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Neuropeptide Y activates a G-protein-coupled inwardly rectifying potassium current and dampens excitability in the lateral amygdala

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ABSTRACT

Neuropeptide Y (NPY) reduces anxiety-related behavior in various animal models. Since activity in the lateral amygdala (LA) seems crucial for fear expression of behavior, we studied the mechanisms of NPY in LA projection neurons using whole-cell patch-clamp recordings in slices of the rat amygdala *in vitro*. Application of NPY activated a membrane K^+ current with inwardly rectifying properties in 92% of tested neurons. Pharmacological properties were indicative of mediation via Y1 receptors. Nonhydrolyzable analogues of guanine nucleotides and SCH23390 blocked the NPY-activated current. Single-cell RT-PCR demonstrated expression of G-protein-coupled inwardly rectifying K^+ channel (GIRK) subunits GIRK1, GIRK2 and GIRK3, suggesting mediation of the NPY response through GIRK type channels. The NPY-activated current depressed action potential firing in LA projection neurons, through membrane hyperpolarization and decreased input resistance. Functionally, the dampening of excitability in projection neurons of the amygdala may contribute to the decrease in anxiogenic behavior during action of NPY.

Introduction

Neuropeptide Y (NPY), one of the most abundant peptides within the CNS (Tatemoto et al., 1982), has been found to be involved in the regulation of various physiological functions, in particular, food intake (Polidori et al., 2000), learning and memory (Thorsell et al., 2000), anxiety-related behavior (Krysiak et al., 2000; Primeaux et al., 2005) as well as pathophysiological conditions such as epilepsy (Vezzani and Sperk, 2004) and alcohol dependence (Kimpel et al., 2007). Central NPY expression is primarily detected within the forebrain of all vertebrates (Hendry, 1993), and high levels of NPY expression can be found within cerebral cortex including hippocampal formation, the inner layer of the olfactory bulb, striatum, septum, the basal forebrain, and the amygdala (Cerda-Reverter and Larhammar, 2000). Together with the other members of the NPY family, peptide YY (PYY) and pancreatic polypeptide (PP), NPY exerts its biological actions through a family of G-protein-coupled heptahelical receptors which includes five cloned members in mammals: Y1, Y2, Y4, Y5, and y6 (Michel et al., 1998). Of these y6 is not expressed in rat brain (Burkhoff, 1998), and the NPY-preferring Y3 receptor has not yet been cloned (Michel et al., 1998). *In situ* hybridization techniques revealed that in rat brain Y1, Y2, Y4, and Y5 are highly expressed within the limbic system, especially in the hypothalamus, piriform and cingulate cortices, olfactory tubercle, hippocampus and the amygdala (Parker and Herzog, 1999).

The amygdala is involved in the interconnection of memory and emotional behavior, which has been studied in good detail using the fear conditioning model of emotional memory (LeDoux, 2000; Maren and Quirk, 2004; Paré et al., 2004). Furthermore, the amygdala has been shown to play a role in pathophysiological alterations of these functions, as for instance posttraumatic stress disorders (Tsoory et al., 2008) and phobia. In addition, it is a critical site for the generation of epileptic discharges in the course of temporal lobe epilepsy (Gloor, 1992). Of the various subnuclei of the amygdaloid complex, the lateral amygdala (LA) acts as an input gate for the integration of polymodal sensory information arriving from thalamus and cortex (for review see Sah et al., 2003; Maren and Quirk, 2004). Administration of NPY into the basolateral nucleus of the amygdala (BLA) in rats resulted in decreased anxiogenic behavior in the social interaction test, and BIBO 3304, a selective Y1 receptor antagonist, blocked NPY-induced anxiolysis (Sajdyk et al., 1999). Furthermore, neuropeptide Y injection into the BLA produced stress-resilient responses to restraint challenge in the social interaction test through a mechanism most likely involving neuronal plasticity (Sajdyk et al., 2008). Rats with NPY overexpression in the amygdala demonstrated reduced anxiety-related behaviors in the elevated plus-maze test (Primeaux et al., 2005). On the other hand, NPY knockout mice displayed an anxiogenic-like phenotype, and hypalgesic effects in the hot plate paradigm (Bannon et al., 2000). Additionally, NPY-deficient mice were more susceptible to seizure induction by pentylenetetrazole (Erickson et al., 1996). Within the amygdala, NPY is expressed predominantly in local GABAergic interneurons (McDonald and Pearson, 1989; McDonald and Mascagni, 2002), although

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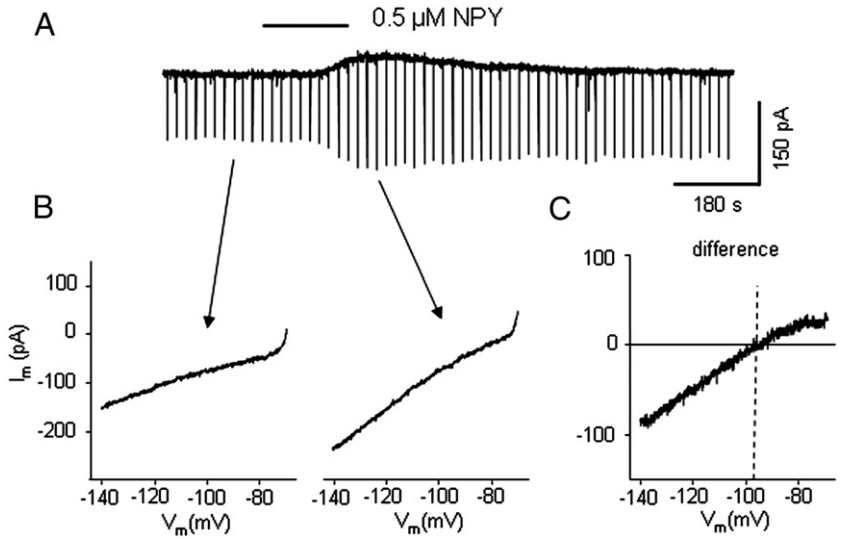

