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Neuroprotective Effects of Neuropeptide Y and Y2 and Y5 Receptor Agonists *In Vitro* and *In Vivo*

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1. Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide, widely distributed in the nervous system where it plays the role of a neurotransmitter and neuromodulator (Chronwall et al., 1985; Gray & Morley, 1986). It belongs to the pancreatic polypeptide family (PP family) together with peptide YY (PYY) and pancreatic polypeptide (PP), which are mainly gut and endocrine regulatory peptides (Tatemoto et al., 1982). All these peptides have a common hairpin-like tertiary structure, consisting of an N-terminal poly-proline helix and a long alpha-helix connected by a beta turn (Allen et al., 1987; Glower et al., 1984). NPY contains 5 tyrosine residues in its primary structure, (Fig.1) therefore, it is named neuropeptide Y or neuropeptide tyrosine, as "Y" is an abbreviation of tyrosine.

Tyr^{1}-Pro-Ser-Lys-Pro-Asp-Asn-Pro-Gly-Glu^{10}-Asp-Ala-Pro-Ala-Glu-Asp-Met-Ala-Arg-**Tyr**^{20}-

Tyr-Ser-Ala-Leu-Arg-His-**Tyr**-IIe-Asn-Leu^{30}-IIe-Thr-Arg-Gln-Arg-**Tyr**^{36}-NH_2

Fig. 1. Primary structure of NPY (human, rat).

NPY is present in the central and peripheral neurons and is described as one of the most abundant peptides in the brain (Chronwall, 1989). In the brainstem, it often coexists with noradrenaline (NA) and adrenaline (A) in some projecting catecholaminergic neurons, and with serotonin in human and rabbit, (but not rat) medullary neurons (Everitt & Hökfelt, 1989). In the forebrain NPY is present mainly in local inhibitory interneurons, where it coexists with GABA and often with somatostatin (Everitt & Hökfelt, 1989). Besides the central nervous system (CNS), NPY occurs also in the periphery: in chromaffin cells of the adrenal medulla (coexisting with A and NA), in sympathetic neurons (coexisting with NA) and in some (few) parasympathetic neurons. NPY was also found in blood cells, spleen, bone marrow, magakaryocytes and thrombocytes (Ericsson et al., 1987; Persson et al., 1989).

NPY is involved in many physiological functions, such as: regulation of blood pressure (in the periphery NPY is a potent vasoconstrictor), cardiorespiratory parameters and body temperature, control of the release of luteinizing hormone releasing hormone (LHRH) and corticotropin releasing factor (CRF), and modulation of feeding, sexual behavior, pain,

