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Induction of Depolarization-Induced Suppression of IPSP by Adenosine and Cannabinoids in The Rat Nucleus Accumbens

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ABSTRACT

A short period of depolarization evokes transient suppression of neurotransmitter release (Depolarization-induced Suppression of IPSP: DSI). Recent studies suggest that cannabinoids are candidates for the retrograde messenger required to induce DSI in the hippocampal CA1 area. A G protein-coupled process and inhibition of N-type calcium channels at the presynaptic terminals are believed to be involved in the induction mechanism. We examined DSI induction in the nucleus accumbens in which inputs from multiple sites in the telencephalon, including the hippocampus and basolateral amygdala, converge. Using whole-cell recording from a horizontal slice preparation of nucleus accumbens from the rat brain, we monitored the effect of endogenous cannabinoids on DSI induction and found that 300 nM AM281, a selective cannabinoid CB1 receptor antagonist, resulted in blockade of DSI. Adenosine is reported to suppress neurotransmitter release by inhibiting N-type calcium channels, which is a similar mechanism to that reported for cannabinoid-induced DSI. We therefore examined the effect of endogenous adenosine on DSI induction using DPCPX, a selective A1 receptor antagonist, and found that DSI induction was blocked in the presence of 100 nM DPCPX. These results suggest that cooperative activation of CB1 and A1 receptors contributes to DSI induction in the rat nucleus accumbens.

Key words : Nucleus accumbens, DSI, Cannabinoid, Adenosine, Whole-cell recording,

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INTRODUCTION

Depolarization-induced suppression of excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs), DSE and DSI respectively, are recently reported short-term plasticities of the synaptic response[1]. In pathological conditions, such as ischemia, depression of presynaptic activity induced by depolarization of the postsynaptic cell membrane could help cell survival by decreasing glutamate-induced neurotoxicity[2]. In the other case, for instance during an epileptic seizure, cells at the focus are simultaneously depolarized and the subsequent increase in extracellular K^+ facilitates the depolarization of neighboring cells. Under such a pathological condition, DSI could prevent excess excitability of the tissue by causing a transient decrease in neurotransmitter release. In physiological conditions, DSI could contribute to the precise regulation of synaptic responses and synaptic plasticity by acting as a negative feedback mechanism.

The mechanism for DSI induction has been mainly investigated in the hippocampus, in which there is abundant information underlying synaptic plasticity[3]. Involvement of cannabinoid as a retrograde signal to induce DSI/DSE in the hippocampus and cerebellum has been recently investigated and it has been demonstrated that cannabinoid induces DSI/DSE by the activation of G-protein and the block of N-type voltage-dependent calcium channels (VDCCs) at the presynaptic sites.

It has been known that adenosine has a protective role on the cell survival in the pathological condition by activating G-protein and blocking of VDCCs. In the present study, on the analogy of the role of adenosine with that of cannabinoid, we examined the role of adenosine on the DSI/DSE. We investigated DSI induction in the nucleus accumbens (NAc), that has a critical role on the control of behavior by precise regulation of synaptic responses from the limbic system, cortex and amygdala[4], since cannabinoid binding sites are abundant in this region of the brain[5].

MATERIALS AND MOTHODS

All experiments were performed according to the guidelines laid down by the Animal Welfare Committee of Kochi Medical School. Slices of the NAc were prepared from young adult SD rats of both sexes (30- to 38-days-old). The rats were anesthetized with diethyl ether, decapitated, and the brain was quickly removed and rinsed with partially frozen solution consisting of (in mM): 124 NaCl, 3.0 KCl, 0.5 $CaCl_2$, 4.0 $MgSO_4$, 1.25 NaH_2PO_4, 22.0 $NaHCO_3$, 10 glucose, pH 7.2-7.3, bubbled with 95% O_2, and 5% CO_2, then horizontal slices (500 μm thick) were prepared using a rotary tissue slicer (Dosaka, Japan; DK-7700). The slices were transferred to an incubation chamber containing extracellular solution consisting of (in mM): 124 NaCl, 3.0 KCl, 2.0 $CaCl_2$, 2.0 $MgSO_4$, 1.25 NaH_2PO_4, 22.0 $NaHCO_3$, 10 glucose, bubbled with 95% O_2 and 5% CO_2, and maintained at room temperature (25±1℃) for at least 2 hours. For each experiment, a slice was transferred to a submersion recording chamber and continuously perfused with extracellular solution (2 mL/min) saturated with 95% O_2 and 5% CO_2.

