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A SUBNANOMOLAR CONCENTRATION OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) PRE-SYNAPTICALLY MODULATES GLUTAMATERGIC TRANSMISSION IN THE RAT HIPPOCAMPUS ACTING THROUGH ACETYLCHOLINE

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Abstract—The neuropeptide PACAP modulates synaptic transmission in the hippocampus exerting multiple effects through different receptor subtypes: the underlying mechanisms have not yet been completely elucidated. The neurotransmitter acetylcholine (ACh) also exerts a well-documented modulation of hippocampal synaptic transmission and plasticity. Since PACAP was shown to stimulate ACh release in the hippocampus, we tested whether PACAP acting through ACh might indirectly modulate glutamate-mediated synaptic transmission at a pre- and/or at a post-synaptic level. Using patch clamp on rat hippocampal slices, we tested PACAP effects on stimulation-evoked AMPA receptor-mediated excitatory post-synaptic currents ($EPSCs_{AMPA}$) in the CA3-CA1 synapse and on spontaneous miniature EPSCs (mEPSCs) in CA1 pyramidal neurons. A subnanomolar dose of PACAP (0.5 nM) decreased $EPSCs_{AMPA}$ amplitude, enhanced EPSC paired-pulse facilitation (PPF) and reduced mEPSC frequency, indicating a presynaptic decrease of glutamate release probability: these effects were abolished by simultaneous blockade of muscarinic and nicotinic ACh receptors, indicating the involvement of endogenous ACh. The effect of subnanomolar PACAP was abolished by a PAC1 receptor antagonist but not by a VPAC receptor blocker. At a higher concentration (10 nM), PACAP inhibited $EPSCs_{AMPA}$: this effect persisted in the presence of ACh receptor antagonists and did not involve any change in PPF or in mEPSC frequency, thus was not mediated by ACh and was exerted postsynaptically on CA1 pyramidal neurons. We suggest that a high-affinity PAC1 receptor pre-synaptically modulates hippocampal glutamatergic transmission acting through ACh. Therefore, administration of PACAP at very low doses might be envisaged in cognitive diseases with reduced cholinergic transmission.

Key words: PACAP, neuropeptide, synaptic transmission, glutamate, AMPA, acetylcholine.

INTRODUCTION

The neuropeptide Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP), initially isolated from ovine hypothalamus (Miyata et al., 1989), is widely diffused in the central nervous system (CNS) as well as in peripheral tissues and exists in two forms with either 38 or 27 amino acid residues (PACAP-38 and PACAP-27). PACAP-38 (PACAP) is the most abundant form in the CNS where it acts as a neurotransmitter, neuromodulator and/or neurotrophic factor (Shioda, 2000; Vaudry et al., 2009) and regulates numerous functions such as circadian rhythms, responses to stress and brain injury, mood and learning (Harmar et al., 2012). PACAP can activate two main receptor types: the first is a PACAP-selective receptor named PAC1, which binds PACAP with nanomolar affinity and the analog peptide vasoactive intestinal peptide (VIP) with a 1,000-fold lower affinity (Pisegna & Wank, 1993). The second family includes VPAC1 and VPAC2 receptors, binding PACAP and VIP with equal nanomolar affinity (Ishihara et al., 1992; Lutz et al., 1993; Sreedharan et al., 1993; Harmar & Lutz, 1994). PAC1, VPAC1 and VPAC2 receptors are G-protein-coupled receptors that potently stimulate adenylate cyclase (AC) and increase intracellular cyclic AMP (cAMP) levels; activation of other intracellular messengers, including calcium and/or phospholipase D, has also been reported particularly for PAC1 receptors (Dickson & Finlayson, 2009).

Various studies indicate that PACAP modulates cognitive functions inducing neurotrophic/

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Abbreviations: ACh, acetylcholine; ACSF, artificial cerebrospinal fluid; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CA1, Cornu Ammonis 1; CA3, Cornu Ammonis 3; cAMP, cyclic adenosine mono phosphate; D-AP5, D-(−)-2-Amino-5-phosphonopentanoic acid; EPSC, excitatory post-synaptic current; EPSCAMPA, AMPA receptor-mediated excitatory post-synaptic current; fEPSPs, field excitatory post-synaptic potentials; LTD, long-term depression; LTP, long-term potentiation; mEPSCs, spontaneous miniature EPSCs; NMDA, N-methyl-D-aspartate; PACAP, Pituitary Adenylate Cyclase-Activating Polypeptide; PPF, paired-pulse facilitation; TTX, Tetrodotoxin.

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neuroprotective effects as well as modulation of neuronal excitability and synaptic transmission (Sauvage et al., 2000; Sacchetti et al., 2001; Otto et al., 2001a; Borbely et al., 2013). Among brain structures playing a key role in learning and memory, the hippocampus contains PACAP-expressing cells and nerve fibers, as shown in the rat by immunohistochemistry (Koves et al., 1991; Piggins et al., 1996; Hannibal, 2002) and in situ hybridization (Skoglosa et al., 1999; Jaworski & Proctor, 2000; Hannibal, 2002).

