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GDPβS Activates Excitatory Synapses in CA1 Pyramidal Cells by Disinhibiting the PKA Activating Pathway

Mohamed Ouardouz*

NRAC, Gatineau, Quebec, Canada

Abstract

Objective: During the early postnatal brain developmental stages, excitatory synapses mediated by AMPA receptors are weak or silent. Activity-dependent insertion of AMPA receptors into synapses depends on the activation of protein kinase A. In this work, we investigated the effect of Guanosine 5'-[βthiol] diphosphate ($GDP_{\beta S}$) on excitatory and inhibitory synaptic currents in CA1 pyramidal cells at postnatal days 9-12.

Methods: Whole-cell patch-clamp recordings from identified hippocampal CA1 pyramidal cells were used. $GDP_{\beta S}$ Was applied through the recording electrode.

Results: $GDP_{\beta S}$ induces an increase in excitatory synaptic current amplitude, but not in the inhibitory synaptic current amplitude. An analysis of the change in excitatory synaptic current amplitude in the presence of $GDP_{\beta S}$ revealed a progressive increase, which is blocked by the protein kinase A inhibitor Rp-3',5'-cyclic monophosphothioatetriethyl-amine (Rp-cAMP), suggesting that $GDP_{\beta S}$ inhibits G-protein with a tonic negative control on a protein kinase A activating pathway. In addition, $GDP_{\beta S}$ has no effect on paired-pulse facilitation, suggesting that the glutamate release machinery is not affected. Moreover, as $GDP_{\beta S}$ was applied to postsynaptic neurons, the increase in excitatory postsynaptic current amplitude is related to changes at the postsynaptic side.

Conclusion: Those results suggest that in developing hippocampal CA1 pyramidal cells, the tonic inhibition of a protein kinase A activating pathway by a G-protein prevents the activation of excitatory synapses.

Keywords: Hippocampus; EPSCs; Rat; Development; G-protein; Protein kinase A

Introduction

Excitatory synaptic transmission in the central nervous system is mainly mediated through the activation of glutamate receptors. In the early development of the rodent hippocampus, excitatory synapses contain mostly N-methyl-D-aspartate(NMDA) receptors, lack α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and are considered silent synapses as they are inactive at the resting membrane potential [1-4]. These synapses can be turned on by the insertion of new AMPA receptors [5-11]. The cascade of events that regulates AMPA receptor insertion into synapses requires phosphorylation by protein kinase A (PKA) [8]. In addition, PKA activation drives AMPA receptors into synapses [6-8,10,12,13].

The mechanisms by which AMPA receptors are prevented from inserting into synapses during the early developmental stage remain unclear. Since the insertion of AMPA receptors depends on phosphorylation by PKA, PKA is possibly inactive at silent synapse sites, thereby preventing the insertion of new receptors at these sites. In the present work, the effect of $GDP_{\beta S}$ G-proteins inhibitor on evoked excitatory postsynaptic currents (EPSCs) in CA1 hippocampal pyramidal cells is investigated. The results suggest the involvement of G-proteins in inhibiting the PKA activating pathway involved in the activation of silent or weak synapses.

Materials and Methods

Male Wistar rat pups (from different litters, n = 18) were obtained from the animal care unit at the University of British Columbia on postnatal day 9-12. Pups were kept with their mother (standard cages) in a 12-hour light/dark cycle with freely available food and water. Animal care and use conformed to the guidelines and the policies of the Animal Care Committee atThe University of British Columbia.

Hippocampal slices (400 µm) were prepared from male Wistar rat (9-12 days old) as previously described [14]. The rat pups were anesthetised using isofluorane (Sigma aldrich) then decapitated, and the brain was quickly removed and placed in cold artificial cerebrospinal fluid containing the following: 120 mM NaCl, 3 mM KCl, 1.8 mM NaH_2PO_4, 2 mM $MgSO_4$, 2 Mm CaCl, 26 mM $NaHCO_3$ and 10 mM dextrose; the fluid was also saturated with 95% O_2 and 5% CO_2. Hippocampal slices were cut in cold ACSF with a vibrating blade microtome Leica (Leica Microsystems) and transferred into a container filled with oxygenated ACSF at room temperature. After an incubation period of 1 h, a slice was placed into a recording chamber mounted in an upright microscope (Zeiss), and continuously perfused with oxygenated ACSF.