The patch clamp technique for thick slices (the ‘blind patch’technique) was employed using a low magnification microscope.

Inhibitory postsynaptic currents (IPSCs) were measured using whole-cell recording in the voltage clamp configuration. Glass patch pipettes with input resistances of approximately 4-5 MΩ were made using a puller (P-97, SUTTER Instrument Co., Novato, CA). The internal solution for the recording pipette consisted of (in mM): 115 $CsCl_2$, 1.0 ethylene glycol-bis(2-aminoethyl)-N,N,N′, N′-tetraacetic acid (EGTA), 10 HEPES, 1.0 NaCl, 2.0 $MgCl_2$, 2.0 ATP, 0.3 GTP, with the pH adjusted to 7.25 using KOH. The osmolality of the internal solution was adjusted to 280 mosm. To induce DSI, a depolarizing step current from −80 to 0 mV lasting 10 sec was applied in the voltage clamp configuration through the patch pipette. The series resistance, normally about 12-20 MΩ, was monitored throughout the experiment; if it changed more than 15% from the original value, the recording was discarded. The current was recorded using an amplifier (Axopatch 200B, Axon Instrument, Foster City, CA) with a 2 kHz low-pass filter. Data were presented as mean ±S.E. Statistical significance of data was evaluated by Mann-Whitney non-parametric test. Differences were considered significant when p values were less than 0.05.

RESULTS

We first examined whether DSI could be evoked in the NAc using the protocol used in the hippocampus[1]. The recording pipette was located in the core of the NAc close to the commissural fibers, and the bipolar concentric tungsten-wire stimulating electrode, with a tip diameter of approximately 25 μm (Rhod Instruments, CA), was located 300-500 μm apart (Fig. 1).

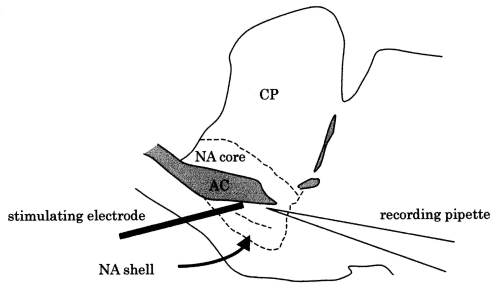

Fig. 1.
Schematic drawing of the experimental arrangement of on a nucleus accumbens slice.
Positions of the stimulating electrode and the recording electrode are indicated in the figure. AC: anterior commissure; CP: caudate putamen; NA core: nucleus accumbens core; NA shell: nucleus accumbens shell.

Monosynaptic IPSCs were evoked continuously with 0.33 Hz stimuli in the presence of 20 μM CNQX in the voltage clamp configuration at −80 mV. IPSC suppression was quantified as the percentage reduction in the mean amplitude of five consecutive IPSPs immediately after depolarization to the baseline responses. The first recording of IPSP after depolarization was not included in the calculation. DSI was successfully induced in the NAc with a time-course similar to that reported in the hippocampus[1] (Fig. 2, 63.7±6.2%, n=7).

We then examined the effect of cannabinoids on the DSI using the CB1 receptor antagonist, AM281. A stock solution of 300 μM AM281 in DMSO was prepared, then diluted in the recording solution immediately before the experiment. DSI was not evoked in the presence of 300 nM AM281 (Fig. 3A, inhibition: 5.8±3.1%, n=5), suggesting that the induction mechanism in the NAc was similar to that in the hippocampus[6]. To verify that CB1 receptor activation contributed to the suppression of IPSCs in the NAc, we applied a

