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ScienceDirect
Neuropharmacology 54 (2008) 87–94
NEURO PHARMACOLOGY
www.elsevier.com/locate/neuropharm

Role of the cyclic-AMP/PKA cascade and of P/Q-type Ca^{++} channels in endocannabinoid-mediated long-term depression in the nucleus accumbens

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Received 12 January 2007; received in revised form 19 April 2007; accepted 24 April 2007

Abstract

Glutamate transmission between prefrontal cortex (PFC) and accumbens (NAc) plays a crucial role in the establishment and expression of addictive behaviors. At these synapses exogenous cannabinoid receptor 1 (CB1R) agonists reversibly inhibit excitatory transmission, and the sustained release of endogenous cannabinoids (eCB) following prolonged cortical stimulation leads to long-term depression (LTD). Activation of presynaptic K^+ channels mediates the effects of exocannabinoids, but the transduction pathway underlying the protracted phase of eCB-LTD is unknown. Here we report that the maintenance of eCB-LTD does not involve presynaptic K^+ channels: eCB-LTD was not affected by blockade of K^+ channels with 4-AP (100 μM) and $BaCl_2$ (300 μM) (fEPSP = 78.9 ± 5.4% of baseline 58–60 min after tetanus, compared to 78.9 ± 5.9% in control slices). In contrast, eCB-LTD was blocked by treatment of the slices with the adenylyl cyclase (AC) activator forskolin (10 μM), and with the protein kinase A (PKA) inhibitor KT5720 (1 μM) (fEPSP = 108.9 ± 5.7% in forskolin and 110.5 ± 7.7% in KT5720, compared to 80.6 ± 3.9% in control conditions). Additionally, selective blockade of P/Q-type Ca^{2+} channels with ω-agatoxin-IVA (200 nM) occluded the expression of eCB-LTD (fEPSP = 113.4 ± 15.9% compared to 78.6 ± 4.4% in control slices), while blockade of N- with ω-conotoxin-GVIA (1 μM) or L-type Ca^{2+} channels with nimodipine (1 μM), was without effect (fEPSP was 83.7 ± 5.3% and 87 ± 8.9% respectively). These data show that protracted inhibition of AC/PKA activity and P/Q-type Ca^{2+} channels are necessary for expression of eCB-LTD at NAc synapses.

Keywords: Endocannabinoid; Long-term depression; Drug abuse; Nucleus accumbens; Metabotropic glutamate receptor 2/3; CB1 receptor; P/Q-Calcium channels

1. Introduction

The nucleus accumbens (NAc) is a primary component of the mesocorticolimbic dopaminergic system that participates to the rewarding and reinforcing properties of most abused drugs (Kelley, 2004). It is thought that homeostatic deficits in glutamate transmission at prefrontal cortex (PFC)-NAc synapses underlie the motivation to seek drugs that classically characterizes addiction (Kalivas and Volkow, 2005). Adaptations in PFC-NAc excitatory transmission following chronic cocaine administration include reduced levels of extracellular glutamate in the NAc (Pierce et al., 1996) and augmented glutamate release from PFC projections, associated with cocaine-primed reinstatement of drug seeking (McFarland et al., 2003). Furthermore, reinstatement responding can be either elicited or blocked by administration of AMPA glutamate receptor agonists or antagonists, respectively, into the NAc (Cornish and Kalivas, 2000). Thus, clarifying the cellular mechanisms that regulate glutamate release at PFC-NAc pathway is important to the understanding of the neurobiological basis of addiction.

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0028-3908/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2007.04.014

Synaptic strength at PFC excitatory inputs onto NAc medium spiny neurons can be bidirectionally modulated, as these synapses express both long-term potentiation (LTP) and depression (LTD). LTP of glutamatergic transmission in the NAc depends on postsynaptic NMDA receptors (Pennartz et al., 1993), whereas LTD can be triggered by different mechanisms, involving postsynaptic NMDA receptors (Thomas and Malenka, 2003), presynaptic glutamate mGlu2/3 receptors (mGluR2/3) (Robbe et al., 2002a,b,c) or presynaptic CB1R (Robbe et al., 2002a,b,c). Interestingly, converging data indicate that acute and/or chronic exposure to drugs of abuse modulate both mGluR2/3- and/or CB1R-dependent regulation of glutamate release and synaptic plasticity in the NAc, suggesting that alteration of the functionality of both presynaptic receptors is involved in the development of addictive behaviors (Mato et al., 2005; Robbe et al., 2002a,b,c; Xi et al., 2002,2006). Whereas mGluR2/3-dependent LTD in the NAc is mediated by a selective reduction in the contribution of P/Q-type Ca^{2+} channels to glutamate release (Robbe et al., 2002a,b,c), the mechanisms of eCB-LTD at these synapses are unknown. We recently reported that in response to chronic Δ^9-Tetrahydrocannabinol (Δ^9-THC), homeostatic processes involving mGluR2/3 rescue LTD and that mGluR2/3-LTD and eCB-LTD mutually occlude, suggesting that these two forms of presynaptic plasticity have at least one common point in their transduction pathways (Mato et al., 2005). Here we searched for the mechanism responsible for eCB/CB1R-mediated LTD in the NAc. We found that eCB-LTD shares the expression mechanism of mGluR2/3-LTD: a selective reduction in the contribution of P/Q-type Ca^{2+} channels to glutamate release.

