

Activation of neuropeptide Y receptors is neuroprotective against excitotoxicity in organotypic hippocampal slice cultures¹

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SPECIFIC AIMS

We recently provided evidence that NPY Y1 and Y2 receptors modulate (inhibit) intracellular calcium changes and glutamate release in the rat hippocampus. Since NPY affects synaptic transmission and neuronal excitability and these effects are significantly mediated by its interaction with glutamatergic neurotransmission, we thought it important to clarify the involvement of different NPY receptors in neuronal protection against excitotoxicity. The present study was designed to investigate the possible neuroprotective role for NPY Y1, Y2, and Y5 receptors against excitotoxic insults in the hippocampus.

PRINCIPAL FINDINGS

1. AMPA and kainate-induced excitotoxicity

Using organotypic slice cultures of rat hippocampus, we investigated the putative neuroprotective role of NPY receptors against excitotoxic glutamate receptor-mediated neurodegeneration. To quantify neuronal damage, we used the fluorescent marker propidium iodide (PtdIns), a polar substance that enters only dead or dying cells with damaged cell membranes and binds to DNA with a bright red, intensified fluorescence (630 nm) when absorbing blue-green light (493 nm). Exposure of hippocampal slice cultures to 2 μ M AMPA for 24 h induced an increase in cellular uptake of PtdIns, particularly evident in the CA1 pyramidal and the dentate granule cell layers (Fig. 1A). Application of a selective AMPA receptor antagonist (LY303070; 15 μ M) allowed us to demonstrate that the neuronal degeneration essentially was due to a selective activation of AMPA receptors, since application of this compound abolished the increased PtdIns uptake induced by AMPA in the dentate granule and the CA1 pyramidal cell layers (Fig. 1A).

Exposure of hippocampal slice cultures to 6 μ M kainate for 24 h induced PtdIns uptake in the CA, particularly in the CA3 pyramidal cell layer, whereas no increase in PtdIns uptake was found in the dentate granule cell layer (Fig. 2A). Neurodegeneration in CA1 and CA3 was mediated by kainate receptors, since the AMPA receptor antagonist was unable to significantly prevent the PtdIns uptake induced by 6 μ M kainate in both cell layers (Fig. 2A).

2. Protection against AMPA-induced neurodegeneration of dentate granule cells and CA1 pyramidal cells by NPY receptor activation

The neuroprotection was evaluated after preexposure (24 h) to the different selective NPY receptors agonists, followed by 24 h of coexposure with 2 μ M AMPA. We found that 1 μ M [Leu³¹,Pro³⁴]NPY, an Y1 receptor agonist, reduced PtdIns uptake in the dentate granule cell layer (Fig. 1B) without having a significant effect on CA1 pyramidal cells (Fig. 1C). Concerning activation of Y2 receptors, neuroprotection induced by the selective agonist (300 nM NPY13–36) was significant, with a reduction of PtdIns uptake in the dentate granule (Fig. 1B) and CA1 pyramidal cell layers (Fig. 1C). In the presence of 500 nM NPY(19–23)-(Gly¹,Ser³,Gln⁴,Thr⁶,Ala³¹,Aib³²,Gln³⁴)-PP, described as an Y5 receptor agonist, AMPA-induced PtdIns uptake was reduced in the dentate granule cell layer (Fig. 1B), whereas no significant inhibition was observed in the CA1 pyramidal cell layer (Fig. 1C).

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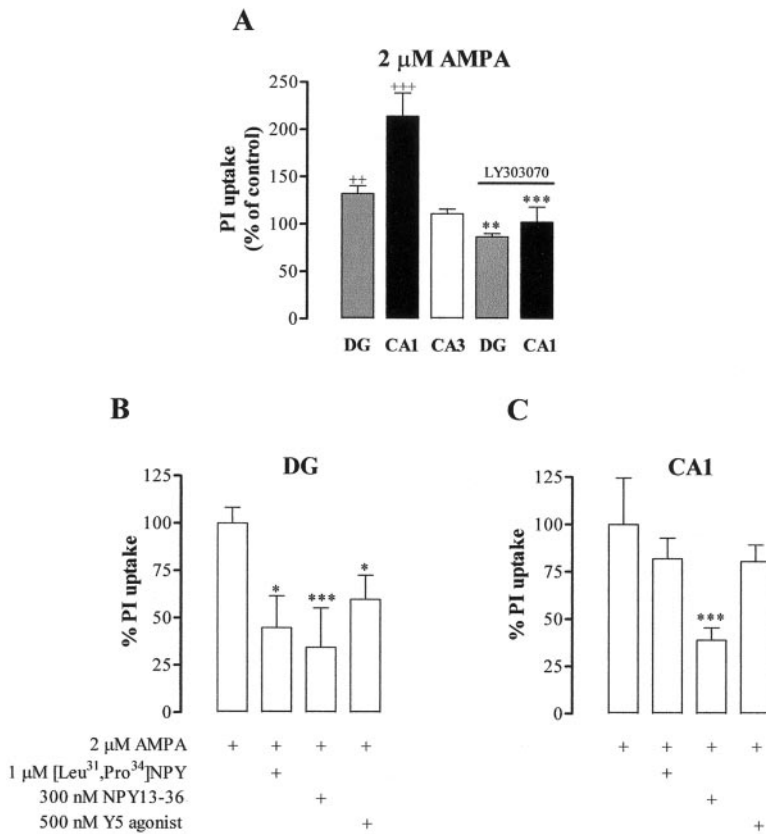