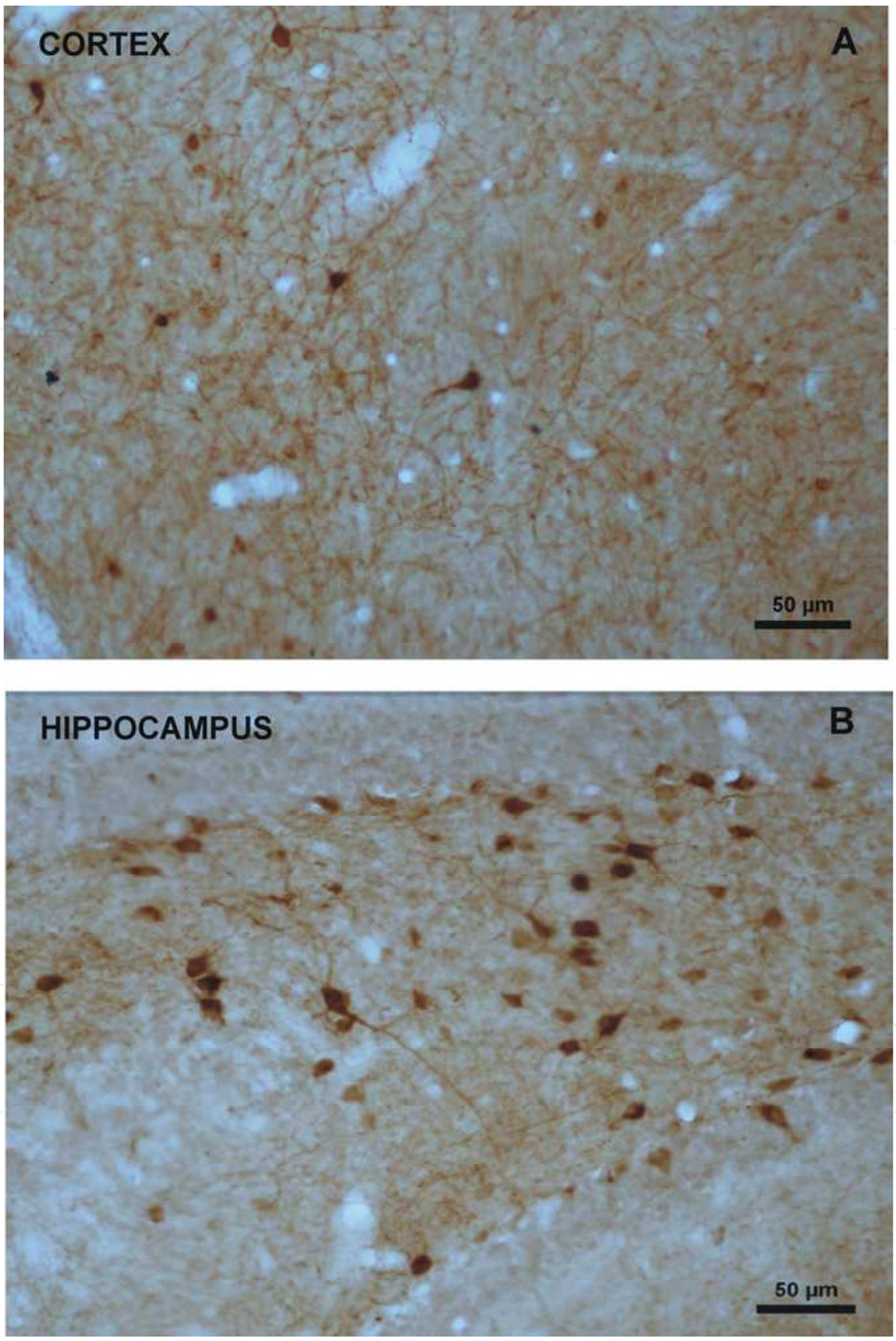

Fig. 2. Microphotographs showing NPY–immunoreactive neurons in the frontal sections of the mouse brain. A: the cerebral cortex; B: the hilar region of the hippocampus. Nerve cell bodies and varicose fibres can be seen. ABC staining.

anxiety (NPY has a strong antianxiety effect), circadian rhythms, memory processing, inhibition of the release of other neurotransmitters (Colmers et al., 1990; Danger et al., 1990; Gray & Morley, 1986; Greber et al., 1994; Wettstein, et al., 1995). NPY plays an important role in the regulation of neuronal activity, it reduces epileptiform activity in the hippocampus (Woldbye et al., 1997) by inhibition of glutamate release (Colmers & Bleakman, 1994; Greber et al., 1994; Silva et al., 2005b; Vezzani et al., 1999).

The numerous and diverse actions of NPY are mediated by specific, G-protein coupled heptahelical receptors, called Y receptors. Six Y receptors have been identified (Y1, Y2, Y3, Y4, Y5, and y6) based on different pharmacological profiles (Dumont et al., 1992). Five of them, except for Y3, have been cloned. The Y1, Y2 and Y5 receptors preferentially bind NPY and PYY, whereas Y4 is activated preferentially by PP. The Y3 receptor has not been cloned till now and was only pharmacologically characterized as having a very low affinity for PYY (ten times weaker than for NPY). In the brain, it was found only in the nucleus of the solitary tract. The y6 receptor functions only in the mouse and the rabbit and is absent in the rat, human and other primates (Michel et al., 1998; Silva et al., 2002, 2005a). Activation of all Y receptor types inhibits adenylate cyclase and in most of cells, that effect is abolished by botulin toxin, which indicates that Gi/Go proteins participate in the signal transduction from Y receptors to adenylate cyclase. The Y receptor activation influences also ion channels but the final effects depend on the type of target cell. It was best characterized for Y1 and Y2 receptors. In neurons, Y1 receptor activation enhanced Ca++ availability via inositol triphosphate (IP3)-mediated mobilization of intracellular Ca++ stores or by enhancing calcium ion entry through L-type channels, causing neuronal excitation. Inhibition of K+ channels after Y1 stimulation has also been described. On the other hand, Y2 receptor activation reduced Ca++ availability (via N-type channels) and inhibited neuronal function. Much evidence supports the excitatory role of Y1 receptors but opposite effects have also been noted, e.g. small neurons of dorsal root ganglia are inhibited by Y1 receptor activation by their hyperpolarization. The Y5 receptors have inhibitory properties and such effects have also been postulated for other Y receptors but were much less studied.

NPY activates all Y receptors with similar potency as PYY except for Y3 which is characterized by a higher affinity for NPY than for PYY and Y4 receptor which binds PP with high affinity, but PYY and NPY with very low affinity. If we want to study a role of particular Y receptor types, a more specific ligands are needed. Agonistic ligands are still only peptides (no smaller non-peptide molecules have been introduced). Y1 receptors are selectively activated by peptides NPY or PYY modified at the C-terminal end i.e. [Pro^{34}]-NPY, [Leu^{31},Pro^{34}]-NPY, [Leu^{31},Pro^{34}]-PYY. Such modification induces a loss of Y2 receptor affinity. It indicates that N-terminal part of the peptide is essential for Y1 receptor binding. In contrast, the binding to Y2 receptors does not require N-terminal sequence but C-terminal part of the peptide is crucial. Thus, the selective agonists of Y2 receptors include NPY (13-36) and some cyclic analogs of NPY. Receptors of Y5 type are activated by NPY(2-36), PYY(3-36), but the peptides stimulate also Y2 receptors. Selective Y5 receptor agonists were synthesized during the last 10 years, and one of them, a chimeric peptide of PP with changed NPY fragment, [cPP^{1-7},NPY^{19-23},Ala^{31},Aib^{32},Gln^{34}]-hPancreatic Polypeptide was described as a very selective Y5 agonist (Cabrele et al., 2000). A better situation is with the availability of non-peptide antagonists of Y receptors. There are several non-peptide Y1 receptor antagonists and one of them BIBO3304 has a good selectivity profile and high

affinity (Brothers & Wahlestedt, 2010; Dumont et al., 2000). Y2 receptors are successfully and specifically blocked by BIIE0246 (Doods et al., 1999) but submicromollar affinities for opioid and alpha1 adrenergic receptors were also noted. Y5 receptors can be antagonized by the compounds called MK-0557, S-2367, L152,804 or 2-36[K4,RYYSA(19-23)]PP, and a very selective compound, CGP 71683 synthesized in the last years showed more than 1000-fold higher selectivity for Y5 over Y1, Y2 and Y4 receptors (Brothers & Wahlestedt, 2010).