2. Methods

2.1. Animal treatment

Animal experiments accomplished the criteria of the European Communities Council Directive (86/609/EEC) and the United States National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Mice (4–6 weeks old male, C57B1/6 strain) were housed, grouped and acclimatized to laboratory conditions (12 h light/dark cycles) 1 week before the experiment and had ad libitum food and water access.

2.2. Slice preparation and electrophysiology

Extracellular field recordings and whole cell patch clamp recordings were made from medium spiny neurons in parasagittal slices of mouse NAc. These methods have been described in detail previously (Robbe et al., 2001). In brief, mouse (male C57BL6, 4–6 weeks housed, grouped and acclimatized to laboratory conditions 1 week before the experiment) were anesthetized with isoflurane and decapitated according to institutional regulations. The brain was sliced (300 μm) in the parasagittal plane using a vibratome (Integraslice, Campden Instruments, UK) and maintained in physiological saline at 4 °C. Slices containing the NAc were stored at least one hour at room temperature before being placed in the recording chamber and superfused (2 ml/min) with artificial cerebrospinal fluid (ACSF) that contained (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 18 $NaHCO_3$, 1.2 NaH_2PO_4, and 11 Glucose, and was equilibrated with 95% O_2/5% CO_2. All experiments were done at room temperature. The superfusion medium contained picrotoxin (100 μM) to block GABA-A receptors. All drugs were added at the final concentration to the superfusion medium. The recording pipette was filled with ACSF.

For extracellular field experiments, the recording pipette was filled with ACSF. Both the field excitatory postsynaptic potential (fEPSP) area and amplitude were measured (graphs depict area). The glutamatergic nature of the extracellular fEPSP was confirmed at the end of the experiments through the application of the non-NMDA ionotropic glutamate receptor antagonist DNQX (20 μM), which completely blocked the synaptic N2 component without altering the non-synaptic N1 component (not shown) (Mato et al., 2005).

For whole cell patch-clamp experiments, medium spiny neurons were visualized using an upright microscope with infrared illumination. Recordings were made with electrodes containing the following (mM): Cesium Methane-Sulfonate (CH_3O_3SCs) or K^+ Gluconate 128, NaCl 20, $MgCl_2$ 1, EGTA 1, $CaCl_2$ 0.3, Na^{2+}-ATP 2, Na^+-GTP 0.3, Glucose 10 buffered with Hepes 10, pH 7.3, osmolarity 290 mOsm. Electrode resistance was 4–6 MOhms. An Axopatch-1D (Molecular Devices, Sunnyvale, CA, USA) was used to record the data, which were filtered at 1–2 KHz, digitized at 5 KHz on a DigiData 1200 interface (Molecular Devices, Sunnyvale, CA, USA) and collected on a PC using pClamp9.2 (Molecular Devices, Sunnyvale, CA, USA). To evoke synaptic currents, stimuli (100–150 μs duration) were delivered at 0.033 Hz through bipolar tungsten electrodes placed at the PFC-NAc border. Recordings were made in the rostral-medial dorsal accumbens close to the anterior commissure. The magnitude of LTD was calculated by comparing averaged responses (55–60 min) after induction to baseline-averaged responses before induction protocol.

2.3. Data analysis and materials

Data were analyzed using pClamp9.2 (Molecular Devices, Sunnyvale, CA, USA). Spontaneous miniature excitatory postsynaptic currents (sEPSCs) were recorded in the whole cell voltage-clamp configuration using Axoscope 9.2 (Molecular Device, Sunnyvale, USA). sEPSC amplitude and inter-interval time were detected and analyzed using Clampfit 9.2 (Molecular Device, Sunnyvale, USA). For this analysis, a template of sEPSCs generated from averaging several typical synaptic events was slid along the data trace one point at a time. At each position, this template is optimally scaled and offset to fit the data and a detection criterion is calculated. The detection criterion is the template-scaling factor divided by the goodness-of-fit at each position. An event is detected when this criterion exceeds a threshold and reaches a sharp maximum.

All values are given as mean ± SEM. The data from all the slices assayed under the same experimental condition were averaged and statistical significance tested with the Mann–Whitney *U*-test ($p < 0.05$ was taken as indicating statistical significance) using Kyplotβ13 (Koichi Yoshioka).

2.3.1. Drugs used

Picrotoxin, forskolin, $BaCl_2$ and nimodipine were purchased from Sigma (St. Quentin Fallavier, France); 4-aminopyridine (4-AP) and KT5720 were from Alexis Corporation (San Diego, CA, USA); ω-Agatoxin-IVA and ω-Conotoxin-GVIA were from Alomone Lab (Israel). SR141716A was a generous gift from Sanofi Recherche (Montpellier, France). Other chemicals were from the highest commercial grade available. All drugs were bath-applied following dilution into the external solution from concentrated stock solutions.

