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Neuropeptide S inhibits release of 5-HT and glycine in mouse amygdala and frontal/prefrontal cortex through activation of the neuropeptide S receptor

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ABSTRACT

Neuropeptide S (NPS) is a neurotransmitter/neuromodulator that has been identified as the natural ligand of G protein-coupled receptors termed NPS receptors (NPSRs). The NPS-NPSR system is involved in the control of numerous centrally-mediated behaviours, including anxiety. As several classical transmitters play a role in fear/anxiety, we here investigated the regulation by NPS of the exocytotic release of 5-hydroxytryptamine (5-HT) and glycine in nerve terminals isolated from mouse frontal/prefrontal cortex and amygdala. Synaptosomes, prelabelled with the tritiated neurotransmitters, were depolarized in superfusion with 12–15 mM KCl and exposed to varying concentrations of NPS. The evoked release of [^{3}H]5-HT in frontal/prefrontal cortex was potently inhibited by NPS (maximal effect about 25% at 0.1 nM). Differently, the neuropeptide exhibited higher efficacy but much lower potency in amygdala (maximal effect about 40% at 1 μM). NPS was an extremely potent inhibitor of the K^+-evoked release of [^{3}H]glycine in frontal/prefrontal nerve endings (maximal effect about 25% at 1 pM). All the inhibitory effects observed were counteracted by the NPSR antagonist SHA 68, indicating that the neuropeptide acted at NPSRs. In conclusion, NPS can inhibit the exocytosis of 5-HT and of glycine through the activation of presynaptic NPSRs situated on serotonergic and glycinergic terminals in areas involved in fear/anxiety behaviours. The possibility exists that the NPSRs in frontal/prefrontal cortex are high-affinity receptors involved in non-synaptic transmission, whereas the NPSRs on amygdala serotonergic terminals are low-affinity receptors involved in axo-axonic synaptic communication.

1. Introduction

Neuropeptide S (NPS) and its receptor (NPSR), first described by Xu et al. (2004), represent a neurotransmitter/neuromodulator system that is localized mainly in the brain. NPS consists of 20 amino acids and exhibits significant sequence-homology in human, mouse and rat (Reinscheid, 2007). NPS-producing neurons are mostly concentrated in a few discrete nuclei of the brainstem, whereas NPSR mRNA shows a widespread expression in the central nervous system (Xu et al., 2007), reflecting the involvement of NPS in multiple physiological functions. As indicated by several studies, the NPS-NPSR system is involved in the control of different centrally mediated behaviours including anxiety and wakefulness (Xu et al., 2004; Jüngling et al., 2008; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008; Fendt et al., 2010; Pape et al., 2010), food intake (Beck et al., 2005; Smith et al., 2006; Cline et al., 2007; Peng et al., 2010), drug abuse (Badia-Elder et al., 2008; Kallupi et al., 2010) and memory (Han et al., 2009).

Little is known about the cellular/molecular mechanisms underlying the behavioural effects of NPS. Most likely, these effects are due to regionally selective interactions of the neuropeptide with various neurotransmitter systems. Neurotransmitter interactions often occur through modulation of release mediated by presynaptic receptors (Raiteri, 2006, for a review). In the case of NPS,

Abbreviations: 5-HT, 5-hydroxytryptamine, serotonin; NPS, neuropeptide S; NPSRs, neuropeptide S receptors; SHA 68, 3-oxo-1,1-diphenyl-tetrahydro-oxazolo[3,4-a]pyrazine-7-carboxylic acid 4-fluoro-benzylamide.

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the neuropeptide could modulate the release of other transmitters through the activation of presynaptic NPSRs. In a previous work (Raiteri et al., 2009), it was found that, in nerve endings isolated from mouse prefrontal/frontal cortex, NPS was able to selectively inhibit the depolarization-evoked exocytotic release of some, but not other, neurotransmitters.

The anxiolytic activities of NPS have attracted most interest. Data obtained in a number of preclinical models predictive of anxiolytic activity (Xu et al., 2004; Leonard et al., 2008; Rizzi et al., 2008; Vitale et al., 2008) indicate that the NPS/NPSR system plays important roles in regulating fear and anxiety (see, for a recent review, Pape et al., 2010). Given that a major neural circuit implicated in the pathophysiology of stress, anxiety and fear include prefrontal/frontal cortex and amygdala (Bishop, 2007; Fox et al., 2010) and that some neurotransmitter systems, including the serotonergic (Graeff et al., 1996; Millan, 2003; Lowry et al., 2005; Holmes, 2008; Pum et al., 2009) and the glutamatergic (Moghaddam, 2002; McCool et al., 2010; Popoli et al., 2012) systems, have been proposed as mediators of stress, anxiety and fear, one possible approach to gain further insights into the anxiolytic mechanisms of NPS is to investigate the modulations exerted by this peptide on the release of appropriate neurotransmitters in preparations from the above brain regions.

In our previous work (Raiteri et al., 2009), it was found that NPS inhibited the release of 5-hydroxytryptamine (serotonin; 5-HT) from purified mouse prefrontal/frontal cortex nerve endings. However, the involvement of NPSRs was only indirectly inferred because, at that time, selective NPSR antagonists, particularly the non-peptidic bicyclic piperazine SHA 68 just discovered by Okamura et al. (2008), were not available in our laboratory.