All PACAP receptor subtypes are expressed in the hippocampus since early developmental stages (Jaworski and Proctor, 2000). PAC1 receptors are located on CA1-CA3 pyramidal cells (Shioda et al., 1997; Joo et al., 2004; Gupte et al., 2015), on mossy fiber terminals in the dentate gyrus (Otto et al., 1999) and, as recently evidenced, also on glial cells (Gupte et al., 2015). VPAC1 and VPAC2 receptors are expressed in all hippocampal regions: in particular, VPAC2 receptors are very abundant on pyramidal cells of the CA1–CA3 regions and on granule cells of the dentate gyrus (Joo et al., 2004).

In hippocampal neurons, PACAP inhibits a slow afterhyperpolarizing membrane ion current (I_{AHP}) (Taylor et al., 2014) and downregulates Kv4.2 channels responsible for I_A, a hyperpolarizing potassium current (Gupte et al., 2015), in line with previous studies showing that PACAP enhances neuronal firing in the hippocampus (Di Mauro et al., 2003; Liu et al., 2003). PACAP also modulates glutamate-mediated hippocampal synaptic transmission: a variety of effects have been described depending on PACAP concentration and on experimental conditions. A high dose of PACAP (1 μM) induced a long-term inhibition of field excitatory post-synaptic potentials (fEPSPs) recorded in the CA1 region of rat hippocampus after stimulation of Schaffer collaterals (Kondo et al., 1997). In similar experimental conditions, another research group confirmed that PACAP (1 μM) inhibited fEPSP amplitude, whereas a very low subnanomolar dose (0.05 nM) of PACAP enhanced fEPSP amplitude and intermediate doses (100–500 nM) exerted biphasic inhibitory/excitatory effects (Roberto et al., 2001). In mouse hippocampus, application of PACAP-27 at a 400 nM concentration induced a long-term inhibition of fEPSPs recorded in the CA1 region (Ster et al., 2009). In our laboratories, we observed that PACAP at a nanomolar dose (10 nM) reduced the amplitude of glutamate-mediated excitatory post-synaptic currents (EPSCs) recorded under patch clamp from rat CA1 pyramidal neurons after stimulation of Schaffer collaterals (Ciranna & Cavallaro, 2003).

Other studies later revealed that PACAP differentially modulates the two main components of fast glutamatergic transmission, respectively mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors for glutamate. PACAP enhances NMDA receptor-mediated synaptic responses in the hippocampal CA1 region through activation of PAC1, VPAC1 and VPAC2 receptors (Yaka et al., 2003; Macdonald et al., 2005). On the other side, the effects of PACAP on AMPA receptor-mediated currents seem more complex (Yang et al., 2010). We observed that excitatory currents induced in rat CA1 pyramidal neurons by direct application of AMPA were enhanced by a subnanomolar (0.5 nM) concentration of PACAP and were instead inhibited by PACAP at a 10 nM dose (Costa et al., 2009). Interestingly, it was recently demonstrated that PACAP inversely regulates the phosphorylation state of two distinct sites on the GluA1 subunit of AMPA receptors, acting in part through an interaction with NMDA receptors (Toda & Huganir, 2015).

PACAP also modulates hippocampal long-term synaptic plasticity: as already mentioned, a PACAP-induced long-term depression (LTD) was described in the CA3-CA1 synapse (Kondo et al., 1997; Roberto et al., 2001; Ster et al., 2009). On the other side, PACAP-deficient mice display reduced long-term potentiation (LTP) in the dentate gyrus (Matsuyama et al., 2003) and learning impairment (Takuma et al., 2014). PAC1 receptor knockout mice show impaired LTP at the mossy fiber-CA3 synapse (Otto et al., 2001a; Otto et al., 2001b; Matsuyama et al., 2003) and selective deficits in associative memory (Otto et al., 2001a). Another recent study shows that PACAP infusion in the hippocampus and in the basolateral amygdala affects memory consolidation and extinction in rats submitted to contextual fear conditioning (Schmidt et al., 2015). Collectively, all these data point out that PACAP exerts multiple modulatory effects on basal glutamatergic excitatory transmission, long-term synaptic plasticity, learning and memory.

Some indication exists that PACAP interacts with the cholinergic system in peripheral tissues and in the CNS: PACAP is co-localized with acetylcholine (ACh) in presynaptic sympathetic nerve terminals in the adrenal gland (Hamelink et al., 2002). A stimulatory effect of PACAP on ACh release was observed in parasympathetic ciliary ganglion neurons (Pugh et al., 2010). In the rat hippocampus, an *in vivo* microdialysis study shows that PACAP stimulates ACh release (Masuo et al., 1993) and PACAP effect on glutamatergic transmission was partially reduced by ACh receptor antagonists (Roberto & Brunelli, 2000).

As indicated above, PACAP effects on the CA3-CA1 synapse are variable and dose-dependent; besides, results from distinct research groups are not always comparable due to different experimental conditions. The mechanisms underlying the large variability of PACAP-induced effects on glutamatergic synaptic transmission still need to be clarified, particularly with respect to: 1) differential modulation of AMPAR- and NMDAR-mediated synaptic ion currents; 2) multiple sites of PACAP action; 3) indirect effects involving the cholinergic system. To clarify these issues, we have tested the effects of PACAP specifically on the AMPAR-mediated component of glutamatergic transmission in the CA3-CA1 synapse, focusing on the site of action (pre- vs post-synaptic) of PACAP effect. Secondly, we have investigated which fraction of PACAP effect might be indirectly mediated by ACh release.

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