3. Results

We have previously reported that the effects of cannabinoid agonists and synaptic plasticity can be observed either when recording in the whole-cell patch-clamp mode or when performing extracellular field recordings (Mato et al., 2004, 2005; Robbe et al., 2001, 2002a,b,c). Thus, in the present study extracellular field recordings were preferred to whole-cell patch-clamp recordings because of their non-invasive nature and because they permit stable recordings over lengthened period of times (typically 1–2 h).

3.1. eCB-LTD does not require CB1R-mediated modulation of K^+ conductances

In NAc slices, perfusion of cannabinoid agonists induces a robust and partially reversible presynaptic inhibition of glutamatergic transmission at PFC-NAc synapses that requires CB1R-mediated modulation of K^+ conductances (Robbe et al., 2001). We first tested the involvement of presynaptic K^+ channels in eCB-LTD. As previously described (Robbe et al., 2001), bath application of 4-amino-pyridine (4-AP, 100 μM) in combination with $BaCl_2$ (300 μM) caused a large increase in the fEPSP size and duration (218 ± 18% of baseline, $n = 17$) (Fig. 1A), due to the blockade of both voltage-dependent and G protein-gated inwardly rectifying K^+ channels. Extracellular calcium was then lowered (from 2.4 mM to 0.5 mM) in the presence of 4-AP and $BaCl_2$ to reduce the size of the evoked fEPSP and correct for the effects of K^+ channel blockers on Ca^{2+}-dependent processes (Hoffman and Lupica, 2000; Lupica and Riegel, 2005; Robbe et al., 2001) (Fig. 1A). Tetanic stimulation of PFC afferences (for 10 min at 13 Hz) reliably induced LTD in slices perfused with 4-AP, $BaCl_2$ and low extracellular Ca^{2+}. The size of the fEPSP measured 58–60 min after tetanus was 78.9 ± 5.4% of baseline under these conditions ($n = 17$), compared to 78.9 ± 5.9% in interleaved control slices perfused with standard ACSF ($n = 10$) (Fig. 1A,B). The CB1R antagonist SR141716A (SR-1, 3 μM) completely prevented LTD in the presence of 4-AP, $BaCl_2$ and low extracellular Ca^{2+} (fEPSP was 104.9 ± 7.3% of baseline, $n = 7$; $p < 0.05$ versus control) (Fig. 1B), showing that in the presence of K^+ channel blockers and low extracellular Ca^{2+}, LTD still depends on CB1R. Collectively, these findings show that CB1R mediate activity-dependent LTD in the NAc independently of the modulation of presynaptic K^+ conductances.

3.2. Role of the cAMP/PKA-signaling cascade in eCB-LTD

One of the major transduction pathways activated by agonist stimulation of CB1R is the inhibition of adenylyl cyclase (AC) via $G_{i/o}$ proteins (Howlett, 2002). We and other previously reported that activation of CB1R or mGluR2/3 $G_{i/o}$-coupled receptors can decrease intracellular cAMP levels and reduce the activity of protein kinase A (PKA), which causes the inhibition of presynaptic voltage-sensitive calcium channels (VSCCs), and the subsequent reduction of evoked synaptic transmission (Hoffman and Lupica, 2000). Moreover, mGluR2/3- and eCB-LTD mutually occlude (Mato et al., 2005). Thus, the involvement of this pathway in eCB-LTD was tested. Bath application of the AC activator forskolin (10 μM) increased of the fEPSP (189 ± 25% of baseline, $n = 8$; Fig. 2A) (Robbe et al., 2002a,b,c) and completely prevented the expression of eCB-LTD (fEPSP = 108.9 ± 5.7% of baseline $n = 8$, vs 80.6 ± 3.9% in control slices, $n = 8$; $p < 0.01$; Fig. 2A, B). Whole cell patch clamp recordings were performed to confirm that the AC activator forskolin acts at a presynaptic site in our preparation as classically described in other neuronal preparations (Chavez-Noriega

