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Forskolin Enhances Synaptic Transmission in Rat Dorsal Striatum through NMDA Receptors and PKA in Different Phases

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The effect of forskolin on corticostriatal synaptic transmission was examined by recording excitatory postsynaptic currents (EPSCs) in rat brain slices using the whole-cell voltage-clamp technique. Forskolin produced a dose-dependent increase of corticostriatal EPSCs (1, 3, 10, and 30 μM) immediately after its treatment, and the increase at 10 and 30 μM was maintained even after its washout. When the brain slices were pre-treated with (DL)-2-amino-5-phosphonovaleric acid (AP-V, 100 μM), an NMDA receptor antagonist, the acute effect of forskolin (10 μM) was blocked. However, after washout of forskolin, an increase of corticostriatal EPSCs was still observed even in the presence of AP-V. When KT 5720 (5 μM), a protein kinase A (PKA) inhibitor, was applied through the patch pipette, forskolin (10 μM) increased corticostriatal EPSCs, but this increase was not maintained. When forskolin was applied together with AP-V and KT 5720, both the increase and maintenance of the corticostriatal EPSCs were blocked. These results suggest that forskolin activates both NMDA receptors and PKA, however, in a different manner.

Key Words: Striatum, Forskolin, PKA, NMDA

INTRODUCTION

Dorsal striatum is the major input nuclei in the basal ganglia. It receives glutamatergic afferent inputs from the cortex and other brain areas, and these synaptic transmissions may play an important role in the generation of voluntary movement (Graybiel et al, 1994). Furthermore, the dorsal striatum appears to play an important role in certain forms of behavioral learning and habit formation, and the change in the synaptic efficiency of the cortical inputs to the dorsal striatum has been considered to contribute to it (Jog et al, 1999; Ronesi & Lovinger, 2005).

Forskolin directly activates adenylyl cyclase, raising cyclic AMP (cAMP) levels, and has been reported to have various effects on intracellular signaling and cellular functions. It also affects cellular development and differentiation, conductance of ion channels, and regulation of neurotransmitter release (Laurenza et al, 1989; Dessauer et al, 1997; Insel & Ostrom, 2003). Studies have shown that forskolin modulates PKA-dependent presynaptic neurotransmitter release (Spencer & Murphy, 2002) and enhances the synaptic efficacy in the hippocampus (Chavez-Noriega & Stevens, 1992). Especially, in the CA1 region of the hippocampus, forskolin induces a chemical long-term potentiation (LTP) that is mediated by NMDA receptors (Otmakhov et al, 2004).

Although the mechanisms of action of forskolin in various brain regions have well been studied, the effects of forskolin on synaptic transmission in the dorsal striatum remain still unclear. Therefore, the aim of this study was to elucidate the effect of forskolin on the corticostriatal glutamatergic synaptic transmission in the dorsal striatum, using *in vitro* whole-cell recording technique in rat brain slices.

METHODS

Slice preparation

Brain slices were prepared from 14- to 20-day-old Sprague-Dawley rats according to the techniques previously described (Cho et al, 2006; Choi et al, 2006). Rats were anaesthetized with pentobarbital (50 mg/kg, i.p.) and killed by decapitation in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Brains were removed and placed in ice-cold, modified artificial cerebrospinal fluid (aCSF) containing (in mM) 194 sucrose, 30 NaCl, 4.5 KCl, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose, pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2. Coronal slices (300 μm thick) were cut using a manual vibrotome (Campden Instruments, Loughborough, UK). Brain slices were transferred to aCSF containing (in mM) 124 NaCl, 4.5 KCl, 2

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ABBREVIATIONS: EPSC, excitatory postsynaptic current; AP-V, (DL)-2-amino-5-phosphonovaleric acid; PKA, protein kinase A; cAMP, cyclic AMP.

$CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.2 NaH_2PO_4, and 10 D-glucose, pH adjusted to 7.4 by bubbling with 95% O_2/5% CO_2 at room temperature. Slices were used for electrophysiological experiments beginning 2 hr after the end of slice preparation. A hemislice containing the cortex and striatum was completely submerged in a recording chamber and continuously superfused with aCSF that was constantly bubbled with 95% O_2/5% CO_2. The flow rate was kept at 2~3 ml/min using a peristaltic pump (Miniplus 3, Glison, France). Temperature of the bath solution was maintained at 30~32°C.

Whole-cell recording

Whole-cell voltage-clamp recordings were performed to record excitatory postsynaptic currents (EPSCs) at the striatal synapses (Choi et al, 2006). Electrical stimuli (0.05 Hz) were delivered through a bipolar, Teflon®-coated tungsten electrode, which was placed in the white matter dorsal to the striatum and close to the recording electrode. Tight-seal whole-cell recordings were obtained using pipettes made from borosilicate glass capillaries pulled on a P-97 micropipette puller (Sutter Instruments, Novato, CA). Pipettes (3~5 MΩ) were filled with internal solution containing (in mM) 120 $CsMeSO_3$, 5 NaCl, 10 tetraethylammonium chloride, 10 HEPES, 5 lidocaine N-ethyl bromide (QX-314) (Br^{2+} salt), 1.1 EGTA, 4 ATP (Mg^{2+} salt), 0.3 GTP (Na^+ salt), pH adjusted to 7.2 with CsOH and osmolarity adjusted to 290~300 mOsm with sucrose. Medium-sized neurons within two or three layers below the surface of the slice were identified using an Olympus BX50WI (Tokyo, Japan) differential interference contrast microscope. Neurons were voltage-clamped at −70 mV, and EPSCs recorded with an Axopatch 1D patch-clamp amplifier (Molecular Devices, Sunnyvale, CA) were filtered at 5 kHz, digitized at 10 kHz using a Digidata 1322A (Molecular Devices) and stored on a personal computer using pClamp 9.2 software (Molecular Devices). Series resistance ranged between 8~25 MΩ and was not compensated. Experiments in which series resistance changed more than 20% were excluded from analysis.

Drugs and chemicals

Forskolin, (DL)-2-amino-5-phosphonovaleric acid (AP-V), and KT 5720 were purchased from Tocris Cookson (Avonmouth, UK). All other chemicals were purchased from Sigma (St. Louis, MO). Drugs were diluted with aCSF immediately before use from the stock solutions prepared according to the manufacturer's instructions and were delivered to the recording chamber with a peristaltic pump.

Data analysis

All averaged data were expressed as means±SEM. The mean values 10 min prior to drug treatment were averaged and defined as control, and subsequent drug responses were compared to this value. The effect of forskolin was divided as early phase and late phase response: The effect during the treatment of forskolin for 10 min was defined as early phase, and the effect during 20~30 min after forskolin treatment was described as late phase. All EPSCs data were normalized to the baseline response. The statistical significance was evaluated using Student's t test or a one-way ANOVA for multiple group comparisons.

RESULTS

Fig. 1 shows that treatment of brain slices with forskolin for 10 min significantly increased corticostriatal EPSCs amplitude in a dose-dependent manner (one-way ANOVA, $p < 0.001$). EPSCs amplitude was increased by 11.2±2.8% ($n=5$, $p<0.05$) of the baseline with 1 μM forskolin,

