Neuropeptide Y-Y1 Receptor Agonist Worsens while Antagonist Improves Survival of Cultured Y1-Expressing Neuronal Cells following Oxygen and Glucose Deprivation

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Astrocytes • Cell culture • Neuronal cells • Neuropeptide Y • Neuropeptide Y receptor • Neuroprotection

Abstract
In this in vitro study, we investigated the influence of neuropeptide Y (NPY) Y1 receptor activation or inhibition on the viability of cultured neuronal or glial cells following oxygen glucose deprivation (OGD). Viability of cultured cells was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. When compared to the vehicle-treated control group, treatment with NPY or [Leu31,Pro34]-NPY (Y1 agonist) reduced viability of cultured SK-N-MC (Y1-expressing) human neuronal cells at 24 h after 1 h of OGD, while BIBP3226 (Y1 antagonist) improved viability. Except at the highest concentration of NPY used in the study, treatment with NPY or NPY3-36 (Y2 agonist) did not influence viability of cultured SH-SY5Y (Y2-expressing) human neuronal cells at 24 h after 1 h of OGD. In addition, treatment with NPY, [Leu31,Pro34]-NPY, NPY3-36, or BIBP3226 did not affect viability of cultured primary astrocytes at 24 h after 4 h of OGD. The present results agree with those of a recent in vivo study. Activation of NPY-Y1 receptors may mediate ischemic pathophysiological processes, and inhibiting the Y1 receptors may be protective. The combination of OGD and cultured neuronal cells may be useful in future studies on the neuroprotective and harmful mechanisms of NPY-Y1 receptor inhibition and activation during ischemia, respectively.

Introduction

Neuropeptide Y (NPY), a highly conserved tyrosine-rich 36-amino acid peptide, is widely distributed throughout the peripheral and central nervous systems [20, 48]. NPY acts on at least six types of G-protein-coupled receptors: the Y1, Y2, Y4 and Y5 receptors are expressed in the rat and human brain, the y6 receptor is inactive in primates and absent in rats, and the putative Y3 receptor has not been cloned [33]. In addition, the Y1 and Y2 receptors predominate over the Y4 and Y5 receptors in the rat and human central nervous system [6, 10, 25, 29, 33]. Apart from its physiological effects on feeding, anxiety, circadian rhythms, reproduction and thermoregulation, NPY has been postulated to play a role in obesity, Huntington’s disease, Alzheimer’s disease, and Parkinson’s disease [19, 22, 46, 49, 50].

Several studies have shown a local increase in the immunoreactivity for NPY within the cerebral cortex around the site of infarct following experimental middle cerebral artery occlusion (MCAO) in rats [3, 16, 18]. In addition, the immunoreactivity for NPY increased...
around the perilesional cortex following excitotoxic damage [15]. The relative infarct volume after 2 h of MCAO plus 70 h of reperfusion was larger following intracarotid injection of a single dose of NPY given at about 1 min after onset of reperfusion or following intracisternal or intracerebroventricular (i.c.v.) injection of a single dose of NPY given at 30 min of onset of ischemia when compared to the vehicle-treated control groups; mild suppression of regional cerebral perfusion was also observed during the first 30 min of reperfusion [12]. Thus, NPY may have a harmful role in ischemic damage. The effects of a single i.c.v. injection of NPY3-36 (an NPY-Y2 receptor agonist), [Leu31,Pro34]-NPY (a Y1 receptor agonist), or BIBP3226 (a Y1 receptor antagonist; full name: N2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-D-arginine amide) given at 30 min of onset of ischemia on infarct volume and hemodynamic parameters in rats undergoing experimental MCAO for 2 h and reperfusion for 70 h were examined in another study [13]. When compared to an infarct volume following injection of NPY3-36, [Leu31,Pro34]-NPY increased the infarct volume whereas BIBP3226 decreased the infarct volume. In addition, injection of [Leu31,Pro34]-NPY tended to further reduce cerebral perfusion during ischemia while injection of BIBP3226 appeared to have the opposite effect [13].

While there is much information on the physiological effects of NPY-Y1 receptor activation, the harmful mechanisms of Y1 receptor activation during ischemia remain unknown and putative. Proposed mechanisms include aggravation of ischemia [12, 13], mobilization of calcium ion from the intracellular stores, inhibition of adenylate cyclase [1, 33], and enhanced production of nitric oxide (NO) [9, 14]. As a potent vasoconstrictor of the cerebral arteries in most animal species [4, 47], NPY has been implicated to play an important role in cerebrovascular regulation under physiological and pathological conditions. The present study was conducted to confirm the direct harmful and protective effects, respectively, of NPY-Y1 receptor activation and inhibition during in vitro 'ischemia/reperfusion injury' in cultured neuronal cells expressing either the Y1 or Y2 receptor as well as in primary glial cell culture.

