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Effect of a selective neuropeptide Y Y_2 receptor antagonist, BIIE0246 on neuropeptide Y release

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Abstract

We have examined the 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) on neuropeptide release from rat hypothalamic slices in vitro. BIIE0246 prevented neuropeptide Y-(13-36)-induced reduction in basal and K^+-stimulated neuropeptide Y release. Addition of BIIE0246 alone enhanced K^+-stimulated neuropeptide release, without affecting basal release. These data are consistent with anatomical and functional studies suggesting a pre-synaptic role for neuropeptide Y Y_2 receptors in regulating rat hypothalamic neuropeptide Y release in the rat.

Keywords: Neuropeptide Y-(13-36); (Rat); Hypothalamus

Presently, five receptors which respond to the peptide neurotransmitter, neuropeptide Y, namely neuropeptide Y Y_1, Y_2, Y_3, Y_4, and Y_5 receptors, have been characterised in rat brain (Michel et al., 1998). Moreover, four of these receptors, (neuropeptide Y Y_1, Y_2, Y_4, and Y_5) have been localised to the hypothalamus, a region containing the highest concentration of neuropeptide Y in the brain where they may play a role in mediating the orexigenic effects of neuropeptide Y in this brain region (Stanley et al., 1986; King and Williams, 1998). Anatomical studies have localised neuropeptide Y Y_2 receptors to neuropeptide Y-containing neurons in the hypothalamic arcuate nucleus (Broberger et al., 1997), suggesting that they act as pre-synaptic autoreceptors, modulating neuropeptide Y release. In addition, mice deficient in neuropeptide Y Y_2 receptors demonstrate increased food intake and body weight which are suggestive of increased hypothalamic neuropeptide Y release as a result of loss in autoreceptor activity (Naveilhan et al., 1999). We have examined the effect of the selective, high-affinity non-peptide 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-oxo-ethyl]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) on neuropeptide Y release from rat hypothalamic slices to examine for evidence of an autoreceptor role for this receptor.

Experiments were performed on male Wistar rats (250–400 g) as described previously (King et al., 1999). Briefly, rats were killed by carbon dioxide inhalation and hypothalamic slices (0.5 mm^2) prepared by cross chopping with a razor blade. The slices were pre-incubated for 60 min each in 0.4 ml warm (37°C) Kreb's Ringer bicarbonate buffer (pH 7.4) which had been previously gassed with a 5% CO_2/95% O_2 mixture. Slices were then incubated in 0.4 ml fresh warm gassed Krebs Ringer bicarbonate buffer for four 15-min periods to establish basal neuropeptide Y release and then stimulated with a high K^+ medium (60 mM KCl) followed by three further recovery periods in normal Krebs Ringer bicarbonate buffer. Neuropeptide Y concentrations were measured by radioimmunoassay (King et al., 1999). The neuropeptide Y Y_2 receptor agonist,

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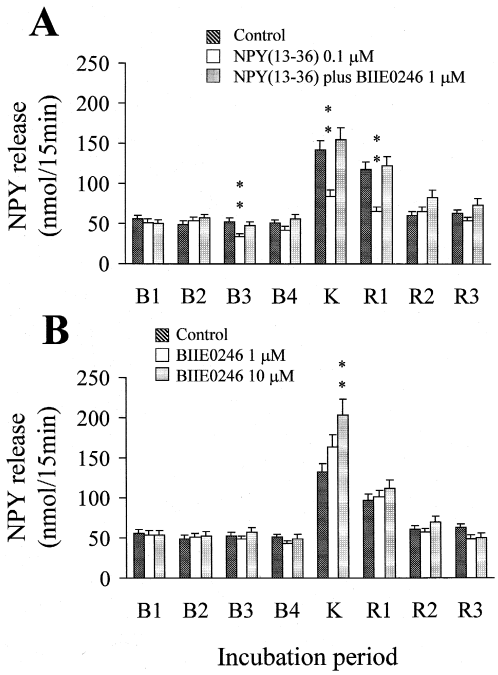

Fig. 1. (A) Basal (B1–B4), K^+-stimulated (K) and recovery (R1–R3) of neuropeptide Y release from hypothalamic slices in the control slices and in the presence of neuropeptide Y-(13-36) (0.1 μM) or neuropeptide Y-(13-36) (0.1 mM) and BIIE0246 (1 μM). Data shown as mean ± S.E.M. for experiments performed on 6 hypothalami. (B) Basal and K^+-stimulated release of neuropeptide Y from hypothalamic slices in the absence or presence of BIIE0246 (1 and 10 μM). Data shown as mean ± S.E.M. for experiments performed on 4–6 hypothalami. $^{**}P < 0.01$ as compared to controls, analysis of variance (ANOVA) with Bonferonni-modified *t*-tests for multiple comparisons.

neuropeptide Y-(13-36) (Bachem UK Saffron Walden, Essex, UK) and BIIE0246 (Boehringer Ingelheim Pharma) were introduced at the beginning of the third basal incubation period and were present throughout the remained of the experiment. Stimulated release was the release measured in period 5 minus the average of basal release during periods 1 and 2.