Figure 1. AMPA-induced cell degeneration in the dentate granule and CA1 pyramidal cell layers and neuroprotection by NPY receptor activation. *A*) Densitometric measurements of propidium iodide (PtdIns) uptake induced by 2 μ M AMPA in the dentate granule (DG), CA1, and CA3 pyramidal cell layers and the effect of LY303070 (15 μ M) on the toxicity induced by AMPA in both cell layers. Effect of Y1 (1 μ M [Leu³¹,Pro³⁴]NPY), Y2 (300 nM NPY13-36) or Y5 receptor agonist (500 nM NPY(19-23)-(Gly¹,Ser³,Gln⁴,Thr⁶,Ala³¹, Aib³², Gln³⁴)-PP) on PtdIns uptake induced by 2 μ M AMPA in the *B*) dentate granule cell layer or *C*) CA1 pyramidal cell layer. *A*) PtdIns uptake determined after 24 h is expressed as % of control. *B*, *C*) PtdIns uptake induced by 2 μ M AMPA was set to 100%. Data are shown as means \pm SE with $n=14-66$. *A*) $^{+}P < 0.01$, $^{+++}P < 0.001$ using *t* test for comparison with control (no drugs exposure), and $^{**}P < 0.01$, $^{***}P < 0.001$ using ANOVA with Bonferroni correction for comparison with the effect of 2 μ M AMPA in DG and CA1, respectively. *B*, *C*) $^{*}P < 0.05$, $^{***}P < 0.001$ using Dunnett's correction for comparison with control (2 μ M AMPA).

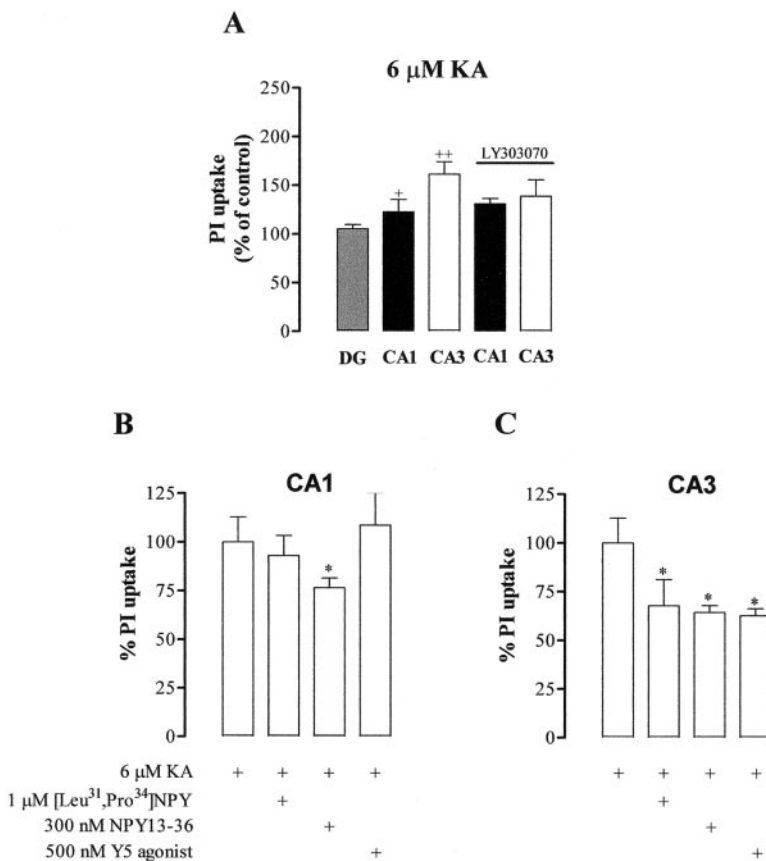
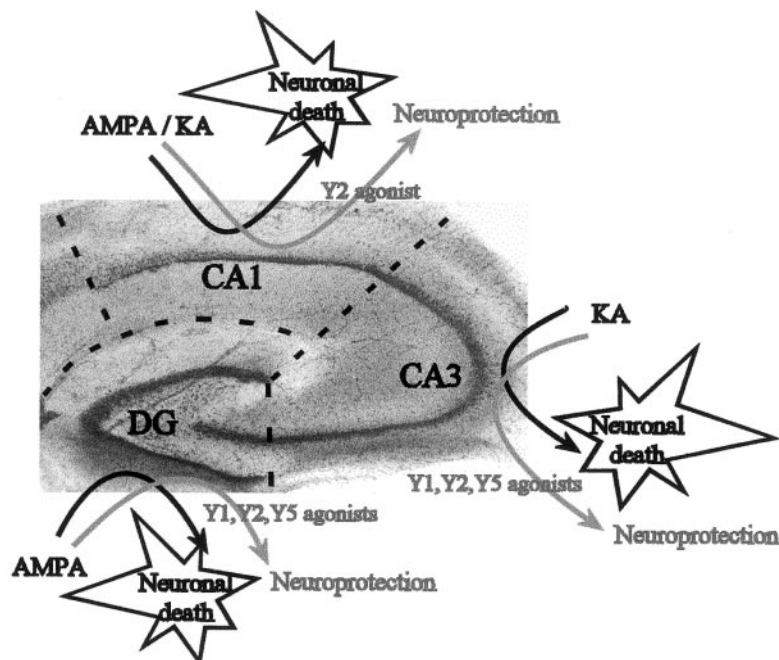


