippocampal membranes.

The corresponding IC_{50} value amounted to 4.0 ± 1.6 nM ($n = 4$). In contrast, BIBO3304, in a concentration up to 10 μM, did not displace the radioligand.

4. Discussion

The presynaptic inhibitory effects of neuropeptide Y on neurotransmitter release of peripheral neuroeffector junctions as well as in the central nervous system have been extensively investigated. Based on studies with neuropeptide Y receptor agonists, it seems that the presynaptic receptor in rat hippocampus has an identical pharmacological profile with the neuropeptide Y Y_2 receptor first characterized at sympathetic neuroeffector junctions in the rat vas deferens (Colmers et al., 1991; Colmers and Bleakman, 1994; Doods and Krause, 1991; Greber et al., 1994; Wahlestedt et al., 1985).

Recently, we described the pharmacological profile of BIIE0246, the first selective and high affinity neuropeptide Y Y_2 receptor antagonist (Doods et al., 1999). We could previously show that BIIE0246, at a concentration that is selective for the neuropeptide Y Y_2 receptor (1 μM), antagonized the neuropeptide Y-mediated inhibition of the twitch response in the isolated, electrically stimulated rat vas deferens. Accordingly, this peripheral presynaptic neuropeptide Y receptor inhibiting the release of noradrenaline from sympathetic nerve terminals is definitely of the neuropeptide Y Y_2 receptor subtype. In order to characterize the presynaptic neuropeptide Y receptor in the hippocampus, we first examined the affinity of BIIE0246 to neuropeptide Y receptors in the rat hippocampus. This novel neuropeptide Y Y_2 receptor antagonist displayed an affinity of $IC_{50} = 4$ nM for the neuropeptide Y Y_2 receptor in rat hippocampus which is in good agreement with the affinity reported for the human neuropeptide Y Y_2 receptor ($IC_{50} = 3.3$ nM). In addition, the binding studies revealed that the selective neuropeptide Y Y_1 receptor antagonist BIBO3304 (Wieland et al., 1998) was not able to displace any specifically labelled [^{125}I]neuropeptide Y from hippocampal membranes, supporting earlier studies that the neuropeptide Y receptor in the rat hippocampus is predominantly of the neuropeptide Y Y_2 receptor subtype (Dumont et al., 1996).

The present study confirms previous reports showing that neuropeptide Y is an effective and potent inhibitor of excitatory glutamate-mediated synaptic transmission in the hippocampus (Colmers et al., 1985, 1991; Bleakman et al., 1992; Greber et al., 1994). The neuropeptide Y Y_1 receptor antagonist BIBO3304, at a concentration at least 100-fold higher than its affinity for the rat neuropeptide Y Y_1 receptor, was not able to modify the response mediated by the neuropeptide Y. In contrast, BIIE0246 effectively antagonized the effect of neuropeptide Y on excitatory synaptic transmission. Our data support earlier findings, based on receptor localization studies and functional stud-

ies employing agonists, that the neuropeptide Y receptor modulating excitatory glutamatergic neurotransmission in the rat hippocampus belongs to the neuropeptide Y Y_2 receptor subtype.

It has been proposed that neuropeptide Y has an important role in the neuronal activity under pathological conditions such as seizures (Vezzani et al., 1999). For instance, it has been reported that in epileptic tissue, the density of presynaptic neuropeptide Y Y_2 receptors is increased (Schwarzer et al., 1998). Since exogenously applied neuropeptide Y has anticonvulsant properties (Smialowska et al., 1996), neuropeptide Y knockout-mice show increased susceptibility to seizures (Erickson et al., 1996) and neuropeptide Y Y_2 receptors seem to be present in human hippocampus (Jacques et al., 1997), the neuropeptide Y Y_2 receptor could be an interesting target for the treatment of human epilepsy. The neuropeptide Y Y_2 receptor antagonist BIIE0246 can play an important role to further characterize and investigate the neuropeptide Y Y_2 receptor in experimental epilepsy/seizure models.

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