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Promotion of Forskolin-Induced Long-Term Potentiation of Synaptic Transmission by Caffeine in Area CA1 of the Rat Hippocampus

Kwok-Tung Lu, Shang-Peng Wu and Po-Wu Gean

Department of Pharmacology
College of Medicine
National Cheng-Kung University
Tainan 701, Taiwan, ROC

Abstract

Caffeine which is present in soft drinks has been shown to increase alertness and allays drowsiness and fatigue. The aim of this study is to investigate whether caffeine could produce a long-term effect on the synaptic transmission using extracellular recording technique in the hippocampal slices. Bath application of caffeine (100 μM) reversibly increased the slope of field excitatory postsynaptic potential (fEPSP). Forskolin (25 μM) by its own did not affect the fEPSP significantly. However, in the presence of caffeine, forskolin induced a long-term potentiation (LTP) of fEPSP. Enprofylline which has been shown to exhibit some actions like caffeine but with a low adenosine antagonistic potency did not affect the normal synaptic transmission or the effect of forskolin at a lower concentration (10 μM). However, when the concentrations were increased to 20 and 50 μM, enprofylline significantly enhanced the fEPSP slope and promoted forskolin-induced LTP. The parallel increase of fEPSP and promotion of LTP observed with enprofylline suggests that adenosine A_1 antagonism is the primary mechanism behind caffeine's effect. This hypothesis was further strengthened by the finding that promotion of forskolin-induced LTP was mimicked by the non-xanthine adenosine antagonist 9-chloro-2-(furyl)[1,2,4]triazolo [1,5-c]quinazolin-5-amine (CGS 15943). The promotion of forskolin-induced LTP provides a cellular basis behind caffeine's increase in capacity for sustained intellectual performance.

Key Words: caffeine, long-term potentiation, forskolin, adenosine, hippocampus, c-AMP

Introduction

Caffeine which is present in soft drinks, coffee, tea, cocoa and chocolate is the most widely used social drug in the world. The ingestion of 85 to 250 mg of caffeine, the amount contained in 1 to 3 cups of coffee, increases alertness and allays drowsiness and fatigue. As the dose of caffeine increased, signs of progressive CNS stimulation appeared, including nervousness, anxiety, restlessness, insomnia, tremors and hyperesthesia. In more severe cases, focal and generalized convulsion may occur (4).

At the cellular level, caffeine has been shown to inhibit cyclic nucleotide phosphodiesterase (23), to antagonize adenosine receptors (8,14) and to interfere with the uptake and storage of Ca^{++} by the sarcoplasmic reticulum in striated muscle (18). However, it is still not known which of these effects is most relevant to its enhancement of cognitive function. Long-term potentiation (LTP) of synaptic transmission is a cellular process thought to underlie some forms of learning and memory (5). In hippocampal CA1 neurons, increase in presynaptic cAMP level by activation of β-adrenergic receptors or adenylyl cyclase only caused a transient enhancement of glutamate release and LTP was not observed consistently. However, when adenosine A_1 receptors were blocked or the metabolism of cAMP was disrupted, activation of adenylyl cyclase by forskolin induced LTP (17). These results suggest that it is adenosine which acts on adenosine A_1 receptors to mask forskolin-induced LTP. In this study, we test

Corresponding author: Dr. Po-Wu Gean, Department of Pharmacology, National Cheng-Kung University, Taiwan 701, Taiwan, ROC. Fax: 886-6-2749296, E-mail: powu@mail.ncku.edu.tw
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the hypothesis that if caffeine can promote forskolin-induced LTP in the hippocampal CA1 neurons and whether this effect is due to blockade of adenosine A_1 receptor.

Materials and Methods

Male Sprague-Dawley rats of 5- to 7-week-old were decapitated and the brains rapidly removed from the skull. Coronal slices of 400-450 μm thick were cut and the appropriate slices were placed in a beaker of artificial cerebrospinal fluid (ACSF). The ACSF was bubbled continuously with 95%O_2-5%CO_2 to maintain the proper pH (7.3-7.5). The composition of the ACSF solution was (in mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgCl_2$ 1.2, $NaHCO_3$ 25, NaH_2PO_4 1.2 and glucose 11. The slices were kept at room temperature for at least one hour before being transferred to the recording chamber where it was held submerged between two nylon nets and maintained at 32±1°C.

Extracellular recordings of fEPSPs were obtained from stratum radiatum using microelectrodes filled with 3 M NaCl (3-8 MΩ). A bipolar stimulating electrode was placed in stratum radiatum for stimulation of Schaffer collateral/commissural pathway. The stimulus duration was 150 É›s and the stimulus intensity was adjusted individually for each experiment to produce fEPSP which were ~30-40% of the maximal responses that could be evoked. Experimental treatments were not initiated until the response had been stable for at least 20 min. The strength of synaptic transmission was quantified by measuring the initial slope of the fEPSP. The fEPSP slopes were measured by linear regression of their initial rising phases, usually during the first 0.4-0.6 ms after their onset. Onset was taken after the afferent volley.

Data were analyzed using pClamp data acquisition and analysis softward (Axon Ins., Foster City, CA, USA) running on a PC586 computer. All data were expressed as mean±S.E.M. Statistical analysis was performed using Student's *t*-test and a *p* value of less than 0.05 was considered to be statistically significant. Forskolin and caffeine were purchased from Sigma Chemicals (St. Louis, MO, USA), and other drugs were obtained from Research Biochemicals International (Natick, MA, USA).