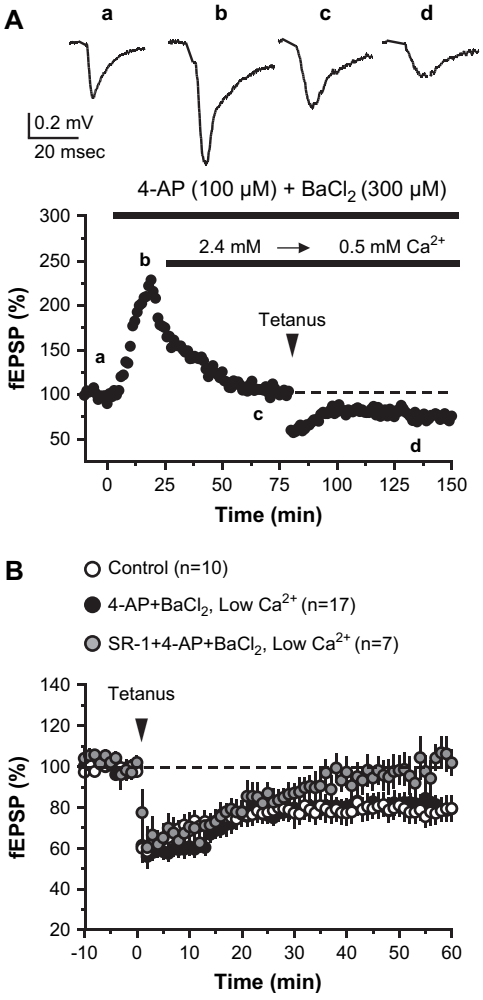

Fig. 1. eCB-LTD does not require the modulation of K^+ conductances. (A) Representative experiment in which blockade of voltage-dependent and G protein-gated K^+ channels with 4-AP (100 μM) and $BaCl_2$ (300 μM) did not prevent the expression of eCB-LTD. Perfusion of 4-AP and $BaCl_2$ in standard ACSF (2.4 mM $CaCl_2$) caused a large increase in the size and duration of the evoked fEPSP, that was compensated by lowering extracellular calcium (from 2.4 to 0.5 mM). In this condition, tetanic stimulation (10 min at 13 Hz given at the time indicated by the arrow) of PFC afferences to the NAc induced a robust LTD. Averaged sample traces (of 10 consecutive fEPSPs) taken during the experiment are depicted above (calibration bar: 0.2 mV/10 ms). (B) Averaged graph depicting 13 Hz-tetanus induced LTD both in standard conditions (open circles) as well as in the presence of the K^+ channel blockers 4-AP and $BaCl_2$ with low extracellular [Ca^{2+}] (black circles). Co-perfusion of the CB1R antagonist SR141716A (SR-1, 3 μM) with 4-AP and $BaCl_2$ in low Ca^{2+} medium completely prevented the expression of 13 Hz-LTD (grey circles).

and Stevens, 1994; Chavis et al., 1998; Tzounopoulos et al., 1998). Similarly to what we observed in our extracellular field recordings (Fig. 2A), forskolin (10 μM) enhanced evoked excitatory post synaptic currents (EPSCs, 174 ± 25% of baseline, $n = 8$; Supplementary Fig. 1A). The forskolin-induced enhancement of EPSCs was accompanied by a clear and statistically significant ($p < 0.05$, paired t-test), reduction of the paired pulse ratio, a classical indicator of presynaptic modifications (Supplementary Fig. 1A, upper panel). Moreover, we also quantified forskolin's effects on spontaneous miniature EPSCs (sEPSCs) and observed that forskolin increased the frequency

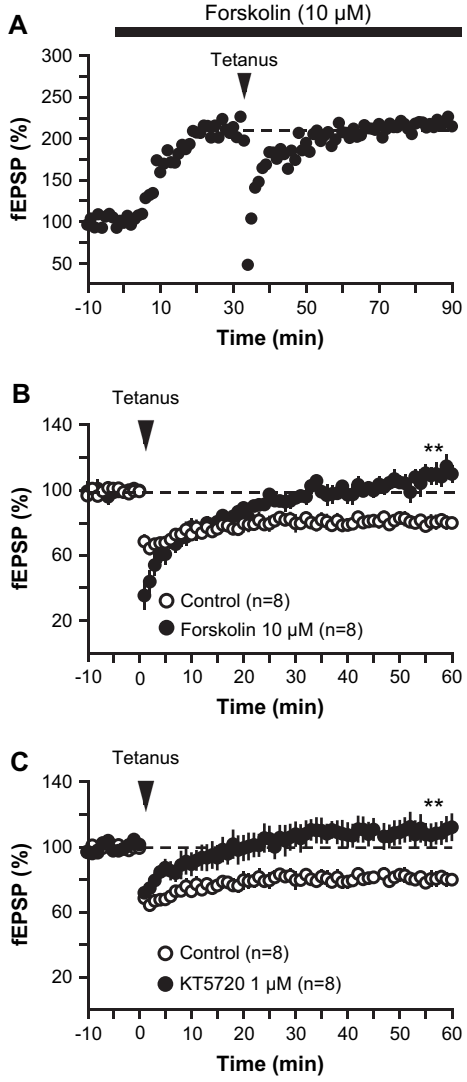

Fig. 2. Role of the cAMP/PKA cascade in eCB-LTD. (A) Representative experiment in which bath application of the AC activator forskolin (10 μM) induced a large augmentation of the fEPSP in NAc slices and completely prevented the expression of eCB-LTD (induced by 10 min at 13 Hz given at the time indicated by the arrow) (B) Averaged temporal courses comparing the effect of 10 min at 13 Hz-tetanus in control conditions (white circles) and in slices perfused with forskolin (black circles). (C) Summary of all the experiments in which slices were preincubated at least 2 h with the PKA inhibitor KT5720 (1 μM, black circles). In this condition, the LTD induced by 13 Hz-tetanus was completely prevented.

but not the amplitude of sEPSCs (Supplementary Fig. 1A, B). Together, these data show that forskolin increased transmitter release at the excitatory synapses of the accumbens.