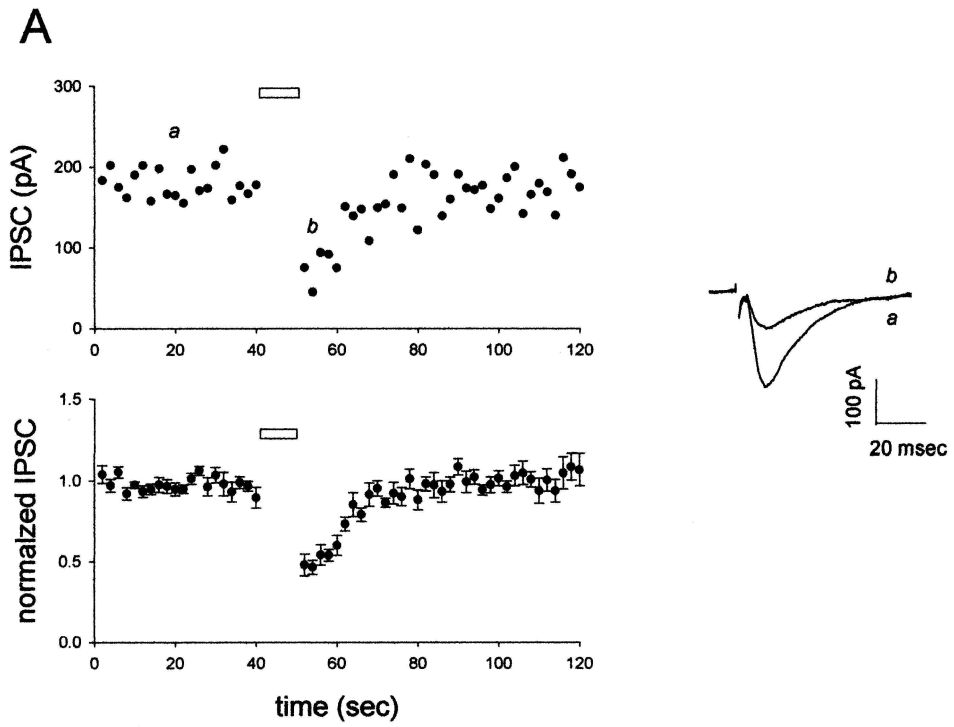

Fig. 2.
DSI is induced in the nucleus accumbens.
Whole cell IPSPs were monitored in the presence of 20 μM CNQX. A depolarization pulse was applied at the time shown by the horizontal bar. The left upper panel shows a typical example of DSI, while the left lower panel shows the averaged DSI (n=8). Representative traces obtained at times *a* and *b* indicated in the left upper panel are shown in the right panel. The scales are shown as an inset.

CB1 receptor-selective agonist, WIN55,212-2 (100 nM), and found that the IPSCs were significantly eliminated (Fig. 3B).

Adenosine is a tonically released endogenous agent which suppresses neurotransmitter release by activating presynaptic A1 receptors[7]. We used the adenosine A1 receptor antagonist, DPCPX, to examine the effect of endogenous adenosine on DSI induction. DSI was almost completely blocked in the presence of 100 nM DPCPX (Fig. 4A, 19.6±5.0%, n=6), suggesting that A1 receptor activity also contributed to DSI induction in the NAc. We further investigated the effects of the A1 agonist on the synaptic response in the NAc and found that addition of 50 nM CHA resulted in remarkable blockade of the synaptic responses (Fig. 4B), suggesting that A1 receptor activity modulates transsynaptic responses in the Nac, presumably by affecting transmitter release. The quantified amplitude of DSI in the presence of DPCX and AM281 are summarized in Fig. 4C.

