Neuropharmacology 79 (2014) 101–111

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Cooperation of taurine uptake and dopamine D1 receptor activation facilitates the induction of protein synthesis-dependent late LTP

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ARTICLE INFO

Article history:
Received 14 June 2013
Received in revised form
28 October 2013
Accepted 30 October 2013

Keywords:
Taurine
Dopamine
NMDA
Hippocampus
Slice
SKF89976A

ABSTRACT

Co-activation of NMDA and dopamine receptors is required for the induction of the late phase of LTP (L-LTP) that is dependent on new protein synthesis. Other neuromodulatory substances may also contribute to this process. Here, we examined whether taurine is one of the neuromodulators contributing to L-LTP induction, since it is known that taurine uptake induces a long-lasting synaptic potentiation dependent on protein synthesis, and taurine uptake inhibition blocks L-LTP induced by tetanization. Experiments were conducted using rat hippocampal slices where field synaptic potentials were evoked and recorded in CA3-CA1 synapses. Taurine (1 mM) applied 10 min before a high frequency stimulation (HFS) train converted a transitory early-LTP (E-LTP) into an L-LTP dependent on protein synthesis. This taurine effect was blocked by a taurine uptake inhibitor. A facilitation of L-LTP induction was also obtained by pre-application of SKF38393, a D1/D5 dopamine receptor (D1R) agonist. In this case, LTP facilitation was not affected by the taurine uptake inhibitor. Nevertheless, when taurine and SKF38393 were simultaneously pre-applied at a concentration that individually did not modify E-LTP, they produced a synergistic mechanism that facilitated the induction of L-LTP with a sole HFS train. This facilitation of L-LTP was blocked by inhibiting either taurine uptake or D1R activation. Taurine and SKF38393 activated different signaling pathways to transform E-LTP into L-LTP. Taurine-induced L-LTP facilitation required MAPK activation, while D1R-agonist-induced facilitation depended mainly on PKA activation and partially on MAPK activation. On the other hand, the synergistic mechanisms induced by the cooperative action of taurine and SKF38393 were impaired by inhibitors against MAPK, PKA and PI3-K. This pharmacological profile resembles that displayed by L-LTP induced by three HFS trains at 10-min intervals. These results indicate that taurine uptake is necessary and cooperates with other neurotransmitter systems in the induction of L-LTP.

1. Introduction

Long-term changes in synaptic efficacy are widely used to gain insight into the cellular basis of learning and memory processes. The direction, magnitude and duration of such synaptic modifications depend on the pattern of synaptic stimulation applied, as different stimulation patterns activate different signaling pathways. For example, the protein synthesis-dependent phase of long-term potentiation (L-LTP) is usually evoked by three to four high frequency trains of synaptic stimuli (HFS) spaced 5–10 min apart (Kandel, 2001). A number of studies have demonstrated that L-LTP induction in the hippocampal CA1 region requires dopamine D1R activation (Frey et al., 1991; Huang and Kandel, 1995; Swanson-Park et al., 1999; Granado et al., 2008), and that this type of receptor has to be co-activated with NMDA receptors to be effective (O'Carroll and Morris, 2004; Navakkode et al., 2007; Stramiello and Wagner, 2008). In addition, other neuromodulatory molecules such as noradrenaline, brain-derived neurotrophic factor and nitric oxide (Bailey et al., 2000) might contribute to the coordination of the

Abbreviations: AP5, D,L-2-Amino-5-phosphonopentanoic acid; D1R, D1/D5 dopamine receptor; E-LTP, early-LTP; fEPSP, field excitatory postsynaptic potential; ERK, extracellular regulated kinase; GAT1, GABA transporter 1; HFS, high frequency stimulation; LLP-TAU, long-lasting potentiation induced by taurine; LTP, long-term potentiation; L-LTP, late-LTP; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; NMDAR, N-methyl-D-aspartate receptor; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; TAUT, taurine transporter.

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http://dx.doi.org/10.1016/j.neuropharm.2013.10.035

different signaling pathways involved in the transition from an early phase of LTP (E-LTP) to a more perdurable potentiation phenomenon. However, the exact identity of these neuromodulators and their interactions resulting in L-LTP have not been elucidated.