Finally, in further support of a role of PKA in eCB-LTD, it was found that preincubation of the slices with the PKA inhibitor KT5720 (1 μM) also abolished eCB-LTD (fEPSP was $110.5 \pm 7.7\%$ of baseline, $n = 8$ vs $80.6 \pm 3.9\%$, $n = 8$; $p < 0.01$; Fig. 2C) (Robbe et al., 2002a,b,c). Together, these data show that the cAMP/PKA transduction pathway is necessary to eCB-mediated LTD expressed at PFC-NAc. Keeping in mind that CB1R are normally negatively coupled the AC/PKA pathway and based on our previous data showing that bath perfusion of a PKA inhibitor reduced excitatory transmission at NAc synapses (Robbe et al., 2002a,b,c) and on the seminal work of Tzonopoulos and colleagues (Tzounopoulos et al., 1998), our interpretation is that PKA inhibition mimicked and occluded eCB-LTD induction while on the contrary, maximal activation of the AC/PKA pathway by forskolin prevented eCB-LTD.

3.3. Role of the VSCCs in eCB-LTD

The inhibition of presynaptic VSCCs is a well documented pathway involved in the CB1R-mediated inhibition of transmitter release (Howlett, 2002). Of particular interest, endocannabinoid-mediated LTD in the amygdala involves the inhibition of P/Q-type Ca^{2+} channels (Huang et al., 2003), whereas in the hippocampus, Wilson and collaborators have shown that cannabinoid-sensitive connections use exclusively N-type Ca^{2+} channels (Wilson et al., 2001). The role of VSCCs in eCB LTD in the NAc was tested by selectively blocking N-, P/Q- or L-type VSCCs with ω-conotoxin-GVIA (1 μM), ω-agatoxin-IVA (200 nM) or nimodipine (1 μM), respectively (Robbe et al., 2001, 2002a,b,c). Bath application of ω-conotoxin-GVIA to selectively block N-type VSCCs strongly inhibited evoked excitatory transmission in the NAc (averaged fEPSP measured after 20 min of ω-conotoxin-GVIA was $52 \pm 3.4\%$ of baseline, $n = 10$; Fig. 3A) but did not prevent the expression of eCB-LTD (fEPSP 58–60 min post-tetanus measured $83.7 \pm 5.3\%$ $n = 10$, vs $78.6 \pm 4.3\%$ in control, $n = 6$; Fig. 3A, B). By contrast, selective blockade of P/Q-type channels with ω-agatoxin-IVA reduced synaptic transmission to $58 \pm 7.7\%$ of baseline ($n = 5$; Fig. 3C) and completely abolished the expression of eCB-LTD ($113.4 \pm 15.9\%$ $n = 5$ vs $78.6 \pm 4.3\%$, $n = 6$; $p < 0.05$; Fig. 3D). Perfusion of nimodipine to selectively block L-type VSCCs slightly reduced the size of the fEPSP ($80.8 \pm 3.8\%$ of baseline, $n = 4$; Fig. 3E), and had no effect on eCB-LTD ($87 \pm 8.9\%$ $n = 4$, vs $78.6 \pm 4.3\%$ in control, $n = 6$; Fig. 3F). Taken together the data strongly suggest that the maintenance of eCB-LTD in the NAc is caused by the long-lasting inhibition of P/Q-type Ca^{2+} channels.

4. Discussion

The specific purpose of this study was to unveil the mechanisms responsible for the expression of retrograde eCB-LTD at PFC-NAc synapses. The data show that, similarly to mGluR2/3-LTD (Robbe et al., 2002a,b,c), the expression of eCB-LTD requires the inhibition of the cAMP/PKA cascade and of P/Q-type Ca^{2+} channels. The present report is in agreement with our previous report that eCB-LTD and mGluR2/3-LTD mutually occlude (Mato et al., 2005), and supports the idea that both presynaptic mGluR2/3 and CB1R use a common pathway for the induction of LTD in the NAc.

An important finding of this study is that, in contrast to the reversible initial phase of synaptic inhibition elicited by exogenous cannabinoid agonists (Robbe et al., 2001), eCB-LTD at PFC-NAc synapses does not require the activation of K^+