Materials and Methods

Animals and Cell Lines

All experimental procedures were conducted according to the institutional guidelines with the protocol approved by the Committee on the Use of Live Animals in Teaching and Research, University of Hong Kong. Pregnant female Sprague-Dawley rats were obtained from the Laboratory Animal Unit, University of Hong Kong. SK-N-MC [American Type Culture Collection (ATCC) No.: HTB-10] and SH-SY5Y (ATCC No.: CRL-2266) neuronal cell lines were purchased from ATCC (Manassas, Va., USA).

Chemicals

NPY (Cat. No. 7180), [Leu31,Pro34]-NPY (Cat. No. 7607), BIBP3226 (Cat. No. 7617), and NPY3-36 (Cat. No. 7615) were purchased from Peninsula Lab (San Carlos, Calif., USA) and dissolved in normal saline containing 5% dimethyl sulfoxide as the vehicle. All cell culture chemicals were bought from Gibco-BRL (Rockville, Md., USA): minimum essential medium (MEM; Cat. No. 11095), L-glutamine (Cat. No. 25030), sodium pyruvate (Cat. No. 11840), non-essential amino acids (Cat. No. 11140), sodium bicarbonate (Cat. No. 25080), fetal bovine serum (FBS; Cat. No. 26140), Ham's F12 medium (Cat. No. 11765), Dulbecco's modified Eagle medium (DMEM) with high glucose (Cat. No. 11965), trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA; 0.5% trypsin and 0.53 M EDTA; Cat. No. 15305), trypsin blue (Cat. No. 15250), glucose-free DMEM (Cat. No. 11966), and penicillin plus streptomycin (Cat. No. 15140). Culture flasks, culture plates, and falcon tubes were purchased from Corning Lab Science (Acton, Mass., USA). Poly-L-lysine (Cat. No. P6893) and phosphate-buffered saline (PBS) were bought from Sigma Chemical (St. Louis, Mo., USA). Rabbit anti-cow glial fibrillary acidic protein (GFAP; No. Z0334), biotinylated goat antibody to mouse/rabbit IgG, strept-avidin-biotin complex/horse-radish peroxidase (streptABC/HRP), and 3,3'-diaminobenzidine (DAB) were purchased from DAKO (Glostrup, Denmark). Mitochondrial assay kit for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylinetrazolium bromide (MTT) was obtained from Boehringer Mannheim (Mannheim, Germany).

Neuronal Cell Line Culture

SK-N-MC and SH-SY5Y cells belong to a family of tumor-derived neuronal-like cell lines. They retain many properties of human neuronal cells and can be easily maintained under culture conditions [8, 38-40, 42]. The SK-N-MC cells selectively express the NPY-Y1 receptors [1, 42], and the SH-SY5Y cells selectively express the Y2 receptors [38, 39]. Such selective expression of the Y1 or Y2 receptors in these cell lines has previously been confirmed (unpubl. data). The stock vial of SK-N-MC or SH-SY5Y cell line was kept above the level of liquid nitrogen. The vial of cells was thawed at 37 °C for 2 min by gentle agitation before the vial contents were transferred to a 75-cm² culture flask containing 12–15 ml of culture medium and cultured for 1–2 weeks [21, 39, 40]. When the cells became confluent, they were dissociated using trypsin-EDTA and subcultured at a dilution of 1:10. The culture medium for SK-N-MC cells was MEM with 10% FBS, containing L-glutamine at 2 mM, sodium pyruvate at 1 mM, non-essential amino acids at 0.1 mM, sodium bicarbonate at 1.5 g/l, and penicillin and streptomycin at 50 units/ml. The culture medium for SH-SY5Y cells was 1:1 mixture of MEM and Ham's F12 medium with 10% FBS, containing L-glutamine at 2 mM, sodium pyruvate at 1 mM, non-essential amino acids at 0.1 mM, sodium bicarbonate at 1.5 g/l, and penicillin and streptomycin at 50 units/ml. The culture medium was changed twice per week. For the oxygen glucose deprivation (OGD) experiments, cultured SK-N-MC or SH-SY5Y cells were reseeded into 96-well culture plates at a density of 2 × 10⁴ cells per well (100 μl); OGD experiments were performed 24 h later. All SK-N-MC and SH-SY5Y cul-