Receptor	Agonists	Antagonists
Y1R	NPY = PYY = Leu^{31},Pro^{34}NPY >> NPY13-36 > PP	BIBP3226 BIBO3304 BMS193885
Y2R	NPY = PYY ≥ NPY13-36 >> Leu^{31},Pro^{34}NPY > PP PYY3-36	BIIE0246 SF 11 JNJ 5207787 JNJ 31020028
Y5R	NPY ≥ PYY ≥ PP [cPP^{1-7},NPY^{19-23},$Ala^{31}Aib^{32}$, Gln^{34}]-hPP BWX 46	CGP 71683 MK-0557 S-2367 L-152,804
NPY – neuropeptide Y, PYY – peptide YY, PP- pancreatic polypeptide		

Table 1. Representative NPY receptor ligands for Y1, Y2 and Y5 receptors

2. Modulation of an excitatory glutamatergic transmission by NPY

Glutamate is the most abundant excitatory neurotransmitter in the mammalian brain and participates in the changes of synaptic transmission underlying learning and memory (Bliss & Collingridge, 1993), but on the other hand, an overactivation of glutamatergic transmission may lead to excitotoxic cell death (Ankarcrona et al., 1995; Choi, 1985; Conn & Pin, 1997). It is generally assumed that excitotoxicity is a common pathological condition to a variety of neuronal degenerations. It comprises supraphysiological stimulation of ionotropic glutamate receptors and an increase in intracellular Ca++ which triggers downstream processes resulting in neuronal death (acc. to Silva et. al., 2005a). Thus, control of extracellular glutamate concentration seems to be critical for both neuronal signaling and survival. One of the important functions of NPY is related to the inhibition of glutamate release and reduction of glutamatergic excitatory activity which may result in neuroprotection. It was found that NPY reduced epileptiform activity in the hippocampus and cortex (Bijak, 1999; Bijak & Śmiałowska, 1995; Klapstein & Colmers, 1997), inhibited glutamate release (Colmers, 1990; Greber et al., 1994; Silva et al., 2001) and reduced kainic acid (KA)-induced seizures (Madsen et al., 1999; Woldbye et al., 1997). Moreover, increases in the synthesis and content of NPY in the limbic structures were found after seizures of different origin (Bellmann et al., 1991; Bendotti et al., 1991; Marksteiner et al., 1990; Schwarzer et al., 1996; Silva et al., 2005a; Śmiałowska et al., 2003) which may indicate a possibility of neuroprotective role of endogenous NPY.

Our earlier studies showed the neuroprotective effects of NPY against KA-induced neurotoxicity both *in vivo* in the rat hippocampus (Śmialowska et al., 2003) and *in vitro* in the hippocampal and cortical neuronal cultures (Domin et al., 2006). Results of other authors were controversial showing both protective and toxic effects of NPY (Chen & Cheung, 2002; Cheung & Cechetto, 2000). The cause of such a discrepancy may be connected with differentiation of Y receptors.

2.1 The role of particular Y receptors in neuroprotection

Among NPY receptors, Y1R, Y2R and Y5R were postulated to play the most important role in the regulation of neurotoxicity and neuroprotection. Since specific agonists and antagonists of those receptors have been synthesized (Aguirre et al., 1990; Cabrele et al., 2000; Doods et al., 1999; Dumont et al., 2000; Fuhlendorff et al., 1990; Rudolf et al., 1994; Wieland et al., 1998) the studies on the role of particular receptors in neuroprotection became possible. Most of evidences demonstrated neuroprotective effects after Y2 and Y5 receptor agonists (Silva et al., 2003b, 2005a; Śmialowska et al., 2009; Wu & Li, 2005). Neuroprotective role of Y2 and Y5 receptor stimulation was also suggested by electrophysiological and behavioral studies in which the activation of these receptors suppressed epileptiform bursting or seizures (Bijak, 1999; Klapstein & Colmers, 1997; Marsh et al., 1999; Woldbye et al., 1997; Vezzani et al., 1999). The role of Y1R in neuroprotection is ambiguous. The activation of Y1 receptors produced neuroprotective effects in kainate excitotoxicity model of rat organotypic hippocampal slice cultures (Xapelli et al., 2007) and in methamphetamine neurotoxicity model in the mouse striatum (Thieriet et al., 2005), but in both models Y1R had a little weaker effect than Y2R agonists. Excitotoxicity induced by AMPA in organotypic mouse hippocampal slice cultures showed that AMPA-induced degenerations of CA1 and CA3 pyramidal neurons were strongly inhibited by Y2 but not Y1 or Y5 receptor agonists (Xapelli et al., 2008). More detailed studies in hippocampal slices have shown that neuroprotective properties of a particular Y receptor may depend on the region where the receptor is located. Thus Y1, Y2 and Y5 receptor agonists were neuroprotective against AMPA or kainate excitotoxicity in dentate granule cells and CA3 region, while CA1 pyramidal neurons were protected only by Y2 agonist (Silva et al., 2003a, 2005a). On the contrary, the excitotoxic effects of Y1 receptor activation in the rat hippocampus were suggested by the results of Gariboldi et al. (1998) in which Y1R stimulation potentiated convulsions, while administration of Y1R antagonist inhibited kainate-induced seizures. Moreover, studies in an ischemic model both in vitro and in vivo, have demonstrated that Y1 receptor activation enhances neurodegeneration, while Y1R antagonists produced neuroprotective effects (Chen & Cheung, 2003, 2004). No protection after a Y1 receptor agonist was also found in our studies both in vitro and in vivo (Śmialowska et al., 2009).