In the present investigation, the inhibitory effect of NPS on the depolarization-evoked release of [^{3}H]5-HT from mouse prefrontal/frontal cortex serotonergic nerve terminals was re-examined in the presence of the NPSR antagonist SHA 68. Moreover, we investigated the effects of NPS on the release of [^{3}H]5-HT from nerve endings isolated from mouse amygdala. Finally, considering that the glutamatergic system and, particularly, the glutamate receptors of the NMDA type play major roles in the pathogenesis of anxiety, the effects of NPS on the release of [^{3}H]glycine, the glutamate coagonist at NMDA receptors, were analyzed.

2. Materials and methods

2.1. Animals

Adult Swiss mice (weighing 20–25 g; Charles River, Calco, Italy) were used. Animals were housed at constant temperature (22 ± 1 °C) and relative humidity (50%) under a regular light/dark schedule (light 7.00 a.m. to 7.00 p.m.). Food and water were freely available. All the experiments were carried out in accordance with the European legislation (European Communities Council Directive of 24 November 1986, 86/609/EEC). All efforts were made to minimize animal suffering and to use the minimum number of animals necessary to produce reliable results.

2.2. Preparation of synaptosomes

Animals were killed by cervical dislocation and the frontal/prefrontal cortex was quickly removed. In order to prepare purified synaptosomes (Dunkley et al., 1988; Nakamura et al., 1993), the tissue was homogenized in 10 vol. of 0.32 M sucrose buffered at pH 7.4 with Tris–HCl, using a glass-Teflon tissue grinder (clearance 0.25 mm, 12 up-down strokes in about 1 min). The homogenate was centrifuged (5 min, 1000*g* at 4 °C) to remove nuclei and debris and the supernatant was gently stratified on a discontinuous Percoll® gradient (2, 6, 10 and 20% v/v in Tris-buffered sucrose) and centrifuged at 33,500*g* for 5 min. The layer between 10% and 20% Percoll® (synaptosomal fraction) was collected, washed by centrifugation and resuspended in a physiological medium (standard medium) having the following composition (mM): NaCl, 140; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 5; glucose, 10; HEPES, 10; pH adjusted to 7.4 with NaOH. All the above procedures were performed at 0–4 °C.

In the experiments of release from amygdala synaptosomes, the brain region was isolated under a dissecting scope, at T 0–4 °C, using landmarks as defined in the Franklin and Paxinos mouse brain atlas (Franklin and Paxinos, 1997), the dissecting area spanning approximately from −0.94 to −2.30 with respect to bregma. Given the small amount of tissue, in order to maximize the yield and vitality of synaptosomes to obtain reliable results and to minimize the number of animals required, a crude P2 synaptosomal preparation was made up. Briefly, tissues were homogenized in 40 vol of 0.32 M sucrose buffered at pH 7.4 with phosphate (final concentration, 0.04 M). The homogenates were centrifuged at 1000*g* for 5 min to remove nuclei and cellular debris, and synaptosomal fractions were isolated from the supernatants by centrifugation at 12,000*g* for 20 min. The synaptosomal pellets were then resuspended in physiological HEPES-buffered medium having the following composition (mM): NaCl, 140; KCl, 3; $MgSO_4$, 1.2; $CaCl_2$ 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 5; HEPES, 10; glucose, 10; pH 7.4. All the above procedures were performed at 0–4 °C.

2.3. Release experiments

Synaptosomes were incubated at 37 °C for 15 min with [^{3}H]5-HT (0.05 μM) or with [^{3}H]glycine (0.04 μM). In the experiments of [^{3}H]5-HT release, incubation was performed in the presence of the noradrenaline uptake inhibitor desipramine (0.1 μM) and of the dopamine uptake inhibitor GBR 12909 (0.1 μM). In the experiments of [^{3}H]glycine release, incubation was performed in the presence of the selective GLYT1 transporter blocker NFPS (0.1 μM). At the end of incubation, aliquots of the synaptosomal suspension (about 25 μg protein) were distributed on microporous filters placed at the bottom of a set of parallel superfusion chambers maintained at 37 °C and superfused with standard medium, at a rate of 0.5 ml/min (Raiteri and Raiteri, 2000). Physiological medium was supplemented with 0.1% Polypep® from t = 10 min of superfusion. After 36 min of superfusion to equilibrate the system, fractions were collected as follows: two 3-min samples (t = 36–39 and t = 45–48 min; basal release) before and after one 6-min sample (t = 39–45 min; evoked release). A 90-s period of depolarization was applied at t = 39 min. Depolarization of synaptosomes was performed with 12 or 15 mM KCl (substituting for an equimolar concentration of NaCl). NPS was added concomitantly with KCl; SHA 68 was added 9 min before KCl. Fractions collected and superfused filters were counted for radioactivity by liquid scintillation counting.

2.4. Calculations

[^{3}H]5-HT and [^{3}H]glycine efflux in each fraction collected was expressed as a percentage of the radioactivity content of synaptosomes at the start of the respective collection period (fractional rate X 100). Depolarization-evoked neurotransmitter overflow, under the different experimental conditions, was calculated by subtracting the transmitter content of the two 3-min fractions, representing the basal release, from that in the 6-min fraction collected during and after the depolarization pulse. Data in the figures have been expressed as percentages of controls (KCl-evoked overflow) and represent mean ± SEM of the number of experiments reported in the respective figure legends. Data have been analysed by