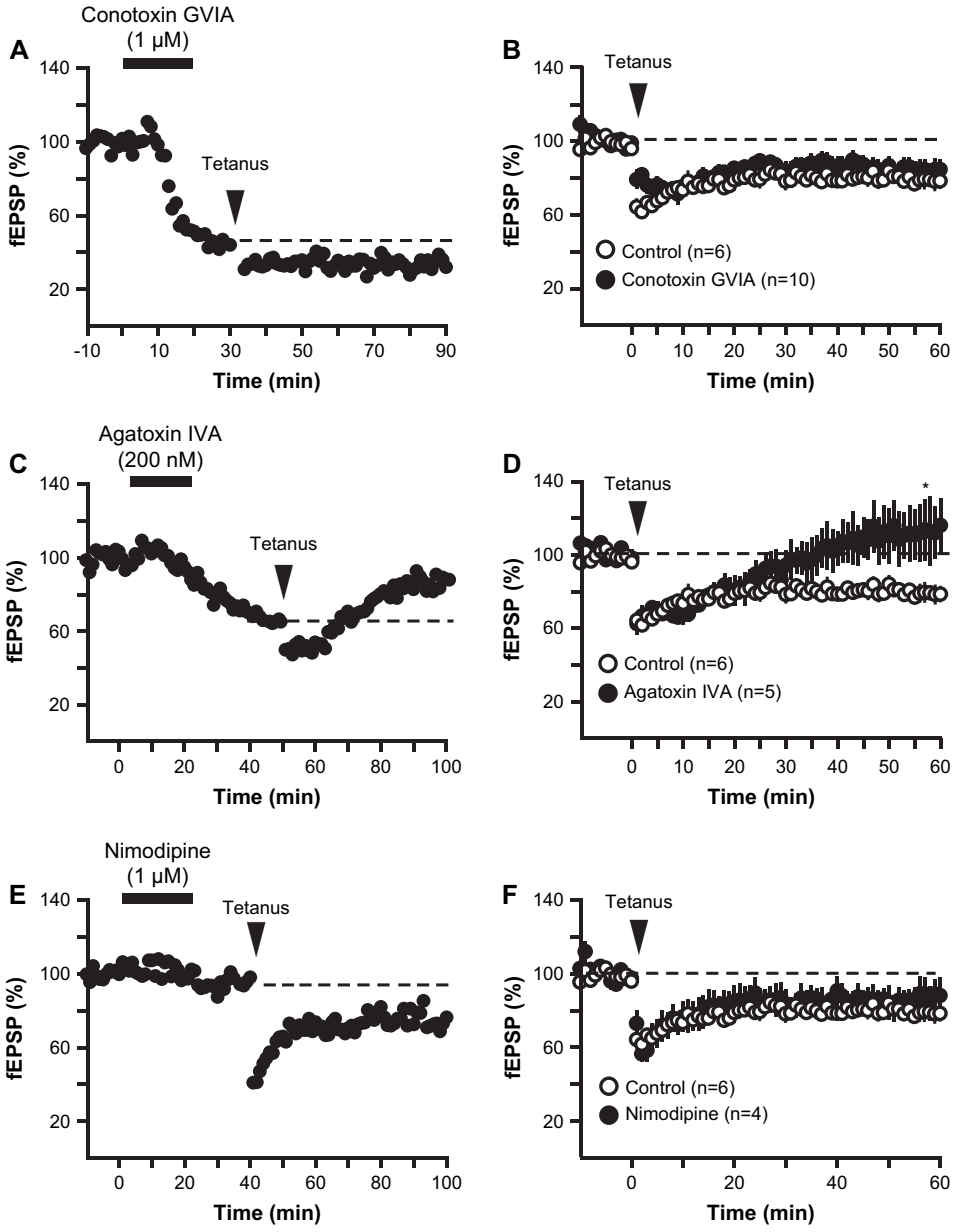

Fig. 3. eCB-LTD is expressed via the selective inhibition of P/Q-type Ca^{2+} channels. (A) Typical experiment in which the slice was tetanized (10 min at 13 Hz at the time indicated by the arrow) after perfusion of ω-conotoxin-GVIA (1 μM). The fraction of excitatory synaptic transmission insensitive to the blockade of N-type Ca^{2+} channels displayed normal eCB-LTD. (B) Summary graph of all the experiments performed with ω-conotoxin-GVIA. (C) Typical experiment in which ω-agatoxin-IVA (200 nM) was perfused before the induction of eCB-LTD. The fraction of synaptic transmission insensitive to the blockade of P/Q-type Ca^{2+} channels did not display LTD. (D) Summary graph of all the experiments performed with ω-agatoxin-IVA. (E) Typical experiment showing that blockade of L-type Ca^{2+} channels by bath application of nimodipine (1 μM) did not prevent from the expression of eCB-LTD. The fraction of synaptic transmission insensitive to the blockade of L-type Ca^{2+} channels displayed normal LTD. (F) Summary graph of all the experiments performed with nimodipine.

conductances. Considering the fact that both the inhibition of transmission mediated by CB1R-agonists and eCB-LTD are absent in CB1R knockout mice (Robbe et al., 2002a,b,c), the most plausible explanation for the present data is that sustained activation of CB1R at PFC-NAc synapses triggers two different transduction pathways: the first one involves activation of K^+ conductances and is responsible for a reversible inhibition of evoked transmission, while the second one implicates the reduction of P/Q-Ca^{2+} currents and underlies eCB-LTD. In agreement with this hypothesis, it is clear from our previous study that the inhibitory effects of the cannabinoid agonist WIN55,212-2 are only partially reversible upon perfusion of the CB1R antagonist SR141716A and that the remaining "irreversible" component is reminiscent of LTD (see Fig. 2A in (Robbe et al., 2001)). Accordingly, we had also observed that although blockade of K^+ channels completely prevents the early/reversible inhibitory effect of exogenous cannabinoid agonist, it does not prevent from a delayed inhibition similar to LTD (see Fig. 7C in (Robbe et al., 2001)). Taken together these data suggest that activation of CB1R induces long-lasting inhibition of evoked transmission independently of the modulation of K^+ currents.