2.1.1 Neuroprotective effect of Y receptor agonists in kainic acid-induced excitotoxicity

The model of kainic acid (KA)-induced excitotoxicity was described as a good and validated simulation of different excitotoxic neurodegenerations, connected with the secondary release of endogenous glutamate (Coyle, 1983; Ferkany & Coyle, 1983; Malva et al., 1998;

Wang et al., 2005). The KA activates kainate receptors, which belong to ionotropic glutamate receptors, but effects after KA develop progressively and endogenous glutamate release enhances degeneration. Therefore, the KA-model seems to be especially suitable for studying the delayed neuroprotection.

The main goal of our studies was to explore a possibility of neuroprotection induced by NPY or specific Y receptor ligands. We focused our attention on Y1, Y2 and Y5 receptors, as it is generally assumed that these receptors play the most important role in the regulation of neurodegeneration and neuroprotection. Moreover, we investigated the efficacy of the treatment delayed even by a few hours while in the majority of other studies NPY and its ligands were applied before or simultaneously with a neurotoxic damage, however, the delayed treatment resembles more closely the situation of patients, who are usually treated some time after an injury.

We performed studies both in vitro and in vivo (Śmialowska et al., 2009). In *in vitro* experiments primary cultures of mouse cortical and hippocampal neurons were used. The cultures were exposed to KA for 24 or 48 h and then Y receptor ligands were added 30 min, 1, 3 or 6 h after starting the KA exposure. Cell death was quantified by measurements of lactate dehydrogenase (LDH) release from damaged cells into the cell culture media. Apoptosis was evaluated by measurements of caspase-3 activation and additionally by fluorescent Hoechst 33342 staining which visualized condensed DNA fragments, characteristic for apoptotic cells. A huge increase in the LDH release and caspase-3 activation was found after the KA and these increases were strongly attenuated by both Y2 receptor agonist (NPY13-36) and Y5 receptor agonist ([cPP$^{1-7}$,NPY1$^{9-23}$,Ala^{31},Aib^{32}Gln^{34}]hPP), added as late as 3 hours after starting KA intoxication. The results indicated neuroprotective and antiapoptotic effects of these peptides. Receptor specificity was confirmed by experiments with antagonists which prevented the neuroprotection. No neuroprotective effects were observed after Y1 receptor agonist [Leu^{31}, Pro^{34}]-NPY given after the KA (for details see Śmialowska et al., 2009). Our *in vitro* results are in line with the studies of other authors showing the protective effect of Y2 and Y5 receptors, but not Y1 in the KA-induced excitotoxicity (Silva et al., 2001, 2003b; Woldbye & Kokaia, 2004; Xapelli et al., 2007).

The same picture of Y receptor efficacy in neuroprotection emerges also from the *in vivo* experiments. Wu and Li (2005) found that NPY and specific Y2 and Y5 receptor agonists rescued mouse hippocampal pyramidal neurons from KA-induced apoptosis (visualized by the TUNEL staining) when the peptides were intracerebroventricularly (icv) injected as late as 2 or 8 h after intraperitoneal KA injection. Also in our *in vivo* studies the neuroprotective effects were found after treatment of the peptides delayed by 30 min or 1 h, but not 3 h, after the KA injection. Our model was different than that of the above-mentioned authors as we used rats and injected KA into the CA1 hippocampal region in a dose and manner which did not induce seizures. The peptides were also microinjected intrahippocampally. Brains were removed and fixed 7 days after the treatment and degeneration and protection were evaluated by stereological counting of neurons in the CA pyramidal layer in the dorsal hippocampus. The results showed the protective effects of Y2 and Y5, but not Y1 receptor agonists (for details see Śmialowska et al., 2009).