Fig. 1. NPY-induced inwardly rectifying K^+ current in an LA projection neuron. (A) In voltage clamp (holding potential −70 mV) NPY application induced an outward current. (B) Representative traces showing the currents elicited by 300 ms voltage ramps from −70 to −140 mV (seen as downward deflections in A) before and after the application of NPY (0.5 μM). (C) Difference currents obtained by subtracting control from NPY currents. Note that inwardly rectifying properties and reversal potential are close to −100 mV.

the expression is not exclusive for this type of neurons, as in the rat LA mRNA encoding NPY has been found not only in a subclass of local interneurons but also in projection neurons (Sosulina et al., 2006). Furthermore, mRNA expression analysis indicated Y1 and Y2 receptor expression in the rat LA (Parker and Herzog, 1999). While these data suggest a functional role of NPY in the amygdala, the mechanisms of action are unknown to date. In various brain regions, NPY enhances a G-protein-coupled inwardly rectifying potassium (GIRK) current via postsynaptic Y1 receptors, as for example in mouse hippocampus (Paredes et al., 2003), rat thalamus (Sun et al., 2001, 2003), rat and mouse arcuate nucleus (Sun and Miller, 1999; Acuna-Goycolea et al., 2005) and mouse hypothalamic hypocretin neurons (Fu et al., 2004).

Therefore, the present study was undertaken to investigate the mechanisms of NPY action in the LA, using whole-cell patch-clamp techniques in slice preparations of the rat amygdala *in vitro*. The focus was on postsynaptic mechanisms in projection neurons in the LA, as they represent likely mediators of NPY-conveyed influences from local interneurons (McDonald and Mascagni, 2002).

Results

Identification of LA projection neurons

The effects of NPY and analogues were investigated in a total of 124 putative projection neurons in the rat LA, which were identified by a multipolar or pyramidal-like shape of the cell body as well as electrophysiological properties (Faber et al., 2001). A representative sample of these neurons (n=28) had a mean resting membrane potential of −70.9±0.9 mV, an input resistance of 478±32.9 MΩ, and showed various degrees of spike frequency adaptation in response to constant depolarizing current injection. Action potential duration (measured at half amplitude) averaged 2.92±0.1 ms when elicited from −60 mV by positive current injection protocols (1 s; 100 pA). These characteristics are in line with previous data on projection neurons in the rat LA (Meis et al., 2005; Sosulina et al., 2006). A representative example is illustrated as part of Fig. 5.

NPY-induced responses: activation of a membrane potassium current with inwardly rectifying properties

At a holding potential of −70 mV, NPY elicited a response in 92% (n=66) of presumed projection neurons. In a representative sample, application of 0.5 μM NPY evoked a transient outward current with an average peak of 21.6±3.3 pA (n=11; Fig. 1A). The effect lasted for several minutes and displayed strong desensitization when application was repeated. Therefore, only first applications were included into analysis and second applications only took place to control general NPY responsiveness in cells which showed no initial response to ≤200 nM NPY. Voltage ramps (0.2 mV/ms, 0.05 Hz, −70 to −140 mV) demonstrated that this outward current was due to an increase in membrane conductance, indicated by increased membrane current flow after NPY application (Fig. 1B). The difference currents, calculated by subtracting voltage ramp-induced currents after NPY application from pre-NPY ones, showed moderate inward rectification and a reversal potential at −100.41±0.89 mV (n=50) (Fig. 1C), which was near the presumed K^+ equilibrium potential of E_K=−104.1 mV, as obtained by the Nernst equation for 2.5 mM extracellular K^+ concentration. These data indicated that the NPY-induced conductance was for potassium.

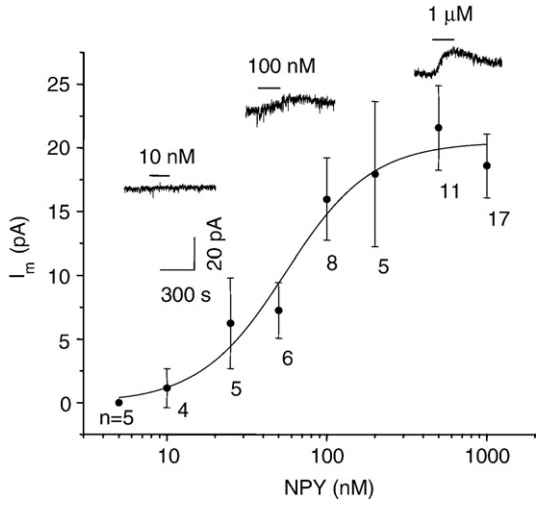

Fig. 2. Concentration-response relationship of NPY-induced current (holding potential at −70 mV). Insets show examples of membrane currents elicited by 10 nM, 100 nM and 1 μM NPY. To an individual cell NPY was only applied once. Numbers of cells examined are given near data points. The curve was fitted to the Hill equation. EC50 was 55.3 nM and the Hill coefficient was 1.6.