DISCUSSIONS

The mechanism underlying DSI induction has been extensively investigated in the

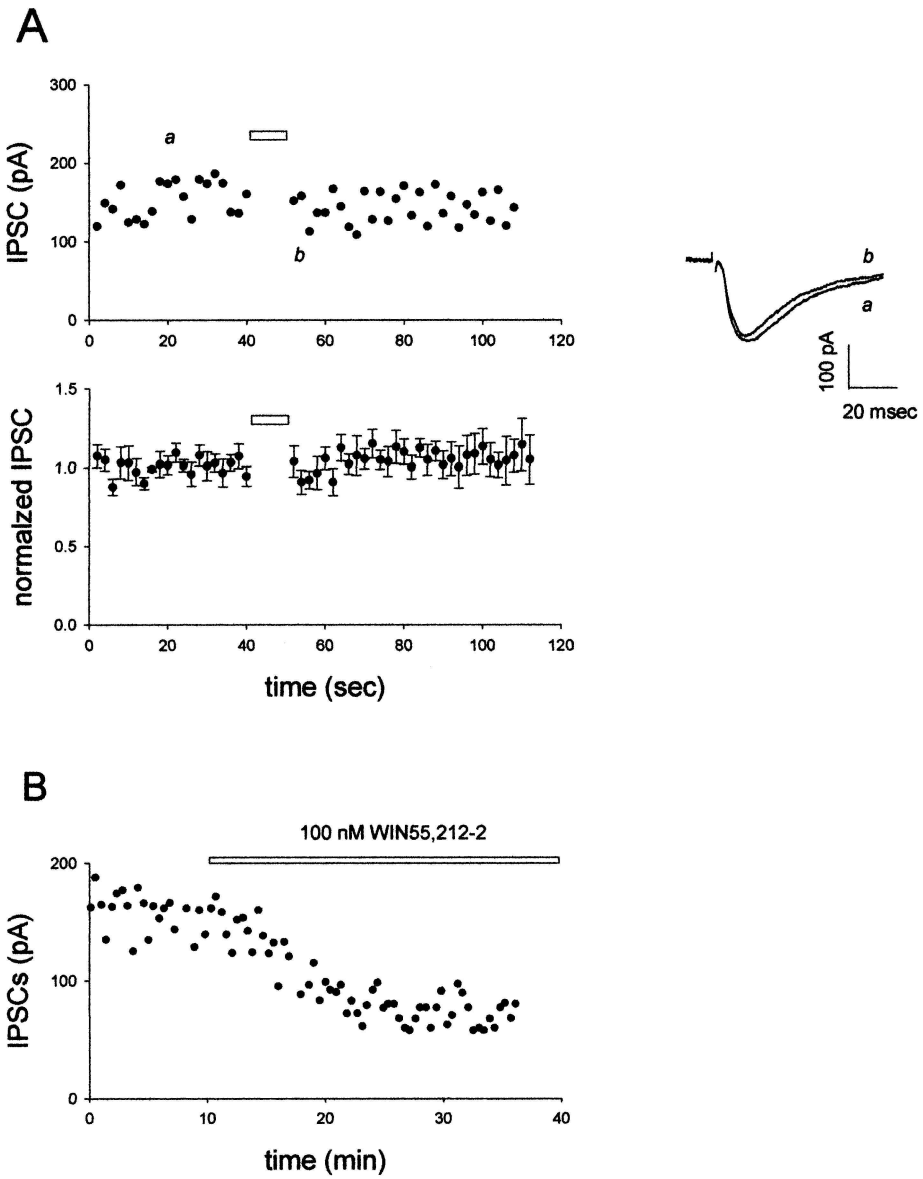

Fig. 3.
DSI is suppressed in the presence of a cannabinoid CB1 receptor antagonist.
(A) DSI was monitored in the continuous presence of the CB1 receptor antagonist, AM281 (300 nM). A depolarization pulse was applied at the time shown by the horizontal bar. The left upper panel shows a typical example of DSI, while the left lower panel shows the averaged DSI (n=5). Representative traces obtained at time *a* and *b* indicated in the left upper panel are shown in the right panel. The scales are shown as an inset. (B) The CB1 receptor agonist, WIN55,212-2 (100 nM), was administered at the time shown by the horizontal bar.