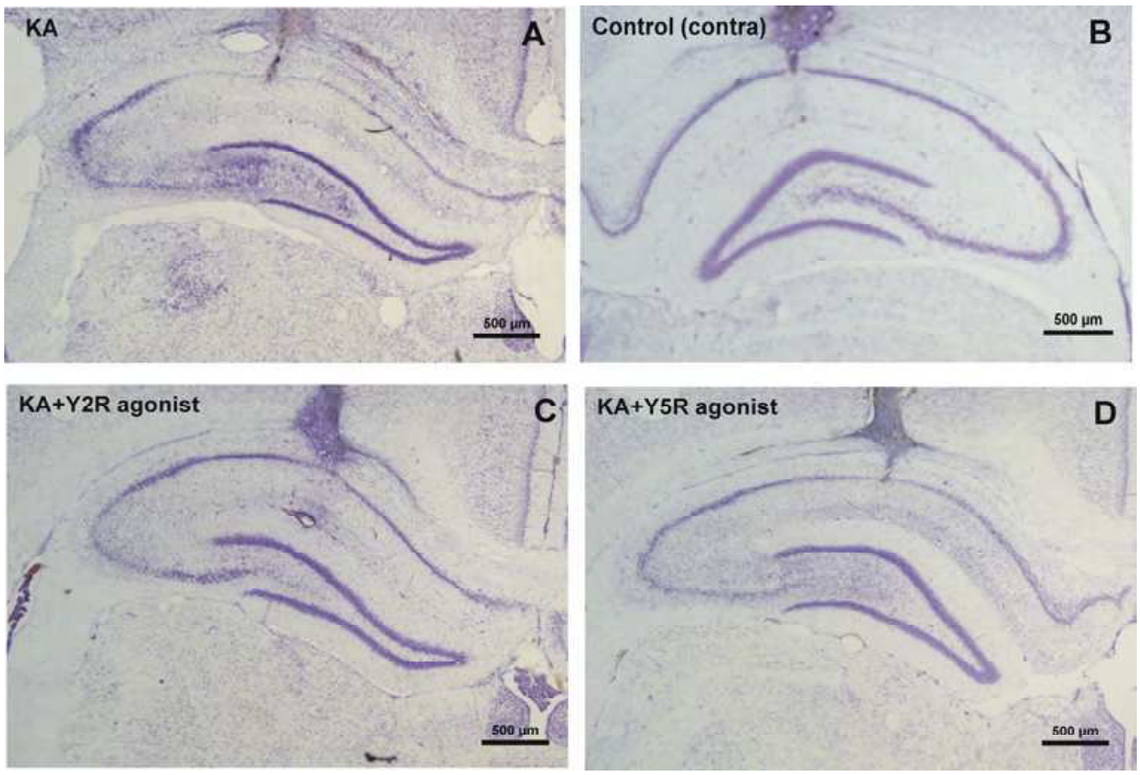

Fig. 3. Microphotographs of frontal sections of the rat brain hippocampi, stained with cresyl violet for visualization of nerve cell bodies. A: The hippocampus after KA injection into the CA1 region. Strong degeneration of pyramidal neurons and extensive gliosis are seen. B: non-degenerated, contralateral hippocampus microinjected with a phosphate buffer used as a control side. C: Neuroprotective effect of Y2 receptor agonist injected 30 min after the KA. The lesion is much smaller. D: A similar neuroprotective effect is seen after Y5 receptor agonist microinjected 30 min after the KA. After Y2 (C) and Y5 (D) receptor agonists pyramidal neurons are much more numerous than after KA alone (A).

2.1.2 The role of NPY and Y receptors in ischemic neurodegeneration

Much less is known about the neuroprotective role of NPY in ischemic degenerations. A possibility of such function of endogenous NPY was postulated on the basis of the observations that NPY-immunoreactivity increased locally in the cerebral cortex around the site of infarct following middle cerebral artery occlusion (MCAO) in rats (Allen et al., 1995, Cheung & Cechetto, 1997; Cheung et al., 1995). On the other hand, an increase in the infarct volume in a similar MCAO model was observed after intravenous or central NPY administration into the rat (Chen & Cheung, 2002). The effect was suggested to be connected with a vasoconstrictive action of NPY, via Y1 receptors, as these authors reported a reduction of the cerebral blood flow after NPY injection. The possibility of neuroprotective function of endogenous NPY was also suggested by the experiments in which ischemic preconditioning induced an increase in NPY-immunoreactivity in the gerbils' hippocampus (Duszczyk et al., 2009). Quite different results were obtained when antisense oligonucleotide to Y1 receptors was given to rats prior to MCAO. Such treatment strongly reduced cortical

Y1 receptor density and evoked a huge increase in the infarct volume (Cheung & Cechetto, 2000). The result suggested a positive role of Y1 receptors in the protection against ischemia as the lack of Y1 receptors resulted in an increased degeneration. However, other studies have demonstrated that Y1 receptor activation enhanced ischemic degenerations. Again in MCAO model in rats intracerebroventricular injection of Y1 agonist increased, while antagonist reduced the infarct volume (Chen & Cheung, 2003). Similarly in *in vitro* model in which neuronal cultures were deprived of oxygen and glucose (OGD model) Y1 receptor agonist worsened while antagonist improved survival of neurons (Chen & Cheung, 2004).

Therefore, the results of studies on the role of NPY and Y receptors in ischemic neurodegenerations obtained so far were divergent and insufficient. Our group also examined this problem. We found that Y2 receptor agonist (NPY13-36) given intracerebroventricularly 30 min after the onset of ischemia significantly diminished the infarct volume in the rat transient MCAO model (Śmialowska et al., 2009). Our preliminary *in vitro* studies on primary cultures of mouse cortical neurons indicated also that in cultures subjected to oxygen and glucose deprivation (OGD model) Y2 receptor agonist induced neuroprotection and moreover, similar protective effect was observed after Y5 receptor agonist but not after Y1 receptor agonist (data in preparation).

2.1.3 The role of NPY and Y receptors in chronic neurodegenerative diseases

An involvement of NPY in chronic neurodegenerations has been postulated on the basis of clinical studies and animal or in vitro experiments.

