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Role of serotonin (5-HT) in the antidepressant-like properties of neuropeptide Y (NPY) in the mouse forced swim test

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Abstract

Neuropeptide Y (NPY) is thought to be implicated in depressive disorders. The mouse forced swim test (FST) is an animal model widely used as a predictor of the efficacy of antidepressant drugs. The present study was undertaken to explore the possible contribution of endogenous serotonin (5-HT) systems in the behavioral effects elicited by NPY in this model. The selective serotonin re-uptake inhibitor (SSRI), fluoxetine, was also tested for comparison. 5-HT was depleted prior to testing by the administration of the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA; 300 mg/kg, i.p., each day for 3 days; control mice received saline-vehicle over the same period). On the fourth day, mice received NPY (3 nmol, I.C.V.), fluoxetine (16 mg/kg, i.p.) or saline injections before testing in the FST. Both NPY and fluoxetine significantly reduced immobility time in saline-treated control animals. Pre-treatment with PCPA significantly blocked the effects of fluoxetine in the FST, confirming the role of endogenous 5-HT. Similarly, pre-treatment with PCPA also significantly attenuated the anti-immobility effects of NPY, thus suggesting a role for 5-HT in the effects of NPY in the FST. Quantitative receptor autoradiography revealed increases in specific [^{125}I][Leu^{31}, Pro^{34}]PYY sites that were sensitive to BIBP3226 (Y_1-like sites) in various brain regions. Specific [^{125}I]GR231118 and [^{125}I]PYY(3–36) binding levels were not changed following PCPA treatment, suggesting that depletion of endogenous 5-HT resulted in an apparent increase in the level of Y_1 sites in their high-affinity state. Taken together, these results suggest a role for 5-HT-related systems in the antidepressant-like properties of NPY.

Keywords: NPY receptor subtypes; Forced swim test; Serotonin depletion; Receptor autoradiography; Mouse brain

1. Introduction

Neuropeptide Y (NPY) is a 36-amino acid peptide isolated from porcine brain more than two decades ago [52]. This peptide is one of the most abundantly found in the mammalian brain, and is widely distributed in the central nervous system (CNS) [1,11]. Following its intracerebroventricular (I.C.V.) administration, it has been shown that this peptide is involved in various biological processes including food intake, neuronal excitability, anxiety-related behaviors, circadian rhythms, neuroendocrine secretion and alcohol consumption (for reviews see for example: [18,26,53,54]). The biological effects of NPY are mediated by the activation of at least five molecularly defined classes of receptors known as the Y_1, Y_2, Y_4, Y_5, and y_6 receptor subtypes [33].

Behavioral studies performed in rodents, as well as some clinical investigations, suggest that NPY may play a role in the pathophysiology of certain mood disorders, including depression and anxiety [18,24,28,44]. For example, repeated

Abbreviations: FrS, frontal cortex superficial layers; FrM, frontal cortex middle layers; FrD, frontal cortex deep layers; ParS, parietal cortex superficial layers; ParM, parietal cortex middle layers; ParD, parietal cortex deep layers; LS, lateral septum; LSD, lateral septum dorsal part; CA1, CA1 subfield of the hippocampus; CA2, CA2 subfield of the hippocampus; CA3, CA3 subfield of the hippocampus; DG, dentate gyrus; Amy, amygdala

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electroconvulsive shock (ECS) stimulation, one of the most effective treatments of major depression, increases the levels of NPY and of its mRNA in the rat CNS [34,50,58]. Moreover, in vivo microdialysis has revealed that ECS treatments result in significant increases in NPY release in the dorsal hippocampus [25]. Similar results have been observed following a chronic treatment with lithium, a therapy often employed for the treatment of bipolar disorders [25,59]. These data suggest that ECS and lithium can stimulate the biosynthesis and release of NPY in animals.

Chronic antidepressant treatments have also been shown to upregulate the levels of NPY in the CNS [3,23]. In the Flinders Sensitive Line (FSL) of rats, a purported genetic animal model of depression that shows behaviors analogous to those seen in depressed patients such as alterations in REM sleep, reduced body weight and increased immobility time in the forced swim test [35], levels of NPY are lower compared to Flinders Resistant Line (FRL) controls [4]. Fluoxetine also increases the levels of NPY in FSL rats [4]. Additional evidence for a role for NPY in depressive disorders was also provided by studies in olfactory bulbectomized (OB) rats, another purported model of depression [30]. I.C.V. administration of NPY attenuated OB-induced increases in ambulation, rearing, grooming and defecation scores in the open field, increased noradrenaline and serotonin levels in the amygdala and hypothalamus, and reversed OB-induced suppression of lymphocyte proliferation [49].