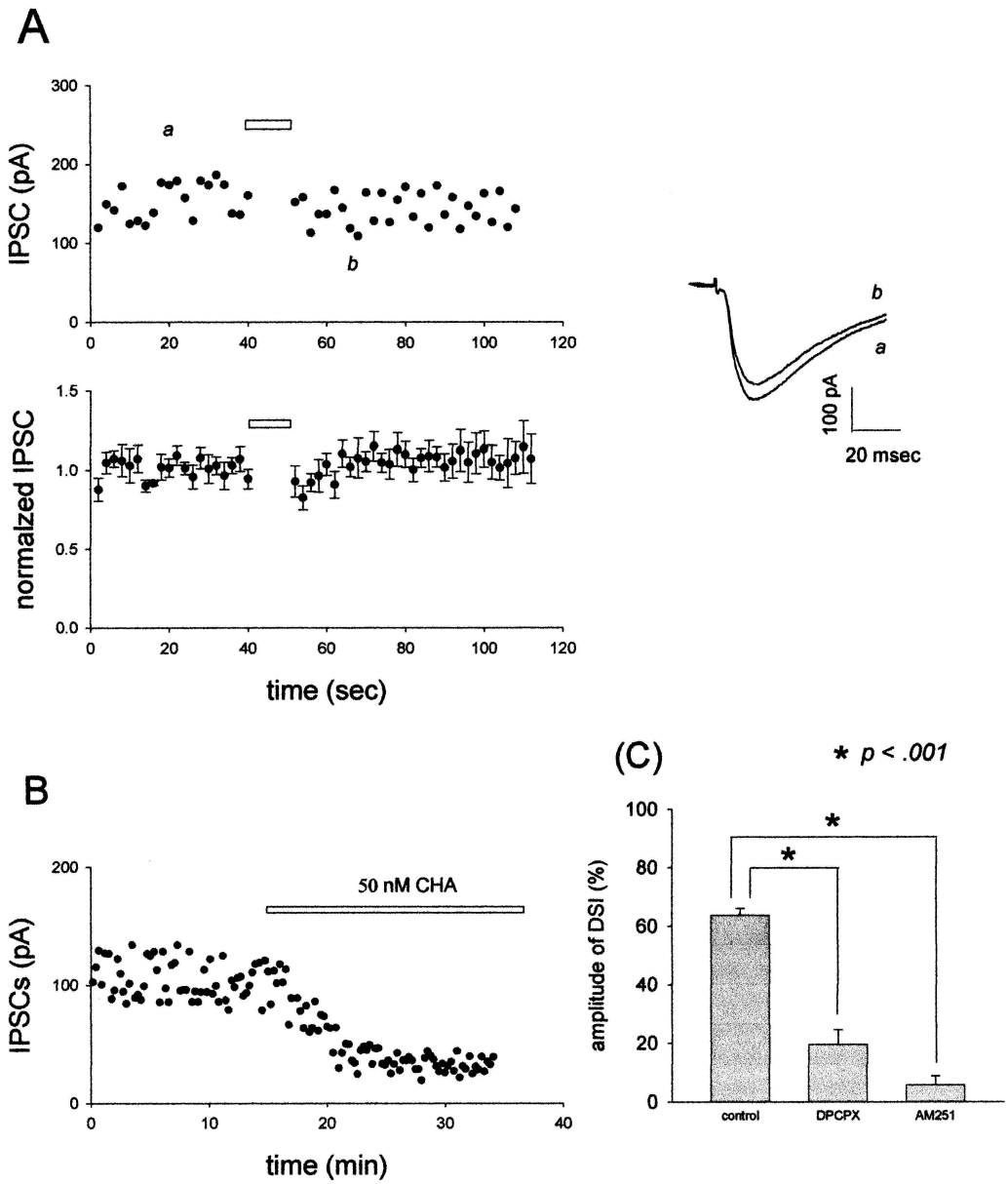

Fig. 4.

DSI is suppressed in the presence of an adenosine A1 receptor antagonist.

(A) DSI was monitored in the continuous presence of the A1 receptor antagonist, DPCPX (100 nM). A depolarization pulse was applied at the time shown by the horizontal bar. The left upper panel shows a typical example of DSI, while the left lower panel shows the averaged DSI (n=6). Representative traces obtained at times a and b indicated in the left upper panel are shown in the right panel. The scales are shown as an inset. (B) The A1 receptor agonist, CHA (50 nM), was administered at the time shown by the horizontal bar. (C) The vertical bars indicate mean ±S.E. of the magnitude of DSI. The DSI amplitude in the presence of DPCPX or AM281 was significantly reduced compared to the control DSI (Mann-Whitney, $p<.001$).

hippocampal CA1 area[1), 3)], for which there is abundant information on the mechanism of synaptic plasticity[8)]. A requirement for a retrograde messenger for DSI induction is suspected, since the induction mechanism is postsynaptic and the expression, presynaptic[9)]. Recent reports have indicated that a possible candidate for the retrograde messenger is the cannabinoid[6), 9), 11)], since the CB1 receptor blocker, AM281, blocks DSI induction by inhibiting N-type voltage-dependent calcium channels through a G-protein coupled mechanism[12), 13)]. Several reports also indicate the involvement of other substances, such as glutamate[14)]. It has been suggested that activation of the group 1 metabotropic receptor (mGluR1) is involved in the induction mechanism of DSI[15),16)]. No other agents have been reported to be involved. We focused on adenosine, since adenosine activates the A1 receptor, which is located presynaptically and suppresses transmitter release by blocking N-type calcium channels via a G protein-coupled mechanism[17)]. We examined the effect of adenosine on DSI because of this similarity in the effects of adenosine and the cannabinoid.