Huntington's disease

Patients with Huntington's disease (HD) have pathological changes mainly in the striatum and cerebral cortex and show characteristic abnormal motor activity (chorea movements), cognitive impairment and emotional disturbances (Colton & Vitek, 2006). HD is one of neurodegenerative diseases in which a pathological protein is expressed in the cells. The hallmark of HD is the protein huntingtin with pathological repeat length and the most affected neurons are medium spiny neurons in the striatum. Loss of these neurons, projecting to the globus pallidus and substantia nigra is the main reason of the motor abnormalities in HD. In contrast, NPY interneurons in the striatum are relatively resistant to degenerations in HD. Many cortical and striatal interneurons contain NPY, usually coexisting with somatostatin (SOM), and it was found that the level of both NPY and SOM increased 3-5 fold in the caudate, putamen, nucleus accumbens and cortex in HD in comparison to control brains (Beal et al., 1988; Dawbarn et al., 1985; Mazurek et al., 1997). The analysis of transgenic mouse models of HD displayed divergent results, both decreases or increases in NPY and SOM and their mRNAs were reported (Figueredo-Cardenas et al., 1994; Kumar, 2004; Luthi-Carter et al., 2000). The degeneration of the striatal medium spiny neurons in HD may be connected with their abundant glutamatergic innervation from the cortex and high density of glutamate receptors (Kumar et al., 1997; Sieradzan & Mann, 2001; Zeron et al., 2002), so excitotoxic cell death is supposed to occur in HD. Therefore, NPY could be neuroprotective but till now a possible role of NPY in HD is unclear. Experiments with transgenic mouse model of HD (R6/2 strain) showed that a single intraventricular injection of NPY improved motor function, increased survival time and diminished cerebral and striatal atrophy. The positive effects were proposed to be connected with an increased

neurogenesis in the subventricular zone (Decressac et al., 2010) induced by NPY and migration of new cells into the striatum, where they differentiate to GABAergic interneurons. On the other hand, the development of the disease in HD patients proceeded in spite of NPY increase, therefore, it may be supposed that protective action of NPY, if happened, is insufficient for preventing the progression of the disease.

Alzheimer's disease

Alzheimer's disease (AD) is a chronic brain neurodegeneration with progressive memory loss, cognitive dysfunction, various neuro-psychiatric and behavioural disturbances that seriously interfere with daily life activity and eventually lead to death. The neuropathological hallmarks of AD are senile plaques (containing a pathological form of amyloid beta) and neurofibrillary tangles (containing hyperphosphorylated tau protein). Advanced stages of AD are characterized by an extensive neuronal loss especially in the hippocampus, cortex and nucleus basalis of Meynert. The damage in the hippocampal and cortical regions was postulated to be associated with dementia. NPY is present in some hippocampal and cortical interneurons and seems to have a role in learning and memory thus the possible involvement of this peptide in AD pathogenesis and/or symptoms has been studied but results are divergent. A number of clinical studies showed a reduced or unchanged level of NPY in the cerebrospinal fluid (Atack et al., 1988; Edvinsson et al., 1993; Heilig et al., 1995; Martignoni et al., 1992; Minthon et al., 1990; Nilsson et al., 2001) or plasma (Koide et al., 1995) of AD patients. Losses of cortical receptor sites for NPY (Nordberg, 1992) and decrease in NPY receptor density in the hippocampus and temporal cortex but not in the putamen have also been reported (Martel et al., 1990). Immunohistochemistry of brain sections evidenced a strong reduction in the number of NPY-immunoreactive neurons in the hippocampal formation and enthorinal cortex and alternation of the morphology of the remaining neurons (Kowall & Beal, 1988). The results obtained from studies of a transgenic mouse model of AD, overexpressing amyloid precursor protein and demonstrating amyloid plaques showed quite different changes in NPY, namely an increased NPY immunoreactivity in the hippocampus and enthorinal cortex (Diez et al., 2000, 2003). However, other models that co-express amyloid plaques and neurofibrillary tangles have altered NPY levels and more resemble changes in human AD (Oddo et al., 2003, 2004).

A possibility of neuroprotective effects of NPY in AD neurodegeneration arises from the neuroprotective potential of NPY in excitotoxicity and its ability to decrease the calcium ion influx into neurons, that is one of the mechanism of this protection. Since amyloid beta (Abeta) peptides were found to disrupt calcium ion homeostasis and increased intraneuronal Ca++ (Fedrizzi & Carafoli, 2011; Pereira et al., 2005; Shirvany et al., 2007), NPY may inhibit this damaging process. Moreover, it was found that NPY increased the production of neurotrophins and protected SY5Y neuroblastoma cells against beta amyloid toxicity with concomitant increase in neurotrophin production (Croce et al., 2011). Additional mechanisms of therapeutic action of NPY in AD might be related to the activation of neurogenesis in the hippocampus (Howell et al., 2005, 2007) as well as to neurogenesis and cell proliferation in other structures (see above in the chapter about HD). A new possibility of neuroprotection based on NPY has also been postulated by Rose et al. (2009), who found protective effects of neuropeptide fragments (mainly NPY 21-36 and 31-36) derived from neprilysin processing in a transgenic mouse model of AD. No studies of the role of specific Y receptors were performed yet.

