Behavioural Brain Research 317 (2017) 27–36

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Calcium homeostasis and protein kinase/phosphatase balance participate in nicotine-induced memory improvement in passive avoidance task in mice

Agnieszka Michalak*, Grazyna Biala

Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, 4a Chodźki Street, 20-093 Lublin, Poland

HIGHLIGHTS

- Nicotine enhances memory via L-type VGCC blockade and via ERK1/2 activation.
- Only short-term memory enhancement induced by nicotine is dependent on CaN inhibition.
- Nicotine affects short- and long-term memory via similar cell signaling substrates.

ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form 4 September 2016
Accepted 11 September 2016
Available online 12 September 2016

Keywords:
Nicotine
VGCC blockers
FK-506
SL-327
Memory

ABSTRACT

Long-term potentiation (LTP) and long-term depression (LTD) depend on specific postsynaptic Ca^{2+}/calmodulin concentration. LTP results from Ca^{2+} influx through the activated NMDA receptors or voltage-gated calcium channels (VGCCs) and is linked with activation of protein kinases including mitogen-activated protein kinase (MAPK). Weaker synaptic stimulation, as a result of low Ca^{2+} influx, leads to activation of Ca^{2+}/calmodulin-dependent phosphatase (calcineurin – CaN) and triggers LTD. Interestingly, both memory formation and drug addiction share similar neuroplastic changes. Nicotine, which is one of the most common addictive drugs, manifests its memory effects through nicotinic acetylcholine receptors (nAChRs). Because nAChRs may also gate Ca^{2+}, it is suggested that calcium signaling pathways are involved in nicotine-induced memory effects.

Within the scope of the study was to evaluate the importance of calcium homeostasis and protein kinase/phosphatase balance in nicotine-induced short- and long-term memory effects. To assess memory function in mice passive avoidance test was used.

The presented results confirm that acute nicotine (0.1 mg/kg) improves short- and long-term memory. Pretreatment with L-type VGCC blockers (amlodipine, nicardipine verapamil) increased nicotine-induced memory improvement in the context of short- and long-term memory. Pretreatment with FK-506 (a potent CaN inhibitor) enhanced short- but not long-term memory effects of nicotine, while SL-327 (a selective MAPK/ERK kinase inhibitor) attenuated both nicotine-induced short- and long-term memory improvement.

Acute nicotine enhances both types of memory via L-type VGCC blockade and via ERK1/2 activation. Only short- but not long-term memory enhancement induced by nicotine is dependent on CaN inhibition.

1. Introduction

Cholinergic signaling dysfunction is one of the most fundamental features of cognitive impairments, which has been proved countless times by using different agonists and antagonists of cholinergic receptors. Acetylcholine signaling pathway via nicotinic acetylcholine receptors (nAChRs) has been identified in learning and memory. Loss of nAChRs, especially α4β2 and α7 subtypes, is observed in Alzhemier's disease [1], and α4β2 subtypes have been showed to be involved in hippocampus-dependent learning and memory [2]. Neuronal nAChRs consist of five subunits arranged around a central pore, which is a ligand-gated ion channel permeable to multiple cations: Na^+, K^+ and Ca^{2+}. Since nAChRs seem to play a crucial role in memory performance and are likely to allow Ca^{2+} influx, calcium signaling pathways are considered to be linked with memory effects of nicotine [1,3].

* Corresponding author.
E-mail address: agnieszka.michalak@umlub.pl (A. Michalak).

http://dx.doi.org/10.1016/j.bbr.2016.09.023
0166-4328/

There is a strong link between learning and memory and drug addiction, which seem to share the same neurotrophic factors, intracellular signaling cascades and specific brain regions such as the hippocampus, the ventral tegmental area (VTA) and the nucleus accumbens (NAc) [4,5]. Memory formation as well as neuroadaptive changes associated with the intake of psychoactive substances, are underpinned by the same phenomenon called neuroplasticity. It is the ability of the brain to be continuously reorganized on a functional and morphological level, expressed through two contradictory processes: long-term potentiation (LTP) and long-term depression (LTD). Learning and memory and drug addiction are both accompanied by LTP and LTD, which are dependent on specific postsynaptic Ca^{2+}/calmodulin concentration. LTP results from Ca^{2+} influx through the activated NMDA receptors [6–8]. Another route for Ca^{2+} to enter the postsynapse can be voltage-gated calcium channels (VGCCs) [9,10]. Growing intracellular Ca^{2+} level activates protein kinases such as Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), resulting in phosphorylation of AMPA receptors. Thus, increased AMPA receptor conductance leads to larger postsynaptic responses and makes neuronal junctions more stable [8,11]. When LTP prolongs activated adenylyl cyclase raises cAMP and activates cAMP-dependent protein kinase (PKA), which is responsible for phosphorylation of cAMP response element binding protein (CREB). Following gene expression leads to protein synthesis and triggers structural changes, essential for long-term memory formation [9]. It has been established that mitogen-activated protein kinase (MAPK) cascade also plays a great role in neuroplasticity, inducing a long-lasting form of LTP [12,13]. There is a clear link between extracellular signal-regulated kinase (ERK) pathway, a member of the MAPK superfamily, and learning and memory formation. ERK is divided into two subtypes, ERK1 and ERK2, and can be activated by MAPK/ERK kinase (MEK), protein kinase C (PKC) or epidermal growth factor receptor (EGFR), which activation is dependent on Ca^{2+} influx through L-type VGCCs [14]. Contrary to LTP, weaker synaptic stimulation results in LTD. Low Ca^{2+} influx through NMDA receptors or VGCC receptors activates Ca^{2+}/calmodulin-dependent phosphatase (calcineurin – CaN). Activated calcineurin increases the activity of protein phosphatase 1 (PP1), which leads to dephosphorylation of synaptic receptors. Consequently, calcineurin counteracts the function of protein kinases, thus is believed to suppress potentiation and to lead to synaptic depression [9–11].