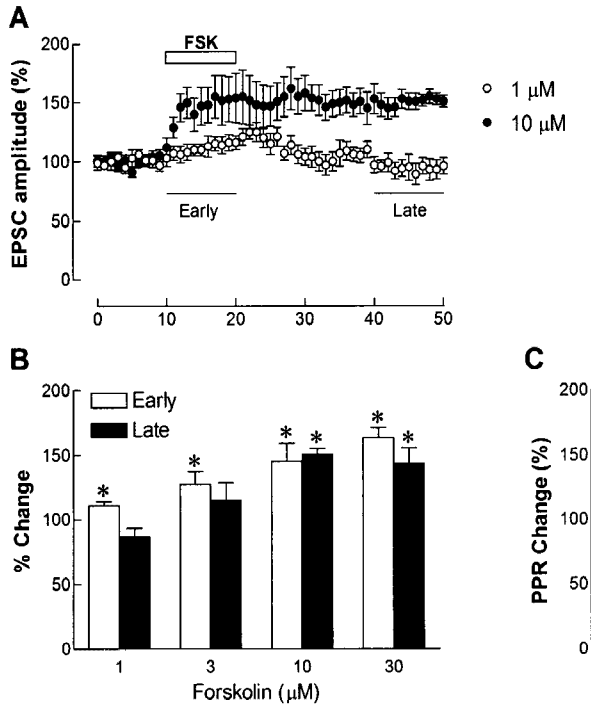

Fig. 1. Forskolin enhances corticostriatal EPSCs in a dose dependent manner. (A) Plot of average EPSCs data shows that forskolin increases EPSCs amplitude (1 and 10 μM). The bar above the plotted graph indicates the period of drug application. (B) Forskolin increases EPSCs amplitude in a dose-dependent manner. Graph shows the average EPSCs change during forskolin treatment (early phase), and after its treatment (late phase) at each concentration. Note that the effect of 10 and 30 μM forskolin was maintained even after its washout. (C) Graph shows the average PPR change by forskolin. *$p<0.05$ when compared to baseline EPSCs.

27.6±9.8% (n=5, $p<0.05$) by 3 μM, 45.4±13.5% (n=7, $p<0.05$) by 10 μM, and 63.3±7.9% (n=4, $p<0.01$) by 30 μM forskolin. The EPSCs increased by 10 μM (50.6±4.8%, $p<0.001$) and 30 μM forskolin (43.8±11.8%, $p<0.05$) were maintained even after its washout, but not at 1 μM (13.2±6.6%, p=0.1164) and 3 μM (15.0±13.9%, p=0.3405). Furthermore, there was no significant change in the PPR by forskolin treatment, suggesting that forskolin might act through a post-synaptic mechanism. As 10 μM forskolin showed the most stable effect, the dose of 10 μM was used in the flowing experiments.

It is well known that NMDA receptors are one of the major glutamate receptors expressed on medium spiny neurons which are the principal neurons of the corticostriatal synapses (Butler et al, 1998; Ozawa et al, 1998), and the induction of LTP is dependent on NMDA receptors (Partridge et al, 2000; Spencer & Murphy, 2000). To elucidate whether the synaptic enhancement induced by forskolin was mediated through NMDA receptors, we tested the effect of forskolin after pre-treatment of brain slices with AP-V (100 μM), an NMDA receptor antagonist. As shown in Fig. 2, the early effect of forskolin was blocked by AP-V (−6.6±3.0%, n=5, p=0.0909). However, the forskolin-induced late response was still observed after its wash out (29.2±5.0%, $p<0.01$).

To determine wether the synaptic enhancement induced by forskolin might be associated with protein kinase A (PKA), KT 5720 (5 μM), a PKA inhibitor, was applied to the recording cells through the patch pipette. The early increase in corticostriatal EPSCs by forskolin was still observed (47.2±10.4%, n=6, $p<0.01$), however, its late enhancement was blocked (13.5±15.4%, p=0.4205; Fig. 3).