Parkinson's disease

Parkinson's disease (PD) is a chronic neurodegeneration characterized mainly by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta with disappearance of their projections into the striatum. It leads to development of motor symptoms such as bradykinesia, rigidity and tremor. Therapies used in PD can temporarily alleviate motor symptoms but do not prevent progression of the disease as the mechanism of PD development is still unclear. There are only a few studies concerning NPY in human PD. A decrease in NPY level in the cerebrospinal fluid (Martignoni et al., 1992), no changes in the cortex and hippocampus (Allen et al., 1985) or decrease in adrenal medulla (Stoddard et al., 1991) were described in PD patients. Besides, it has been postulated that NPY may play some role in the pathogenesis and/or symptoms of PD as both nerve cell bodies and terminals containing NPY are quite abundant in extrapyramidal structures engaged in the disease and, moreover, a reciprocal interactions were found between dopaminergic and NPY neurons. In animal experiments, a decrease or disappearance of dopaminergic innervation in the striatum (such situation occurs in PD) induced a significant increase in NPY synthesis and content in the striatal interneurons (Engber et al., 1992; Kerkerian et al., 1986, 1988; Midgley et al., 1994; Śmialowska, 1995). In situ hybridization immunohistochemical studies of postmortem human brain structures showed an increase in NPYmRNA in the striatum especially in the ventral caudate nucleus and the nucleus accumbens (Cannizzaro et al., 2003) in PD.

A regulatory function of NPY in DA transmission was demonstrated in rats, as an NPY injection into the striatum increased the DA turnover in that structure (Beal et al., 1986). More detailed studies on the role of particular Y receptors in the rat striatum have shown that Y2 receptor activation enhanced dopamine synthesis while Y1 and Y5 receptor stimulation attenuated the DA synthesis (Adewale et al., 2005). Since no genetic mouse model of PD currently exists, studies have focused primarily on the models with lesioned nigro-striatal dopaminergic systems. The lesions were performed by means of dopaminergic toxins 6-hydroxydopamine or MPTP. Recently, Decressac et al., (2011a) described a neuroprotective effect of NPY in PD models. They found that NPY protected dopaminergic neuroblastoma cells (SH-SY5Y) from 6-hydroxydopamine induced toxicity. The neuroprotective effect of NPY was also confirmed in vivo, in the rat and mouse model of PD in which striatal NPY injection inhibited dopaminergic degenerations induced by the toxins and improved motor performance. Moreover, detailed studies using Y receptor ligands evidenced a protective action of Y2 but not Y1 receptors both on dopamine neurons and the behavioral impairments (Decressac et al., 2011a). Therefore, the authors suggested a possibility of therapeutic use of NPY ligands in the future.

2.2 How does NPY modulate neurodegenation and neuroprotection?

NPY appears to be particularly promising for neuroprotection, since it has a significant modulating effect on excitatory neurotransmission. As mentioned above, many studies have shown that NPY inhibits glutamatergic transmission (Bijak, 2000; Colmers et al., 1987; Greber et al., 1994; Klapstein & Colmers, 1992; McQuiston & Colmers 1996; Śmialowska et al., 1996;) through presynaptic inhibition of glutamate release from nerve terminals (Greber et al., 1994; Silva et al., 2003a). It is assumed that such inhibition is associated with the

inhibition of N-type calcium channels (Qian et al., 1997; Silva et al., 2001; Silva et al., 2003a). Since NPY receptors activate signaling pathways involved in the regulation of intracellular Ca^{2+} homeostasis (Silva et al., 2002), one of the possible mechanism of neuroprotective action of NPY consists in the inhibition of calcium channels thereby inhibiting the influx of calcium ions into the cell (Thiert et al., 2005; Xapelli et al., 2007). In our studies, NPY as well as Y2R and Y5R agonists inhibited the toxic effects of KA, decreased the KA-induced caspase-3 activation and the number of apoptotic bodies (Śmialowska et al., 2009). It is known that KA disturbs the homeostasis of calcium ions in cells, cytochrome c release and caspase-3 activation, which leads to cell death (Wang et al., 2005). Therefore, it has been proposed that the antiexcitatory and antiapoptotic properties of the peptides under study are connected with a decrease in the input of calcium ions into neurons and a decrease in glutamate release.

As mentioned above, the role of Y1 receptors in neuroprotection is unclear. It is supposed that the neurotoxic effect of Y1R activation observed in our and other studies may be due to the fact that these receptors are situated mainly postsynaptically, and their activation induces calcium influx and inhibition of potassium channels, which increases neuronal excitability (Dumont et al., 1992; Gobbi et al., 1996). Stimulation of calcium release from the endoplasmic reticulum (Aakerlund et al., 1990) and enhancement of nitric oxide production (Bitran et al., 1999; Chen et al., 2002) have also been proposed as possible mechanisms of neurotoxicity after Y1R activation. On the other hand, it has been found in the brain that some Y1 receptors are located presynaptically (Kopp et al., 2002) and the putative neuroprotective mechanism of action Y1R agonist in some models might be due to the presynaptic inhibition of glutamate release by inhibition of Ca++ entry (McQuiston et al., 1996; Silva et al., 2001; Silva et al., 2003a, 2003b). Such presynaptic inhibitory Y1 receptors were found, for instance, in the hippocampal dentate region (McQuiston et al., 1996; Silva et al., 2001, 2003b), rat cerebral cortex (Wang, 2005) or arcuate nucleus (Sun & Miller, 1999). The Y1 receptor stimulation may have also positive effects in neurodegenerations by quite different mechanism. It has been shown that NPY promotes neurogenesis and proliferation of progenitor cells in the rat dentate gyrus of the hippocampal formation and the effects are mediated by Y1 receptors as they were also observed after Y1R but not after Y2R agonists (Descressac et al., 2011b; Howell et al., 2005; Kopp et al., 2002; Xapelli et al., 2006). Moreover, the proliferation was reduced in Y1 receptor knockout mice, or after co-administration of Y1 antagonist. Similarly, Y1 receptor-mediated stimulation of neurogenesis and proliferation by NPY was also found in the mouse olfactory epitelium (Hansel et al., 2001). As mentioned above, NPY increased neurogenesis in the telencephalic subventricular zone (Decressac et al., 2010) but no receptor specificity was studied. In rat retinal cell cultures, NPY stimulated neural cell proliferation mainly via Y2 and Y5 receptors but some role of Y1R was also postulated as Y1 antagonist decreased the cell proliferation induced by NPY (Alvaro et al., 2008).