We found that taurine, a natural amino acid acting as a $GABA_A$ and glycine receptor agonist, when applied to rat hippocampal slices, induced a long-lasting potentiation of synaptic efficacy in the CA1 area, by a process independent of the activation of these receptor types (Galarreta et al., 1996). Moreover, LLT-TAU induction was also independent of NMDAR activation (Galarreta et al., 1996; Chepkova et al., 2002). In fact, taurine did not seem to activate glycine site on postsynaptic NMDAR (Suárez and Solís, 2006). Later studies revealed that taurine-induced potentiation requires a taurine uptake process (Sergeeva et al., 2003; del Olmo et al., 2004), which somehow triggers several mechanisms similar to those involved in the maintenance of L-LTP, such as PKA activation and de novo protein synthesis (del Olmo et al., 2003). We previously described that L-LTP induced with multiple high frequency trains of synaptic stimulation was impaired in the presence of an inhibitor of taurine uptake, and this blockade was overcome when the inhibitor was concomitantly applied with taurine (del Olmo et al., 2004). These results demonstrate the possibility that taurine is a key activator of the mechanisms required for L-LTP. Here, we report that pre-application of either taurine before a sole HFS train, which by itself only induces E-LTP, causes a perdurable L-LTP requiring protein synthesis.

2. Material and methods

The care and use of animals were carried out in accordance with the European Communities Council Directive (86/609/EEC). Protocols were approved by "Comité Ético de Bienestar Animal" at "Hospital Universitario Ramón y Cajal" (animal facilities ES280790002001). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.1. Slice preparation

Experiments were performed on 400 μm-thick transverse hippocampal slices obtained with standard methods, from male Sprague-Dawley rats (180–240 g). Briefly, rats were decapitated after anesthesia with isoflurane, and the brain was quickly removed and dropped into ice-cold standard Krebs-Ringer bicarbonate solution containing (in mM): 119 NaCl, 26.2 $NaHCO_3$, 2.5 KCl, 1 KH_2PO_4, 1.3 $MgSO_4$, 2.5 $CaCl_2$ and 11 glucose. This solution was pre-gassed with 95% O_2 and 5% CO_2. The hippocampi were sliced with a manual tissue chopper (Stoelting, Illinois, U.S.A.) and placed in an interface holding chamber for at least 3 h at room temperature (20–25 °C). Then, a single slice was transferred to a submersion-type recording chamber, where it was continuously perfused (2 mL/min) with standard solution. Experiments were carried out at 31–32 °C.

2.2. Solutions

Compounds applied by addition to the standard perfusion solution included: D,L-2-amino-5-phosphonopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX), taurine, picrotoxin, SKF38393, emetine and H89 from Sigma (Madrid, Spain); SCH23390 hydrochloride, SKF89976A hydrochloride, CGP55845, wortmannin and UO126 from Tocris (London, UK). The drugs were prepared as stock solutions, stored frozen in the dark and diluted to their final concentrations in the perfusion solution immediately before use. Stock solutions of AP5 (25 mM), CNQX (20 mM), picrotoxin (5 mM), SKF38393 (15 mM), emetine (150 mM), H89 (10 mM), SKF89976A (25 mM) and SCH23390 (1 mM) were prepared in distilled water. Stock solutions of CGP55845 (2 mM), wortmannin (50 mM) and UO126 (20 mM) were prepared in dimethyl sulfoxide (final concentration 0.1%). Taurine was dissolved directly in the perfusion solutions at its final concentration. To prevent epileptiform discharges in those experiments in which the $GABA_A$ receptors were blocked with picrotoxin, a cut was made between the CA1 and CA3 areas, and the concentrations of Ca^{2+} and Mg^{2+} were increased to 4 mM. The osmolarity of the perfusion solutions was tested using a micro-osmometer (Advanced Instruments Mod.3MO, Norwood, MA, USA).