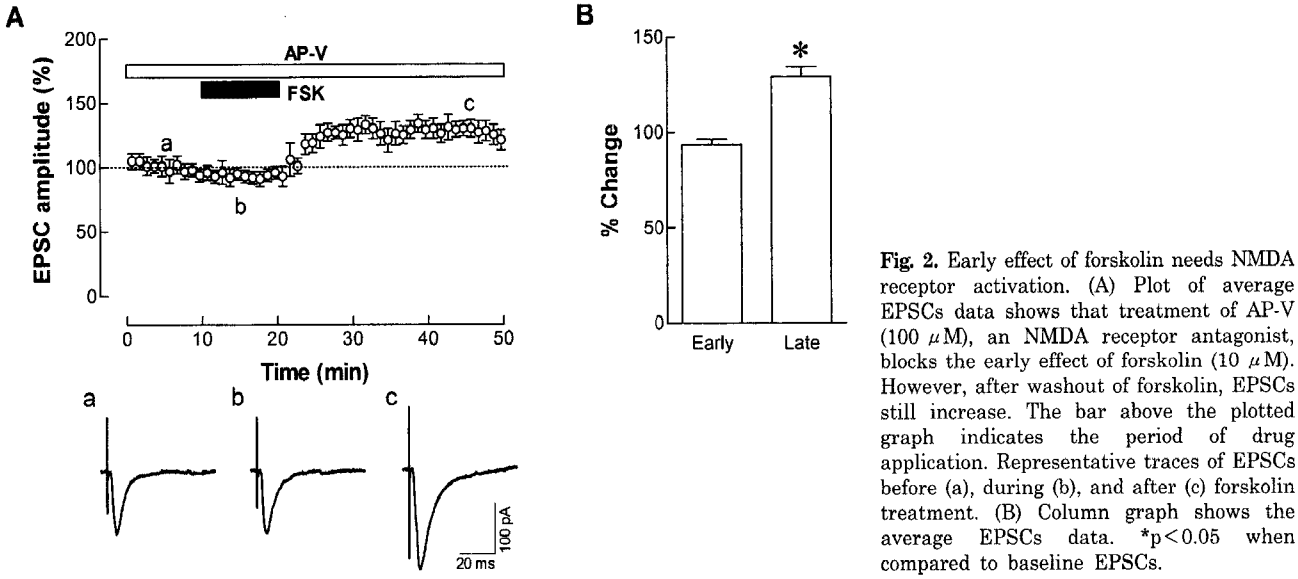

Fig. 2. Early effect of forskolin needs NMDA receptor activation. (A) Plot of average EPSCs data shows that treatment of AP-V (100 μM), an NMDA receptor antagonist, blocks the early effect of forskolin (10 μM). However, after washout of forskolin, EPSCs still increase. The bar above the plotted graph indicates the period of drug application. Representative traces of EPSCs before (a), during (b), and after (c) forskolin treatment. (B) Column graph shows the average EPSCs data. *$p<0.05$ when compared to baseline EPSCs.