Electrophysiological recordings were made in a whole-cell patch-clamp using an Axopatch 200A (Molecular Devices, Foster City, CA, USA). The recording pipette contained the following: 145 mM K-gluconate, 10 mM HEPES, 10 mMKCl, 1 mM K4-bis-(2-aminophenoxy)-N,N,N`,N`-tetra acetic acid (BAPTA, Sigma Aldrich), 5 mM Mg-ATP, 0.1 mM CaCl2, 0.4 mM Na3-GTP. The pH was adjusted to 7.2-7.3 with KOH. Na3-GTP was replaced by $GDP_{\beta S}$ (Sigma Aldrich) to assess the role of the G-protein on synaptic responses. Recordings were acquired at 5 KHz and filtered

***Corresponding author:** Mohamed Ouardouz, NRAC, 39 du météore Gatineau, Quebec, J9A 3B5, Canada, E-mail: ouardouz@bell.net

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at 2 KHz and digitized using digidata 1300 and windows based programme Pclamp 9 (molecular devices). Series resistance and holding currents were monitored during all recordings.

Inhibitory postsynaptic currents were evoked in the presence of of 6,7-dinitroquinoxaline-2,3-dione(DNQX, 10 μM, Tocris) and (+2)-amino-5-phosphonovaleric acid (APV, 50 μM, Tocris). Excitatory synaptic currents were evoked by electrical stimulation using a bipolar electrode placed in stratum radiatum. The connection between CA3 and CA1 was cut.

$GABA_B$ receptor-mediated postsynaptic currents were evoked by a train of 4 pulses (0.1-0.2ms, 200-400 μA) at 200 Hz applied in stratum radiatum in the presence of ionotropic glutamate receptor antagonists, DNQX, (10 μM), APV (50 μM) and $GABA_A$-receptor antagonist: bicuculline methiodide (30 μM, Tocris).

Data are presented as mean + sem and were analysed using Student's t-test and paired t-test (Microsoft Excel); n represents the number of cells. The difference between groups is considered significant if $p<0.05$.

Results

Experiments were performed in visually identified pyramidal cells in the CA1 area of the hippocampus using an upright Zeiss microscope. To verify that $GDP_{\beta S}$ applied through the patch pipette, blocks G-proteins, we tested its effect on $GABA_B$ receptor, a member of G-protein activated receptors. Electrical stimulation of stratum radiatum in the presence of DNQX (10 μM), AP-5 (50 μM) and bicucculine (30 μM) evoked $GABA_B$ inhibitory synaptic potentials in older animals (3 weeks). In the presence of $GDP_{\beta S}$ in the patch solution, the amplitude of $GABA_B$ IPSPs significantly decreased with time (to 37.2 ± 6.4 % after 10 min of recording; n = 5, P = 0.004; Figure 1). In contrast, $GDP_{\beta S}$ had no effect on $GABA_A$ inhibitory synaptic currents (IPSC) (Figure. 2B). $GABA_A$ IPSCs were evoked via electrical stimulation in the stratum radiatum in the presence DNQX, 10 μM) and APV, 50 μM) at -30 mV. The mean amplitude of the IPSCs is not significantly affected by $GDP_{\beta S}$ (53.8 ± 3.5 pA after breaking into the cell and 46.7 ± 3.4 pA after 10 minutes of recording (Figure. 2B, n = 3, p = 0.12, paired t-test).

Excitatory synaptic currents were evoked in the absence of DNQX and APV at -70 mV (Figure 2). The mean amplitude of EPSCs significantly increased from 98.2 ± 9.6 pA after breaking into the cell to 230.5 ± 30.2 pA after 10 minutes of recordings (Figure 2C-D, n = 17, p = 0.00003, paired t-test). The mean amplitude of EPSCs significantly increased with time with $GDP_{\beta S}$ in the recording solution as compared to the control solution (Figure 2). Under the control conditions, the increase in mean EPSC amplitude is not statistically significant (i.e., 114.3 ± 14.4 pA after breaking into the cell and 154.4 ± 30.7 pA after 10 minutes of recording; n = 14, p = 0.5, paired t-test). This increase in EPSC amplitude induced by $GDP_{\beta S}$ is not accompanied by changes in EPSC kinetics. The EPSC rise time and decay time are not affected by $GDP_{\beta S}$ (i.e., the rise time after breaking into the cell was 4.9 ± 0.5 ms, and the rise time after 10 minutes of recording in the presence of $GDP_{\beta S}$ was 4.2 ± 0.3 ms, p = 0.1, paired t-test; the EPSC decay time after breaking into the cell was 57.0 ± 6.5 ms, and the decay time after 10 minutes of recording in the presence of $GDP_{\beta S}$ was 51.9 ± 3.2 ms, p = 0.5, paired t-test).