Our results showed that the activities of both A1 and CB1 receptors were required for DSI induction in the NAc. The relationship between events downstream of A1 and CB1 receptor activity has not been well investigated, but our results indicate that these two types of receptor share a common induction mechanism for DSI. It is notable that Murillo-Rodriguez E. et al., using microdialysis, showed that anandamide increases extracellular levels of adenosine and that this effect is mediated by CB1 receptors[18)], raising the possibility that the effect of the cannabinoid could be at least partially mediated through A1 receptor activation due to an increase in adenosine levels. This raises the question whether adenosine could be released by depolarization[19)].

NMDA receptor activation promotes trans-synaptic feedback inhibition of glutamatergic synaptic transmission via adenosine release[20)], suggesting that the release of adenosine is dependent on the intracellular calcium concentration. Induction of DSI is also dependent on the intracellular calcium concentration, as an increase in calcium due to depolarization or photolysis induces DSI[21), 22)]. These facts indicate that adenosine might be one of the endogenous regulators involved in DSI induction.

DSI induction has been reported in the hippocampus, cerebellum, and substantia nigra[23)-25)]. This is the first report showing that DSI is also evoked in the NAc. The cannabinoid has been well investigated as the retrograde signal mediator. We propose that adenosine is also a candidate for the retrograde messenger, although its interaction with cannabinoid receptor activity remains to be elucidated.

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REFERENCES

1. Ohno-Shosaku T, Sawada S, Yamamoto C: Properties of depolarization-induced suppression of inhibitory transmission in cultured rat hippocampal neurons. Pflugers Arch 1998; 435: 273-279
2. Beau FE, Alger BE: Transient suppression of GABAA-receptor-mediated IPSPs after epileptiform burst discharges in CA1 pyramidal cells. J Neurophysiol 1998; 79: 659-669