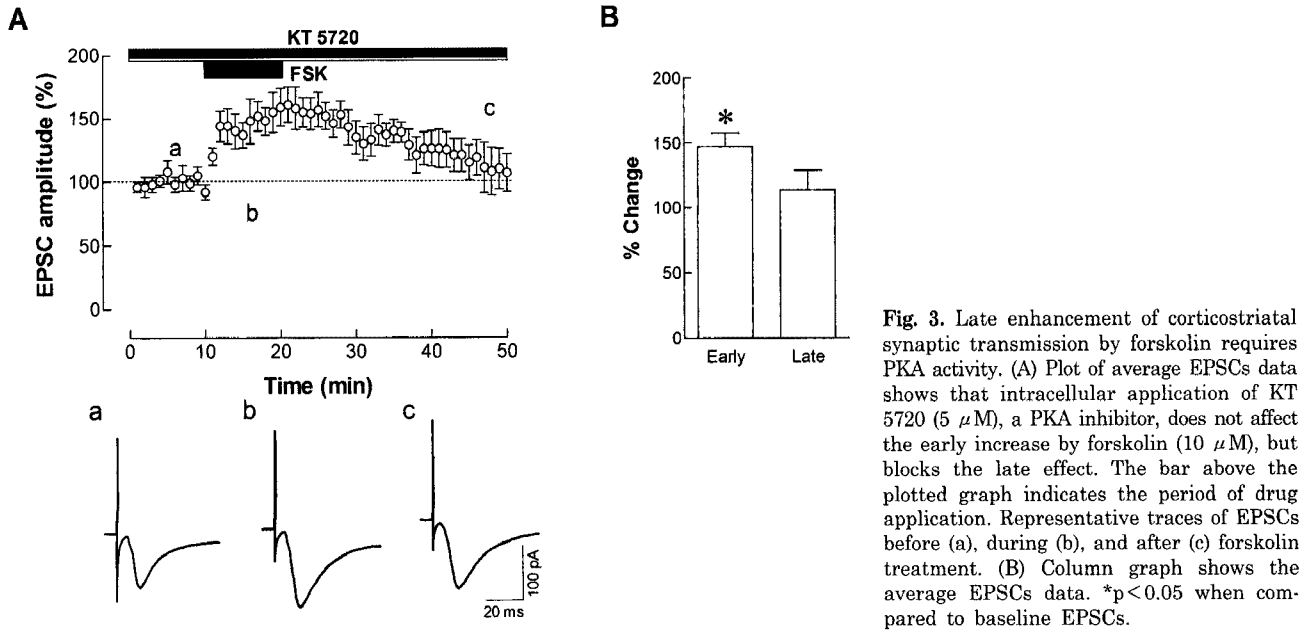

Fig. 3. Late enhancement of corticostriatal synaptic transmission by forskolin requires PKA activity. (A) Plot of average EPSCs data shows that intracellular application of KT 5720 (5 μM), a PKA inhibitor, does not affect the early increase by forskolin (10 μM), but blocks the late effect. The bar above the plotted graph indicates the period of drug application. Representative traces of EPSCs before (a), during (b), and after (c) forskolin treatment. (B) Column graph shows the average EPSCs data. *$p<0.05$ when compared to baseline EPSCs.

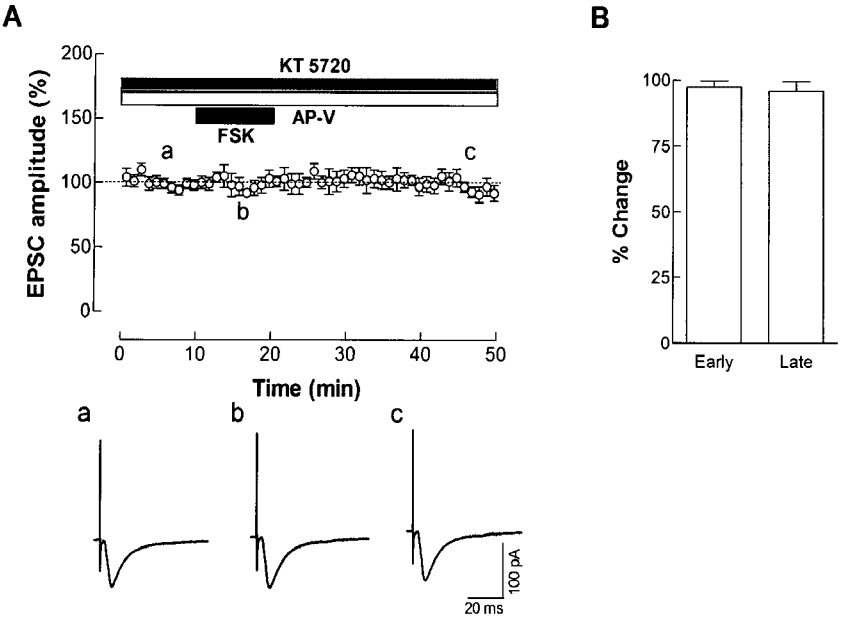

Fig. 4. Forskolin exerts no effect on corticostriatal synaptic transmission when both NMDA receptor and PKA activation are blocked. (A) Plot of average EPSCs data shows that the effect of forskolin (10 μM) on corticostriatal synaptic transmission was blocked when AP-V (100 μM) and KT 5720 (5 μM) were applied together. The bar above the plotted graph indicates the period of drug application. Representative traces of EPSCs before (a), during (b), and after (c) forskolin treatments. (B) Column graph shows the average EPSCs data.

Finally, as shown in Fig. 4, forskolin exerted no effect on corticostriatal synaptic transmission neither in the early response (−2.5±2.4%, n=4, p=0.3738), nor in late response (−4.0±3.6%, p=0.3455), when both NMDA receptors and PKA activity were blocked with each antagonist. These results suggest that forskolin might potentiate corticostriatal synaptic transmission through activation of NMDA receptors and PKA.