$GDP_{\beta S}$ is known to block all G-proteins, including the ones that inhibit the production of second messengers, like cyclic adenosine mono-phosphate (cAMP) (e.g., Gi-proteins), which activate PKA. PKA is known to modulate AMPA receptors [15,16]. To test the involvement of PKA in EPSC potentiation by $GDP_{\beta S}$, we used Rp-3',5'-cyclic monophosphothioatetriethyl-amine (Rp-cAMP), an analogue of cAMP with a selective and competitive antagonist effect on PKA [17]. The application of Rp-cAMP (100 μM, in the recording solution) completely blocks changes in EPSC amplitude induced by $GDP_{\beta S}$ (i.e., 103.8 ± 41.5 pA after breaking into the cell and 99.3 ± 38.1 pA after 10 minutes of recording; n = 5, p = 0.65, paired t-test)

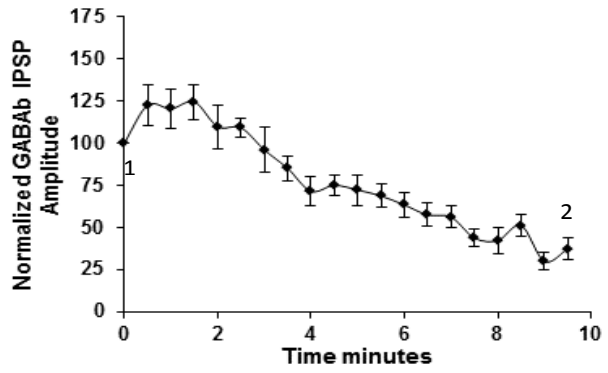

Figure 1: $GDP_{\beta S}$ blocks $GABA_B$ receptor-mediated inhibitory postsynaptic potentials. $GABA_B$ receptor mediated synaptic potentials (IPSPs) were evoked by a train of electrical stimulations (4 pulses at 200Hz). $GDP_{\beta S}$ Applied through the recording electrode induces a progressive reduction of $GABA_B$ IPSPs.

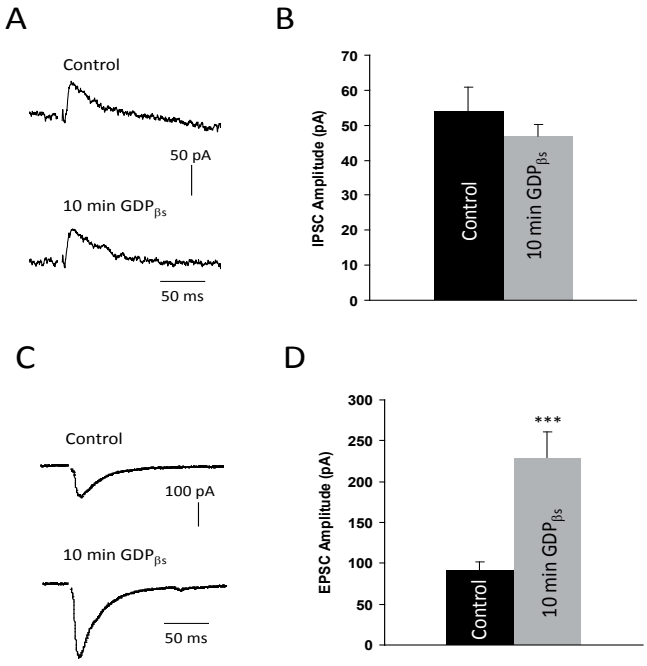

Figure 2: $GDP_{\beta S}$ induces an increase in excitatory postsynaptic current (EPSC) amplitude but does not affect inhibitory synaptic currents (IPSC) amplitude. A: Representative traces of IPSCs recorded at -30 mV just after breaking into the cell (i.e., the control) and after 10 minutes of recording in the presence of Guanosine 5'-[βthiol]diphophate ($GDP_{\beta S}$, 10 minutes $GDP_{\beta S}$). B: Data summary histogram showing no significant effect of $GDP_{\beta S}$ on IPSC amplitude. C: Representative traces of EPSCs recorded at -70 mV just after breaking into the cell (i.e., the control) and after 10 minutes of recording in the presence of $GDP_{\beta S}$ (10 minutes $GDP_{\beta S}$). D: Data summary histogram showing a significant increase in EPSC amplitude by $GDP_{\beta S}$. Error bar represent the sem, *** statically significant P<0.001).