3. Hampson RE, Zhuang SY, Weiner JL, Deadwyler SA: Functional significance of cannabinoid-mediated, depolarization-induced suppression of inhibition (DSI) in the hippocampus. J Neurophysiol 2003; 90: 55-64
4. Horvitz JC: Dopamine gating of glutamatergic sensorimotor and incentive motivational input signals to the striatum. Behav Brain Res 2002; 137: 65-74
5. Shah YB, Prior MJ, Dixon AL, Morris PG, Marsden CA: Detection of cannabinoid agonist evoked increase in BOLD contrast in rats using functional magnetic resonance imaging. Neuropharmacology 2004; 46: 379-387
6. Wilson RI, Kunos G, Nicoll RA: Presynaptic specificity of endocannabinoid signaling in the hippocampus Neuron 2001; 31: 453-462
7. Mitchell JB, Lupica CR, Dunwiddie TV: Activity-dependent release of endogenous adenosine modulates synaptic responses in the rat hippocampus. J Neurosci 1993; 13: 3439-347
8. Bliss TV, Collingridge GL, Morris RG: Long-term potentiation and structure of the issue. Philos Trans R Soc Lond B Biol Sci 2003; 358: 607-611.
9. Alger BE, Pitler TA, Wagner, JJ, Martin LA, Morishita W, Kirov SA, Lenz RA: Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol 1996; 496: 197-209
10. Maejima T, Ohno-Shosaku T, Kano M: Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. Neurosci Res 2001; 40: 205-210
11. Ohno-Shosaku T, Tsubokawa H, Mizushima I, Yoneda N, Zimmer A, Kano M: Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. J Neurosci 2002; 22: 3864-3872
12. Lenz RA, Wagner JJ, Alger BE: N- and L-type calcium channel involvement in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol 1998; 512: 61-73
13. Pitler TA, Alger BE: Depolarization-induced suppression of GABAergic inhibition in rat hippocampal pyramidal cells. G protein involvement in a presynaptic mechanism. Neuron 1994; 13: 1447-1455
14. Morishita W, Alger BE: Evidence for endogenous excitatory amino acids as mediators in DSI of GABA(A)ergic transmission in hippocampal CA1. J Neurophysiol 1999; 82: 2556-2564
15. Morishita W, Kirov SA, Alger BE: Evidence for metabotropic glutamate receptor activation in the induction of depolarization-induced suppression of inhibition in hippocampal CA1. J Neurosci 1998; 18: 4870-4882
16. Ohno-Shosaku T, Shosaku J, Tsubokawa H, Kano M: Cooperative endocannabinoid production by neuronal depolarization and group I metabotropic glutamate receptor activation. Eur J Neurosci 2002; 5: 953-961
17. Chernevskaya NI, Obukhov AG, Krishtal OA: NMDA receptor agonists selectively block N-type calcium channels in hippocampal neurons. Nature 1991; 349: 418-420
18. Murillo-Rodriguez E, Blanco-Centurion C, Sanchez C, Piomelli D, Shiromani PJ: Anandamide enhances extracellular levels of adenosine and induces sleep: an in vivo microdialysis study. Sleep 2003; 26: 943-947
19. Saransaari P, Oja SS: Mechanisms of adenosine release in the developing and adult mouse hippocampus. Neurochem Res 2002; 27: 911-918
20. Harvey J, Lacey MG: A postsynaptic interaction between dopamine D1 and NMDA receptors promotes presynaptic inhibition in the rat nucleus accumbens via adenosine release. J Neurosci 1997; 17: 5271-5280
21. Lenz RA, Alger BE: Calcium dependence of depolarization-induced suppression of inhibi-

tion in rat hippocampal CA1 pyramidal neurons. J Physiol 1999; 521: 147-157
22. Wang J, Zucker RS: Photolysis-induced suppression of inhibition in rat hippocampal CA1 pyramidal neurons. J Physiol 2001; 533: 757-763
23. Diana MA, Levenes C, Mackie K and Marty A: Short-term retrograde inhibition of GABAergic synaptic currents in rat Purkinje cells is mediated by endogenous cannabinoids. J Neurosci 2002; 22: 200-208
24. Yanovsky Y, Mades S, Misgeld U: Retrograde signaling changes the venue of postsynaptic inhibition in rat substantia nigra. Neuroscience 2003; 122: 317-328
25. Yoshida T, Hashimoto K, Zimmer A, Maejima T, Araishi K, Kano M: The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. J Neurosci 2002; 22: 1690-1697

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ラット側座核のIPSPに対するアデノシンとカンナビノイドによる脱分極誘導抑制の誘導

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要　　旨

短時間の脱分極は一過性に伝達物質の放出を抑制する（脱分極誘導 IPSP 抑制:DSI)。最近の研究によればカンナビノイドは海馬 CA1 領域における DSI 誘導に関わる逆行性伝達物質であることが示されている。誘導のメカニズムとしてはG蛋白に結合した過程やN型のカルシウムチャンネルが関与していると考えられている。我々は、海馬・基底外側扁桃体などを含んだ終脳における多くの部位からの入力を統合している場所である側座核において、DSI の誘導を検討した。側座核の水平断薄切標本を用いて whole cell recording を行い、DSI 誘導に関する内因性カンナビノイドの影響を調べたところ、選択的 CB1 カンナビノイド受容体阻害薬である 300nM の AM281 が DSI の誘導を阻害した。アデノシンはN型カルシウムチャンネルを阻害することによって伝達物質の放出を抑制することが報告されているが、この効果はカンナビノイドの効果と類似している。それ故我々は選択的な A1 アデノシン受容体の阻害薬である DPCPX を用いて、DSI 誘導に関する内因性アデノシンの効果を調べてみた。その結果、100nM の DPCPX が DSI の誘導を阻害することがわかった。これらの結果から CB1 と A１受容体活性が側座核における DSI の誘導に関わっていることが示された。

キーワード：側座核、DSI、カンナビノイド、アデノシン、whole-cell recording