DISCUSSION

The present study demonstrated that forskolin enhanced corticostriatal synapse transmission through NMDA receptors and PKA in different phases. Many recent studies reported that forskolin potentiated synaptic transmission in various brain regions (Cherubini et al, 1988; Chavez-Noriega & Stevens, 1992; Colwell & Levine, 1995; Rosenberg & Li, 1996; Chen & Regehr, 1997; Lu & Gean, 1999; Lu et al, 1999). Especially, the increase of synaptic transmission in the striatum by forskolin was shown to be mediated by the activation of adenylyl cyclase (Spencer & Murphy, 2002), and the activation of adenylyl cyclase increases cAMP levels as well as PKA activity (Colwell & Levine, 1995). Both these reports demonstrated that forskolin activates glutamatergic synaptic transmission in the corticostriatal synapses, in agreement with our present results that forskolin enhanced corticostriatal synaptic transmission (Fig. 1).

However, there have been some different interpretations on the action of forskolin; forskolin showed a long-term enhancement in corticostriatal synapses through activation of pre-synaptic PKA (Spencer & Murphy, 2002), whereas forskolin enhanced synaptic transmission in the striatum through a post-synaptic mechanism (Colwell & Levine, 1995). In the present study, we used the whole-cell recording technique, therefore, the PKA inhibitor KT 5720 was applied directly to the cell through the patch pipette, and the late phase increase was blocked. Additionally, there was no significant change in the PPR. Taken together, our results indicate that forskolin might possibly exert a post-synaptic mechanism.

There are some conflicting results about the relationship between forskolin and NMDA receptors over different synapses; the chemical LTP induced by forskolin was blocked by an NMDA receptor antagonist in the hippocampus (Otmakhov et al, 2004; Grey & Burrell, 2008), or NMDA receptors had no function in the effect of forskolin in the striatum (Spencer & Murphy, 2002). Therefore, we also tested the role of NMDA receptors in the effect of forskolin using AP-V, an NMDA receptor antagonist. Pre-treatment of brain slices with AP-V blocked the early phase effect of forskolin, suggesting that NMDA receptors might be involved in the enhancement of corticostriatal synaptic transmission by forskolin. However, after the application was over, the late phase enhancement in the corticostriatal EPSCs reoccurred, dissimilar to the previous study from the stiratum (Spencer & Murphy, 2002). The main difference between our present study and this previous work appears to be that the method of approach used in the present study was whole-cell recordings which are different from field potentials.

Studies with the hippocampus showed that the glutamatergic synaptic transmission could be increased by PKA, which is stimulated by activation of adenylyl cyclase increaseing the level of cAMP (Simonds, 1999; Jay, 2003). Since forskolin can directly activate andenylyl cyclase and raise cAMP levels (Laurenza et al, 1989; Dessauer et al, 1997; Insel & Ostrom, 2003), this mechanism might possibly be involved in how KT 5720 blocked the late enhancement induced by forskolin at corticostriatal synapses (Fig. 3). These results suggest that activation of PKA alone may increase the synaptic transmission in the rat dorsal striatum. However, NMDA receptors are also activated by forskolin and increase corticostriatal EPSCs. A recent study suggested that AMPA receptor trafficking might be involved in the action of forskolin (Grey & Burrell, 2008). However, this study was focused on invertebrate synapses, therefore, it might not show similar mechanism to vertebrate synapses. Additional studies are required to clarify

the different roles of NMDA receptors as well as the involvement of receptor trafficking in the action of forskolin.

Finally, under certain pathological conditions such as Parkinson's disease or Huntington's disease, the synaptic plasticity of the striatum is different, compared to normal conditions. LTP is evoked rather than long-term depression (LTD) or LTD expression is suppressed (Dalbem et al, 2005; Picconi et al, 2005). Our results may contribute to better understanding of the enhancement of synaptic transmission in the dorsal striatum and may provide an additional model for pathological studies of the striatal diseases.

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