Figure 2. Kainate-induced cell degeneration in the CA1 and CA3 pyramidal cell layers and neuroprotection by NPY receptor activation. *A*) Densitometric measurements of propidium iodide (PtdIns) uptake induced by 6 μ M KA in the dentate granule (DG), CA1, and CA3 pyramidal cell layers and the effect of LY303070 (15 μ M) on the toxicity induced by kainate in CA1 and CA3 pyramidal cell layers. *B*, *C*) Effect of Y1 (1 μ M [Leu³¹,Pro³⁴]NPY), Y2 (300 nM NPY13-36) or Y5 receptor agonist (500 nM NPY(19-23)-(Gly¹,Ser³,Gln⁴,Thr⁶,Ala³¹, Aib³²,Gln³⁴)-PP) on PtdIns uptake induced by 6 μ M KA in *B*) CA1 and *C*) CA3 pyramidal cell layers. *A*) PtdIns uptake recorded after 24 h is expressed as % of control. *B*, *C*) PtdIns uptake induced by kainate was set to 100%. Data are shown as means \pm SE with $n=18-33$. *A*) $^{+}P < 0.05$, $^{2+}P < 0.01$ using *t* test for comparison with control (no drugs exposure). *B*, *C*) $^{*}P < 0.05$ using Dunnett's correction for comparison with control (6 μ M KA).

Figure 3. Schematic diagram illustrating the neuroprotective role of NPY Y1, Y2, and Y5 receptors in the CA1, CA3, and dentate gyrus (DG) subregions of the rat hippocampus. In the CA1 subregion, 2 μ M AMPA and 6 μ M KA both induced neuronal death, which was prevented only by activation of the Y2 receptors. In the CA3 and DG subregions, 6 μ M KA or 2 μ M AMPA induced neuronal death, respectively, which was prevented by Y1, Y2, and Y5 receptor activation in both cases.



3. Protection against kainic acid-induced neurodegeneration of CA3 and CA1 pyramidal cells by NPY receptor activation

In CA1, only activation of Y2 receptors inhibited the kainic acid-induced PtdIns uptake (Fig. 2B). In contrast, selective activation of Y1, Y2, and Y5 receptors prevented the increase in kainic acid-induced PtdIns uptake in the CA3 pyramidal cell layer (Fig. 2C). Exposure to 1 μ M [Leu³¹,Pro³⁴]NPY, 300 nM NPY13–36, or 500 nM NPY(19–23)-(Gly¹,Ser³,Gln⁴,Thr⁶,Ala³¹,Aib³²,Gln³⁴)-PP reduced the kainic acid-induced PtdIns uptake.

CONCLUSIONS

Several studies show that NPY can inhibit epileptiform activity in the rat hippocampus. Indeed, epileptic sei-

zures result in extensive increase in extracellular glutamate and NPY mRNA expression, indicating that NPY-mediated effects may be activated under epileptogenesis and excitotoxicity. In this study we have demonstrated the neuroprotective effect of Y1, Y2, and Y5 receptors activation against AMPA- and kainate-mediated neurodegeneration in hippocampal subregions (Fig. 3). A knowledge of which NPY receptors mediate the actions of NPY in the dentate granule, CA1, and CA3 pyramidal cell layers may be important for pharmacological targeting in several pathologic conditions associated with glutamate receptor hyperactivation. Moreover, identification of the molecular mechanisms involved in the inhibition of glutamate release, calcium influx, and neuroprotection may lead to the development of new NPY-based pharmacological therapies important for treating neurodegenerative diseases, epilepsy, and stroke. FJ