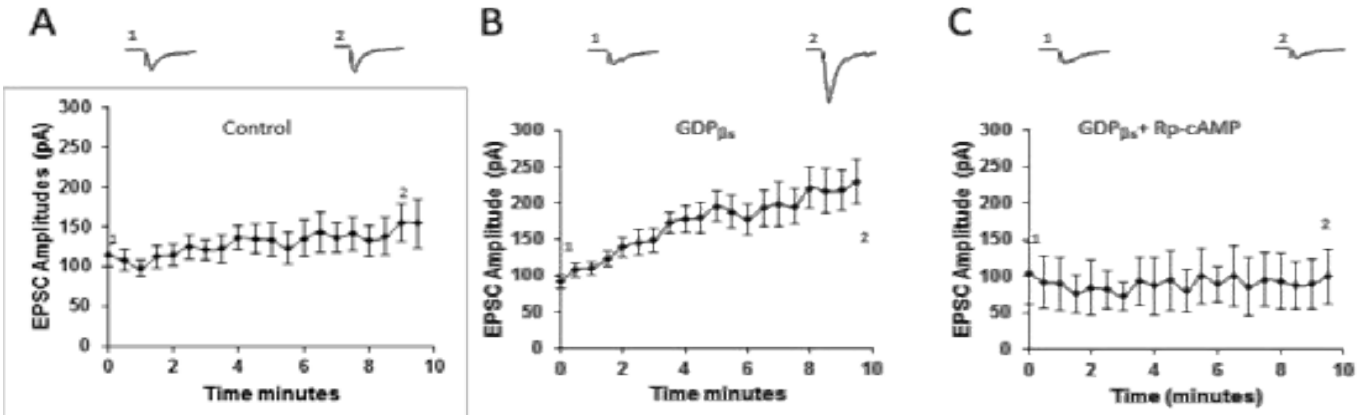

Figure 3: Guanosine 5'-[βthiol]diphophate ($GDP_{\beta S}$) induced potentiation of the excitatory postsynaptic current (EPSC) amplitude is blocked by protein kinase A (PKA) inhibitor Rp-3',5'-cyclic monophosphothioate triethyl- amine (Rp-cAMP). A: Graph of EPSC amplitude during the first 10 minutes of recording showing no significant change in the amplitude with time. Insets are representative traces of EPSCs in the beginning (1) and after 10 minutes (2) of recording. B: Graph of the amplitude of EPSC recorded with a solution containing $GDP_{\beta S}$ versus time, revealing a significant increase in EPSC amplitude in the presence of $GDP_{\beta S}$. Insets in the top are representative traces of EPSCs in the beginning (1) and after 10 minutes (2) of recording. C: Rp-cAMP antagonises the effect of $GDP_{\beta S}$, and the EPSC amplitude change with time that occurs without Rp-cAMP does not occur in the presence of the $GDP_{\beta S}$. Insets in the top are representative traces of EPSCs in the beginning (1) and after 10 minutes (2) of recordings. Error bar represent the sem.

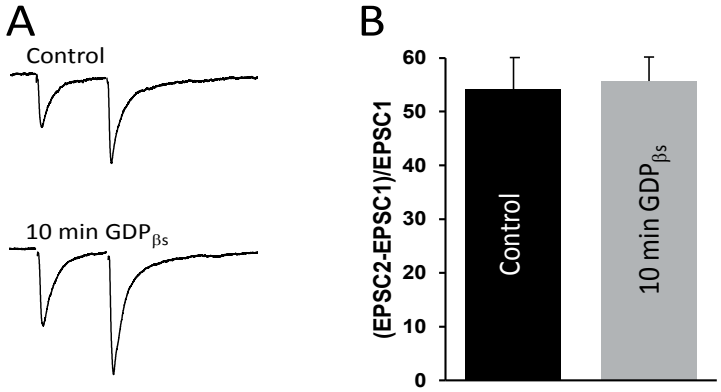

Figure 4: Guanosine 5'-[βthiol]diphophate ($GDP_{\beta S}$) applied through the recording pipette had no effect on paired pulse facilitation of excitatory postsynaptic currents (EPSCs). A: Paired EPSCs evoked at 100 ms intervals resulted in an increase of the second EPSC amplitude as compared to the first one in control condition. B: Paired EPSCs evoked at 100 ms intervals after 10 minutes of recording in the presence of $GDP_{\beta S}$. C: Summary histogram showing the % increase of EPSC2 amplitude as compared to EPSC1 in the control and in the presence of $GDP_{\beta S}$. Error bar represent the sem.

(Figure 3). These results suggest that $GDP_{\beta S}$ blocks a Gi-protein that inhibits adenylatecyclase and cAMP production. The disinhibition of adenylatecyclase increases the production of cAMP and the activation of PKA, which potentiates EPSCs.