The inhibitory coupling of CB1R to presynaptic VSCCs is well established (Howlett, 2002), and this mechanism has been shown to be involved in eCB-mediated short-term plasticity at both excitatory and inhibitory synapses (Wilson et al., 2001). In the hippocampus, synaptic activation of CB1R mediates short-term inhibition of GABAergic transmission via the direct reduction of N-type Ca^{2+} currents (Wilson et al., 2001), but the possibility that eCB-mediated long-term plasticity at excitatory synapses also involves the modulation of VGCCs has been largely unexplored. Recent work suggest that activation of post-synaptic L-type Ca^{2+} channels in medium spiny neurons is necessary for the release of eCBs and subsequent expression of LTD in the rat striatum (Kreitzer and Malenka, 2005). Our present study shows that activation of L-type Ca^{2+} channels is not required to induce or express eCB-LTD at PFC-NAc inputs but instead suggests the involvement of P/Q-type Ca^{2+} channels as downstream targets of presynaptic CB1R. Thus, all evidences so far point to a prominent role of Ca^{2+} channels in long-term eCB-mediated plasticity, similar to what has been observed with shorter-lasting eCB-mediated plasticities (i.e. Depolarization induced Suppression of Excitation or Inhibition, for review (Chevaleyre et al., 2006)). Whether eCB-mediated LTD observed at other central synapses (Chevaleyre et al., 2006) also involves the long-lasting inhibition of P/Q-type Ca^{2+} channels remains to be determined. In support of this possibility, depressed function of P/Q-type Ca^{2+} but not L- or N-type Ca^{2+} channels has been involved in amphetamine-induced CB1R-dependent LTD in the amygdala (Huang et al., 2003). Additionally, metabotropic glutamate mGlu7 receptor-dependent LTD at mossy fiber synapses onto stratum lucidum interneurons in the hippocampus also requires inhibition of presynaptic P/Q-type Ca^{2+} channels (Pelkey et al., 2006). At the light of previous data (Huang et al., 2003; Pelkey et al., 2006; Robbe et al., 2002a,b,c), the present report strengthens the idea that reduced calcium entry through P/Q-type Ca^{2+} channels plays a prominent role in presynaptically expressed LTD at excitatory synapses.

A possible limitation when interpreting our data showing that both elevation of cAMP and inhibition of PKA abolish eCB-LTD at NAc-PFC synapses is that, although forskolin effect in our preparation is mainly presynaptic (Supplementary Fig. 1A), the PKA inhibitor KT5720 might inhibit eCB-LTD by acting postsynaptically. Nevertheless, this possibility seems unlikely for several reasons. First, consistent data suggest a presynaptic locus of action for PKA inhibitors on glutamatergic transmission. First, previous studies indicate that activation of PKA enhances transmitter release (Chavez-Noriega and Stevens, 1994; Chavis et al., 1998; Trudeau et al., 1996). Second, the PKA inhibitor KT5720 reduces transmission at many different central synapses, including those between PFC and NAc (Robbe et al., 2002a,b,c; Tzounopoulos et al., 1998). Finally, to the best of our knowledge, PKA has not been implicated in postsynaptic mechanisms mediating the synthesis and release machinery of eCBs mediating synaptic plasticity in central synapses. Therefore, the inability to generate eCB-LTD in the presence of forskolin/KT5720 is entirely consistent with the hypothesis that the triggering of LTD requires CB1R-mediated inhibition of cAMP/PKA activity.

Inhibition of VSCCs by receptors coupled to Gi/o proteins can occur via direct action of Gβγ subunits (Herlitze et al., 1996; Ikeda, 1996) or by a second messenger cascade involving reduction of AC activity (Fukuda et al., 1996; Huang et al., 1998). Because eCB-LTD in the NAc is prevented by blockade of the cAMP/PKA transduction pathway as well as by inhibitors of P/Q-type Ca^{2+} channels, it is hypothesized that CB1R-mediated inhibition of cAMP/PKA activity is involved in the reduction of calcium entry through P/Q-type Ca^{2+} channels that leads to LTD. Indeed, both the inhibitory coupling of CB1R to the inhibition of cAMP/PKA cascade (Howlett, 2002; Howlett et al., 2002) and the modulatory role of this transduction pathway on VSCCs, are well established (Arias-Montano et al., 2007; Kaneko et al., 1998; Yang et al., 2001). It can be therefore speculated that CB1R-mediated inhibition of the cAMP/PKA cascade leads to a reduction in the activity of P/Q-type Ca^{2+} channels that triggers LTD. This proposed mechanism is in apparent contrast with a previous report showing that, in the hippocampus, CB1R-mediated DSI of GABAergic transmission involves a direct action of Gβγ subunits on N-type Ca^{2+} channels (Wilson et al., 2001). Collectively, these data suggest that endogenous activation of CB1R can trigger different transduction pathways that ultimately lead to synaptic plasticity via inhibition of various subtypes of VSCCs. Preferential coupling to either transduction pathway may reflect heterogeneity in the release machinery of cannabinoid-sensitive terminals among cell types (GABAergic versus glutamatergic neurons) and brain areas (hippocampus versus NAc), or even a different sensitivity of VSCCs subtypes to the modulation by Gi/o protein-coupled receptors. Indeed, a feature of cannabinoid-sensitive GABAergic inputs in the hippocampus is that they use exclusively N-type Ca^{2+} channels for neurotransmitter release (Wilson et al., 2001), which have been reported to be less susceptible to the cAMP/PKA modulation than Q-type channels (Kaneko et al., 1998). Additionally, different stimulation protocols triggering short- versus long-term synaptic depression might lead to different transductional profiles in response to CB1R activation.