Taking into consideration all mentioned above within the scope of the study was to evaluate the impact of calcium homeostasis and protein kinase/phosphatase balance in nicotine-induced short- and long-term memory effects. On the grounds of Biala et al. [15] from the great range of L-type VGCC blockers three the most promising that may affect memory performance were chosen i.e., amlodipine, nicardipine and verapamil. In order to examine protein kinase/phosphatase activities in nicotine memory-related effects FK-506 (a potent calcineurin inhibitor) and SL-327 (a selective MEK1/2 inhibitor) were used. The results should contribute to better understanding of the mechanisms underlying the cognitive effects of nicotine, and neuroplasticity as a phenomenon mutual to memory and addiction. In the long-term perspective the results of this study may help to evolve new therapeutic targets in treatments for cognitive disorders and drug addictions.

2. Materials and methods

2.1. Animals

All experiments were conducted on naive male Swiss mice (Farm of Laboratory Animals, Warsaw, Poland) weighing 20–25 g. The animals were supplied with standard laboratory chow and free access to tap water *ad libitum*. Room temperature of $21 \pm 1\,^{\circ}C$ was maintained during a 12/12 h light–dark cycle. Each experimental group consisted of 8–11 mice and each mouse was used only once after being acclimatized to the laboratories for at least one week after shipment. The experiments were performed in strict accordance with the guidelines of National Research Council 2003, European Community Council directive of 22 September 2010 (2010/63/EU), and approved by the local ethics committee.

2.2. Drugs

The drugs included: nicotine hydrogen tartrate (0.05, 0.1 and 0.5 mg/kg), amlodipine besylate (2.5, 5.0, 10.0 and 20.0 mg/kg), nicardipine hydrochloride (2.5, 5.0, 10.0 and 20.0 mg/kg), verapamil hydrochloride (2.5, 5.0, 10.0 and 20.0 mg/kg) purchased from Sigma-Aldrich (St. Louis, MO, USA); and FK-506 (1.0, 5.0 and 10.0 mg/kg) and SL-327 (3.0, 10.0 and 30.0 mg/kg) purchased from Tocris Bioscence (Bristol, UK). Except for nicotine all drugs were suspended in sterile saline with a drop of Tween 80. Nicotine was dissolved in sterile saline and then the pH of the solution was adjusted to 7.0. All solutions were freshly prepared immediately before use.

2.3. Passive avoidance

2.3.1. Apparatus

To assess memory function passive avoidance test (PA test) was used. The test is based on the association formed between an aversive stimulus (a foot shock) and a specific environmental context. The apparatus consisted of two-compartment light-dark box. The light compartment ($10 \times 13 \times 15$ cm) was illuminated with fluorescent light (8 W) while the dark one ($25 \times 20 \times 15$ cm) was equipped with energized grid floor. The compartments were separated by a guillotine door. The entrance of animals to the dark box was punished by an electric foot shock.

2.3.2. Procedure

24 h before the training session the mice were allowed to explore freely the apparatus for 3 min (habituation). On the training day each mouse was placed separately in the center of light compartment facing away the guillotine door. After 10 s of adaptation period the light was on and the guillotine door was opened exposing the dark compartment [16]. When the mouse entered the dark box with all four paws, the guillotine door was closed, and the foot shock (0.2 mA, 1 s duration) was delivered. The test session was carried out 2 h (short-term memory) or 24 h (long-term memory) after the training. On the test day the mouse was returned to the lighted compartment and the procedure was repeated except that no shock was delivered. Each time the latency to enter the dark compartment was recorded. Mice whose latency on the training session exceeded 60 s were excluded from the experiment in order to minimize the deviation of baseline data. If the animal did not enter the dark compartment during the test within 300 s, the trial was finished and the final score was established as 300 s [17]. After each session the apparatus was cleaned using 70% ethanol.

2.4. Treatment

The first study was to investigate the acute influence of nicotine, amlodipine, nicardipine, verapamil, FK-506 and SL-327 on memory-related effects. Nicotine (0.05, 0.1 and 0.5 mg/kg, sc) was administered 30 min before the training. Amlodipine (2.5, 5.0, 10.0 or 20.0 mg/kg, ip), nicardipine (2.5, 5.0, 10.0 or 20.0 mg/kg, ip) and verapamil (2.5, 5.0, 10.0 or 20.0 mg/kg, ip) were injected 15 min before, while FK-506 (1.0, 5.0 and 10.0 mg/kg, ip) and SL-327 (3.0, 10.0 and 30.0 mg/kg, ip) 1 h before the training session. The