To further characterize the mechanisms by which $GDP_{\beta S}$ induces an increase in EPSC amplitude, we analysed the changes in spontaneous EPSC amplitude and frequency between the first minute after breaking into the cell and the eighth to tenth minute of recordings. As reflected by the inter-event interval, the frequency of spontaneous EPSCs significantly increased with time in the presence of $GDP_{\beta S}$ (i.e., inter-event interval: 3.98 ± 0.45 s during the first min after breaking into the cell and 1.91 ± 0.31 s during the eighth to tenth minute of recordings; n = 4; p = 0.0057 in a paired t-test). Additionally, the spontaneous EPSC amplitude did not significantly change (i.e., 4.7 ± 0.3 pA during the first minute of recording, and 4.3 ± 0.5 pA during the eighth to tenth minute of recordings; n = 4, p = 0.5 in a paired t-test) (Figure 4). The increase in spontaneous EPSC frequency may be due to changes in the probability of glutamate release from the presynaptic terminal.

To assess the effect of $GDP_{\beta S}$ even if it was applied via the recording pipette on glutamate release, we used paired pulse stimulation at 100 and 200 ms intervals. Under the control conditions, the amplitude of the second EPSC increases by 39.2 ± 4.8 % (n = 12, at 200 ms interval) and as compared to the amplitude of the first EPSC. After 10 minutes of recording in the presence of $GDP_{\beta S}$, the percentage increase in the amplitude of the second EPSC as compared to the first is not statistically different from that of the control (27.2 ± 3.5 %; n = 13, p>0.05 at 200 ms interval), thereby suggesting that $GDP_{\beta S}$ does not affect the probability of glutamate release.

Discussion

Silent excitatory glutamatergic synapses lacking AMPA receptors are present in CA1 pyramidal cells during the first postnatal days [1-4]. In this study, we report that $GDP_{\beta S}$, which is known to block G-protein activity, potentiates the evoked EPSCs and the effect is blocked by the PKA inhibitor Rp-cAMP.

The increase in evoked EPSC amplitude by $GDP_{\beta S}$ depends on

PKA activation as the application of its inhibitor Rp-cAMP reverses the effect of $GDP_{\beta S}$. The G-proteins involved in the activation of adenylatecyclase are named Gs, and the one involved in its inhibition are named Gi. $GDP_{\beta S}$ inhibits both Gi and Gs-proteins [18-21]. The inhibition by $GDP_{\beta S}$ of Gi-proteins will remove the negative control over adenylatecyclase and increase the production of cAMP, hence increasing the activity of PKA. Evoked EPSCs were recorded at a holding membrane potential of -70 mV and are mainly mediated by AMPA receptors since most NMDA receptors are inactive at this membrane potential. The increase in evoked EPSC amplitude by $GDP_{\beta S}$ is mostly due to the modulation of AMPA receptors. In fact, previous studies have shown that an increase in the activity of PKA modulates AMPA receptors mediated current [15,16] and plays an important role in AMPA receptor insertion [6-8,13,22-24].

The increase in EPSC amplitude by $GDP_{\beta S}$ is accompanied by an increase in spontaneous EPSC frequency without a change in the spontaneous EPSC amplitude. The increase in spontaneous EPSC frequency may be due to a change in the probability of glutamate release at the presynaptic terminals. Under our experimental conditions, the probability of glutamate release is not affected by $GDP_{\beta S}$ in the recording solution as revealed by the lack of a $GDP_{\beta S}$ effect in the paired pulse facilitation of EPSCs. In addition, $GDP_{\beta S}$ was applied into the postsynaptic cell. Therefore, the change in evoked EPSC amplitude and spontaneous EPSC frequency is likely due to a change on the postsynaptic side. The most probable scenario is the insertion of AMPA receptors and the activation of silent synapse as described for long-term potentiation in CA1 pyramidal cells [5,9,22-24]. $GDP_{\beta S}$ induces an increase in evoked EPSC amplitude via the activation of silent synapses through the insertion of AMPA receptors after phosphorylation by PKA.

In conclusion, our data highlighted the presence of a Gi-protein-dependent system that prevents the activation of excitatory synapses though the modulation of PKA activity. This may be done through the inhibition of adenylatecyclase, which produces cAMP; by the activation of Gi-protein dependent receptors. It will be interesting to determine which of those systems is involved in the sustained inhibition of the PKA activation pathway and hindering the insertion of AMPA receptors into synapses.

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