Recent studies have also reported on the antidepressant-like effects of NPY in rats [51] and mice [42] using the forced swim test (FST). This effect is possibly mediated at least partly by the activation of the Y_1 receptor subtype [42]. Considering that dysregulations of the serotonergic (5-HT) and/or noradrenergic (NA) systems have been implicated in the pathology of affective disorders [36], it has been postulated that the antidepressant-like effects observed following I.C.V. administration of NPY may be through a mechanism involving monoaminergic neurotransmission [42,51]. We have thus investigated the effect of *p*-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor known to deplete 5-HT in the brain [29], on NPY and fluoxetine responses in the FST. Our results suggest that the effects of NPY and fluoxetine in this model are significantly inhibited following PCPA treatment. Moreover, quantitative receptor autoradiography demonstrated the up-regulation of the Y_1 receptor subtype in its high-affinity state.

2. Materials and methods

2.1. Animals

Naive male CD-1 mice (Charles River, St. Constant, Quebec, Canada), weighing 20–24 g were used throughout this study. They were singly housed under standard laboratory conditions (12 h light/12 h dark cycle, lights on at 7:00 a.m., food and water ad libitum). Each experimental group consisted of 10 randomly chosen mice and animals was only used once. Animal care was provided according to protocols and guidelines approved by McGill University and the Canadian Council of Animal Care.

2.2. Drugs

NPY, [Leu^{31}, Pro^{34}]PYY and PYY(3–36) were synthesized as previously described [21]. Fluoxetine and *p*-chlorophenylalanine were purchased from Sigma Chemical (St. Louis, MO, USA). ((*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N2-(diphenylacetyl)-argininamide trifluoroacetate), code name BIBO3304 (Y_1 antagonist) and ((*S*)-N2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6h)-oxodibenz[b,e] azepin-11-yl]-1-piperazinyl]-2-oxoethyl] cyclopentyl] acetyl]-*N*-[2-[1,2-dihydro-3,5 (4H)-dioxo-1,2-diphenyl-3H-1,2,4-triazol-4-yl]ethyl]-argininamide), BIIE0246 (Y_2 antagonist) were generously provided by Boerhinger Ingelheim (Germany), while homodimeric Ile-Glu-Pro-Dpr-Tyr-Arg-Leu-Arg-Tyr-$CONH_2$ (GR231118) was a gift from Glaxo Wellcome (Research Triangle Park, NC, USA). Bovine serum albumin (BSA) and Iodine-125 were obtained from ICN Pharm. Canada Ltd. (Montréal, Que., Canada) and bacitracin was purchased from Sigma Chemical. Schleicher and Schuell #32 glass filters were obtained from VWR Canada (Montréal, Que., Canada). Kodak MR films and ^{125}I-microscale standards were purchased from Amersham (Mississauga, Ont., Canada). All other chemicals were of analytical grade and obtained from Fisher Scientific (Montreal, Que., Canada) or Sigma Chemical (St. Louis, MO, USA).

2.3. Animal treatments

Mice were injected intraperitoneally (i.p.) with either saline or PCPA 300 mg/kg once a day for 3 days. On the fourth day, mice received NPY (3 nmol, I.C.V.), fluoxetine (16 mg/kg, i.p.) or saline injections 30 min before testing in the FST. Immediately after testing, three mice per group, randomly chosen, were sacrificed and their brain was rapidly removed and frozen for receptor autoradiography as previously described [17]. All molecules were dissolved in 0.9% isotonic saline.

2.4. Measurement of immobility time

The forced swimming test employed was essentially similar to that described elsewhere [38]. Briefly, mice were dropped individually into glass cylinders (height: 25 cm, diameter: 10 cm) containing 10 cm of water, maintained at 23–25 °C, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position, and made only small movements to keep its head above water. The duration of immobility was recorded during the last 4 min of the 6-min testing period.