Neuropeptide Y release during K^+-induced depolarisation increased twofold over basal release, which fell back to basal levels over the following three recovery periods (Fig. 1A). Neuropeptide Y-(13-36) (0.1 μM) reduced basal neuropeptide Y release by 24% ($P < 0.05$) and reduced K^+-induced neuropeptide Y release by 51% as compared to control slices. Co-administration of BIIE0246 (1 μM) with the neuropeptide Y-(13-36) (0.1 μM) completely prevented the reduction in both basal and K^+-stimulated neuropeptide Y release (Fig. 1A). Incubation of the slices with BIIE0246 alone at 10 μM significantly increased K^+-induced neuropeptide Y release by 103% above levels measured in control tissue. There was also a non-significant 64% increase in neuropeptide Y release with 1 μM of BIIE0246 (Fig. 1B). However, neither 1 nor 10 μM of BIIE0246 significantly altered basal release of neuropeptide Y, as compared to control slices.

We have demonstrated that the moderately selective neuropeptide Y Y_2 receptor agonist, neuropeptide Y-(13-36) can substantially inhibit K^+-induced release of neuropeptide Y from rat hypothalamic slices as has been described previously (King et al., 1999) and that this effect can be blocked by the selective neuropeptide Y Y_2 receptor antagonist, BIIE0246. Although the weak neuropeptide Y Y_2 receptor antagonist, T4-[neuropeptide Y-(33-36)]$_4$ (Grouzemann et al., 1997) has been previously shown to block the effects of neuropeptide Y-(13-36), it was ineffective in altering release when administered alone (King et al., 1999). Here, we demonstrate that BIIE0246, which exhibits more than 100-fold higher affinity at neuropeptide Y Y_2 receptors over T4-[neuropeptide Y-(33-36)]$_4$ can substantially increase neuropeptide Y release from hypothalamic slices following depolarization of the tissue. Although, relative high amounts of BIIE0246 were required to increase stimulated neuropeptide Y release (1–10 μM), as compared to its high affinity at neuropeptide Y Y_2 receptors (3 nM), this may reflect a relatively poor penetration of the drug into the synaptic cleft, where the autoreceptors are possibly located. Only at higher concentrations of the drug is there sufficient accumulation of the drug at the synaptic cleft where activity is predicted to reside. These data suggests that synaptic concentrations of neuropeptide Y have a strong influence on further neuropeptide Y release, acting through pre-synaptic neuropeptide Y Y_2 autoreceptors, located on hypothalamic neuropeptide Y-ergic neurons. This hypothesis is consistent with data obtained from neuropeptide Y Y_2 knockout mice, where increased food intake leading to increased body weight was observed, possibly leading to increased neuropeptide Y-ergic activity as a result of a loss in autoreceptor negative feedback of neuropeptide Y release (Naveilhan et al., 1999). We suggest that blockade of neuropeptide Y Y_2 autoreceptors with drugs, such as BIIE0246, may be novel approach to stimulating appetite in certain wasting diseases, such as cancer cachexia.

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References

Broberger, C., Landry, M., Wong, H., Hokfelt, T., 1997. Subtypes Y_1 and Y_2 of neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology 66, 393–408.

Doods, H., Gaida, W., Wieland, H.A., Dollinger, H., Schnorrenberg, G., Esser, F., Engel, W., Eberlein, W., Rudolf, K., 1999. BIIE0246: A selective and high affinity neuropeptide Y Y_2 receptor antagonist. Eur. J. Pharmacol. 384, R3–R5.

Grouzemann, E., Buclin, T., Martire, M., Cannizzaro, C., Dorner, B., Razaname, A., Mutter, M., 1997. Characterization of a selective antagonist of neuropeptide Y at the Y_2 receptor. Synthesis and pharmacological evaluation of a Y_2 antagonist. J. Biol. Chem. 272 (12), 7699–7706.

King, P.J., Williams, G., 1998. Role of arcuate neuropeptide Y neurons in energy balance. Drug News & Perspect. 11, 402–410.

King, P.J., Widdowson, P.S., Doods, H.N., Williams, G., 1999. Regulation of neuropeptide Y release by neuropeptide Y receptor ligands and calcium channel antagonists in hypothalamic slices. J. Neurochem. 73, 641–646.

Michel, M.C., Beck-Sickinger, A., Cox, H., Doods, H.N., Herzog, H., Larhammer, D., Quirion, R., Schartz, T., Westfall, T., 1998. XVI. International Union of Pharamcology recommendations for the nomenclature of neuropeptide Y peptide YY, and pancreatic polypeptide receptors. Pharm. Rev. 50 (1), 143–150.

Naveilhan, P., Hassani, H., Canals, J.M., Ekstrand, A.J., Larefalk, A., Chhajlani, V., Arenas, E., Gedda, K., Svensson, L., Thoren, P., Ernfors, P., 1999. Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y_2 receptor. Nat. Med. 5 (10), 1188–1193.

Stanley, B.G., Kyrhouli, S.E., Lampert, S., Leibowitz, S.F., 1986. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7, 1189–1192.