Neurogenesis, proliferation and differentiation of neurons may be also regulated by neurotrophic factors, and a cross-talk between NPY and brain derived neurotrophic factor (BDNF) in the mammalian brains has been suggested. Seizures and excitotoxicity induced a significant increase in BDNF in the injured region (Ballarin et al., 1991; Dugich-Djordjevic et al., 1992; Humpel et al., 1993). As the increase in BDNF always precedes the NPY expression

(Reibel et al., 2000) the BDNF overexpression after excitotoxicity may be neuroprotective by inducing NPY production. Many studies have shown that BDNF enhanced an expression of NPY in the striatum, cortex and the hippocampus (Barnea & Roberts, 2001; Barnea et al., 2004; Nawa et al., 1994; Reibel et al., 2000; Takei et al., 1996; Wirth et al., 2005; Yoshimura et al., 2009). In the mouse organotypic hippocampal slice cultures exposed to glutamate receptor agonist, degeneration of CA1 and CA3 pyramidal neurons was diminished by Y2 receptor agonist and such protection strongly inhibited the stimulating effect of AMPA on the BDNF level (Xapelli et al., 2008). It was also found that NPY treatment decreased BDNF and increased nerve growth factor production in the rat hypothalamus (Gelfo et al., 2011). All these results indicate mutual relations between NPY and BDNF (or maybe other trophic factors).

Nitric oxide (NO) a reactive free radical, gas produced enzymatically from L-arginine by NO synthases is a very important molecule connected with the NPY role in excitotoxicity and neuroprotection. In normal physiological state, NO concentration in the brain amounts to less than 10 nM and NO plays an important role in vascular tone, blood pressure and neuronal signaling (Garthwaite, 1991) but during cerebral ischemia NO production increased which is an important factor inducing ischemic damage, as NO quickly forms highly toxic radicals (Matsui et al., 1999). It was found that in the rat MCAO model of ischemia, the brain NO concentration increased and intracerebroventricular injection of NPY or Y1R agonist enhanced this increase, while, in contrast, a Y1R antagonist strongly inhibited the NO formation. The results indicated harmful effect of Y1R stimulation via an increase in toxic NO formation. On the other hand a possibility of neuroprotection in ischemia by agents blocking Y1 receptors may be suggested.

3. Conclusions

Neuropeptide Y (NPY) reveals many important regulatory function in the brain both under physiological and pathological conditions. The present paper reviews the data on the role of NPY and its receptors in neuroprotection. The peptide counteracts neuronal glutamatergic excitation, inhibits seizures and has significant neuroprotective effects which are produced mainly via Y2 and Y5 receptors. The activation of Y1 receptors gives ambiguous results, usually induces harmful effects but in some situations and some brain regions may also be protective via both inhibition of glutamate release and the induction of repair mechanisms (cell proliferation, trophic factors or others). The results obtained by our group and by a few other authors suggest that NPY and Y2 or Y5 receptor agonists may reveal their protective activity not only when they are given before or simultaneously with a traumatic event but also as the delayed treatment, 30 min or even few hours after the onset of the damaging action. A possible role of NPY in some chronic neurodegenerative disorders is also discussed.

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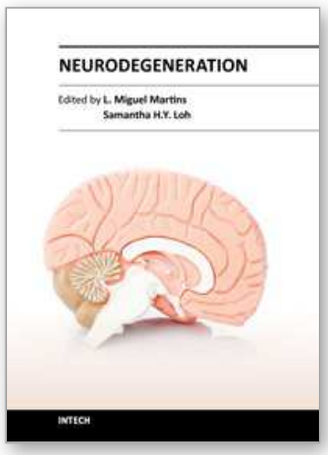

Neurodegeneration

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Currently, the human population is on a collision course for a social and economic burden. As a consequence of changing demographics and an increase in human individuals over the age of 60, age-related neurodegenerative disorders are likely to become more prevalent. It is therefore essential to increase our understanding of such neurodegenerative disorders in order to be more pro-active in managing these diseases processes. The focus of this book is to provide a snapshot of recent advancements in the understanding of basic biological processes that modulate the onset and progression of neurodegenerative processes. This is tackled at the molecular, cellular and whole organism level. We hope that some of the recent discoveries outlined in this book will help to better define the basic biological mechanisms behind neurodegenerative processes and, in the long term, help in the development of novel therapeutic approaches.

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