Although the role of protein synthesis participates to certain types of LTD (Huang et al., 2005; Huber et al., 2000), the importance of this process for the expression of eCB-LTD at PFC-NAc synapses is currently unknown. At the rat corticostriatal synapse, the expression of eCB-LTD is independent of transcription in the presynaptic cell but rather depends on local protein translation (Yin et al., 2006). Although the pathway from CB1R activation to local protein synthesis and the identity of the newly-synthesized proteins remain to be clarified, at the light of the present data, it can be hypothesized that, at corticostriatal synapses, inhibition of the cAMP-PKA cascade might be upstream or downstream of the induction of protein synthesis required for eCB-LTD. In support of this latest hypothesis, previous data indicate that inhibition of neurotransmitter release from developing presynaptic terminals may occur by mechanisms requiring ongoing protein synthesis and leading to the inhibition of cAMP-dependent PKA (Ghirardi et al., 2004).

The present data, along with our previous demonstration that mGluR2/3-LTD involves inhibition of both P/Q-type Ca^{2+} channels and the cAMP/PKA cascade (Robbe et al., 2002a,b,c) suggest a prominent role of this signaling pathway in the long-term inhibitory regulation of PFC-NAc inputs. Available data indicate that these two forms of LTD are not induced in response to the same pattern of synaptic activity (Mato et al., 2005; Robbe et al., 2002a,b,c). Thus, the coexistence of both mGluR2/3 and CB1R coupled to the same effectors on these terminals may allow the induction of LTD in response to a wide range of cortical activity. Noteworthy, neuroadaptations affecting the regulation of glutamate release at PFC-NAc inputs are believed to play an important role in the development and expression of addictive behaviours. Changes in PFC-NAc glutamate transmission following chronic cocaine administration include reduced basal levels of extracellular glutamate (Pierce et al., 1996), as well as increased release associated with the reinstatement of drug seeking (McFarland et al., 2003). Additionally, both CB1R- and mGluR2/3-dependent LTDs in the NAc seem to be affected by acute and/or chronic administration of drugs of abuse: CB1R-dependent LTD is blocked by a single in vivo exposure to Δ^9-THC or to cocaine (Fourgeaud et al., 2004; Mato et al., 2004), and mGluR2/3-dependent LTD is abolished after one week withdrawal from chronic morphine treatment (Robbe et al., 2002a,b,c). Furthermore, experimental data suggest that both CB1R and mGluR2/3 might be useful targets for the development of anti-relapse medication. Restoration of mGluR2/3-dependent inhibition of glutamate release at PFC-NAc synapses inhibits the reinstatement of cocaine- and heroin-seeking (Bossert et al., 2006; Moran et al., 2005). By contrast, both systemic CB1R blockade and local antagonism of CB1R in the NAc attenuate relapse to cocaine seeking (De Vries et al., 2001; Moran et al., 2005). Thus, understanding the mechanisms by which CB1R inhibit glutamate release at PFC-NAc synapses and how these mechanisms are regulated by chronic administration of abused drugs may help clarify the biological basis of addiction, as well as the potential use of cannabinoid antagonist for the prevention of relapse to drug seeking. In this sense, recent data suggest that CB1R tonically inhibit glutamate release at PFC-NAc synapses under cocaine-extinction conditions (Moran et al., 2005). The increase in NAc glutamate secondary to CB1R blockade is believed to activate presynaptic mGluR2/3 that subsequently inhibit cocaine-enhanced glutamate release and reinstatement of drug seeking. At the light of the present data, it will be of interest to clarify if, reminiscent of eCB- and mGluR2/3-dependent LTD, tonic CB1R-mediated inhibition of glutamate release following chronic cocaine administration also involves the reduction of the cAMP/PKA cascade and P/Q-type Ca^{2+} channels function.

Acknowledgments

Work in O.J.M.'s laboratory is supported by grants from INSERM, Région Aquitaine and "ANR 2006 Neurosciences, Neurologie et Psychiatrie n° RPV06092GSA".

Appendix A. Supplementary information

Supplementary figure for this manuscript can be downloaded at doi: 10.1016/j.neuropharm.2007.04.014.

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