2.3. Recording of evoked synaptic potentials

Evoked field excitatory postsynaptic potentials (fEPSP) were recorded in the CA1 stratum radiatum with tungsten microelectrodes (1 MΩ) connected to an AI-401 amplifier (Axon Instruments, Foster City, CA, USA) plugged into a CyberAmp 320 signal conditioner (Axon Instruments). These field responses were evoked by stimulating two independent groups of Schaffer collateral-commisural fibers with biphasic electrical pulses (20–60 μA; 100 μs per phase; 0.033 Hz) delivered through bipolar tungsten insulated microelectrodes (0.5 MΩ) placed in the CA1 mid-stratum radiatum. The stimulation electrodes were placed on opposite sides of the recording electrode. The independence of both pathways was assessed by the lack of paired-pulse facilitation between them, i.e. when two stimuli were applied, one on each pathway at 50-ms intervals, and the second response was identical to that evoked by an unpaired pulse applied 10 s after the paired stimulation. Electrical pulses were supplied by an A.M.P.I. Mod. Master 8 pulse generator (Jerusalem, Israel).

Induction of E-LTP and L-LTP was achieved in one of the two stimulation pathways by applying one and three trains, respectively, of high-frequency stimulation (HFS: 100 Hz, 1 s) at 10-min intervals at the same pulse duration applied during baseline stimulation.

2.4. Electrophysiological data analysis

Evoked responses were digitized at 25–50 kHz using a Digidata 1320A board (Axon Instruments), and stored on a computer using pCLAMP 8.0.2 software (Axon Instruments). The synaptic strength was calculated using the initial rising slope phase of the fEPSP to avoid contamination of the response by the population spike. We used pCLAMP-8.0.2 software for these calculations. Traces shown are averages of eight consecutive responses. Data were normalized with respect to the mean values of the responses during the final 20 min of the basal recording period.

2.5. Statistical analysis

Data are expressed as means ± SEM. Mean values of the fEPSP slope given throughout the text correspond to averages of 5-min periods. Statistical differences were assessed by one- or two-way analysis of variance followed by two-tailed *t*-tests; a value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Taurine converts E-LTP into L-LTP

The application of a single HFS train, which normally produces only E-LTP, induces L-LTP in the presence of a D1R agonist (Swanson-Park et al., 1999). We wondered whether taurine could mimic this dopamine-induced facilitation. To this end, we carried out a group of experiments where an HFS train was applied in the presence of 1 mM taurine. To eliminate the effects of taurine through GABA receptors, these experiments were conducted in the presence of 100 μM picrotoxin, a $GABA_A$ receptor blocker, and 2 μM CGP55845, a $GABA_B$ receptor antagonist. We confirmed that, under these experimental conditions, a single HFS train effectively generated E-LTP (Fig. A1; 2 h post-HFS, fEPSP values were indistinguishable from the basal values; $n = 6$). Interestingly, when the HFS was applied in the presence of 1 mM taurine (perfused 10 min before until 10 min after the HFS), the synaptic potentiation lasted at least 3 h post-tetanization (139 ± 5% in the presence of taurine, $n = 7$, compared to 103 ± 5% in control conditions; $p < 0.001$; Fig. A1).

To examine whether this taurine effect on LTP duration presents an efficacious time window, we carried out a series of experiments in which we used a brief taurine application (5 min) at different intervals preceding the HFS train. Taurine pre-application 10 min before the HFS was equally effective at increasing LTP maintenance as when the tetanus was given during taurine perfusion (Fig. 2; 140 ± 3% with the pre-application of taurine, $n = 7$, vs. 139 ± 5% during the perfusion of taurine, both at 3 h post-HFS; $p > 0.05$). As taurine application was increasingly separated from the tetanus, the effect was less marked, until it disappeared at a 30 min interval (Fig. 2 106 ± 2% at 2 h post-HFS compared to 108 ± 3% in control experiments; $p > 0.05$). It should be noted that the application of taurine 1 min after tetanization ($n = 5$) did not have any effect on E-LTP maintenance (Fig. 2B; $p > 0.05$, compared with the control situation without taurine at 2 h post-HFS).

To rule out the possible influence of the experimental conditions (i.e., GABA antagonists and high concentrations of calcium and magnesium) on the obtained results, we repeated some of these experiments in a standard solution. The E-LTP obtained under