Results

The effect of caffeine on the fEPSP slope as a function of time is illustrated in Figure 1. After the evoked responses were stable for 20-30 min, caffeine was bath applied. At a concentration of 100 μM, caffeine increased the fEPSP slope by an average of 86±14% (n=7, $p<0.001$). The effect of caffeine was

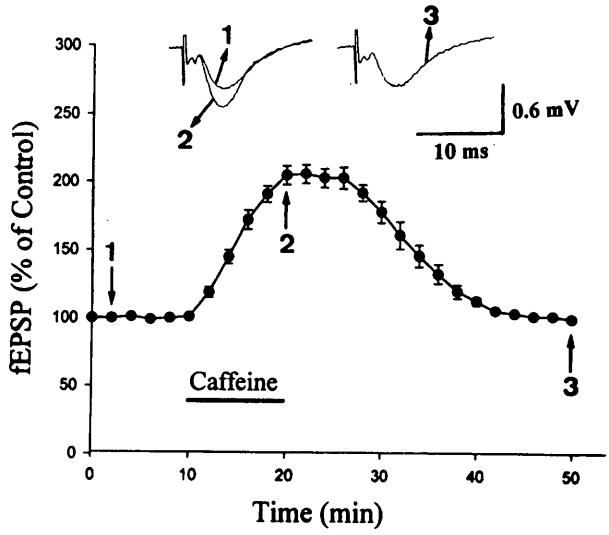

Fig. 1. Reversible enhancement of fEPSP by caffeine. The slope of fEPSP was plotted as a function of time. Inset shows the records taken before and during the application of caffeine (100 μM).

reversible which returned to baseline level within 30 min of washing with control ACSF.

Figure 2 shows that forskolin at the concentration of 25 μM had no significant effect on the fEPSP, a result consistent with previous reports (9). However, in the presence of caffeine, forskolin (25 μM) induced LTP of the fEPSP slope in 8 out of 9 slices tested. The slope of fEPSP remained 167±12% of baseline (n=8, $p<0.01$) 60 min after washout of forskolin.

Caffeine could exert its effect by inhibiting phosphodiesterase (23), blocking adenosine A_1 receptors (8, 14) and releasing Ca^{++} from intracellular stores (13, 18). To determine which effect accounts for the promotion of forskolin-induced LTP, we made use of enprofylline which has been shown to exhibit some actions like caffeine but with a low adenosine antagonistic potency (11, 19). Figure 3 shows that low concentration (10 μM) of enprofylline did not either affect the normal fEPSP (108±4% of baseline, n=7) or the effect of forskolin (108±10% of control, n=7). However, when the concentrations were increased to 20 and 50 μM, enprofylline significantly increased the fEPSP by 46±15% (n=7, $p<0.01$) and 80±7% (n=7, $p<0.001$) respectively, and subsequently promoted the forskolin-induced LTP. The slopes of fEPSP were 128Ñb11% (n=7, $p<0.01$) and 184±11% (n=7, $p<0.001$) of control 60 min following washout of the drugs (Fig. 3).

We speculated that the enhancement of fEPSP and promotion of forskolin-induced LTP by enprofylline was due to its antagonism of adenosine A_1 receptor by testing the effect of enprofylline on the A_1 receptor-induced synaptic depression. 2-chloroadenosine (2-CA), a selective adenosine A_1

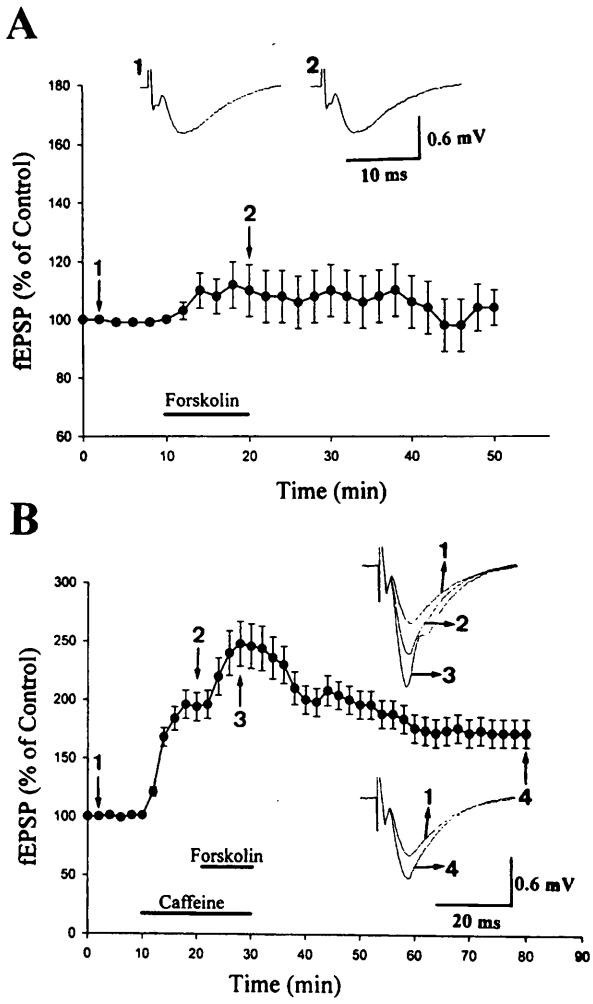

Fig. 2. Caffeine promotes forskolin-induced LTP. A, Effect of forskolin on the fEPSP. The slope of fEPSP was plotted as a function of time. Inset shows the records taken before and during the application of forskolin (25 μM). B, Application of forskolin in the presence of caffeine resulted in a long-term enhancement of fEPSP. Superfusion of caffeine (100 μM) increased the fEPSP slope. Subsequent addition of forskolin (25 μM) in the presence of caffeine resulted in LTP. Inset is superimposed traces taken at different times as indicated.

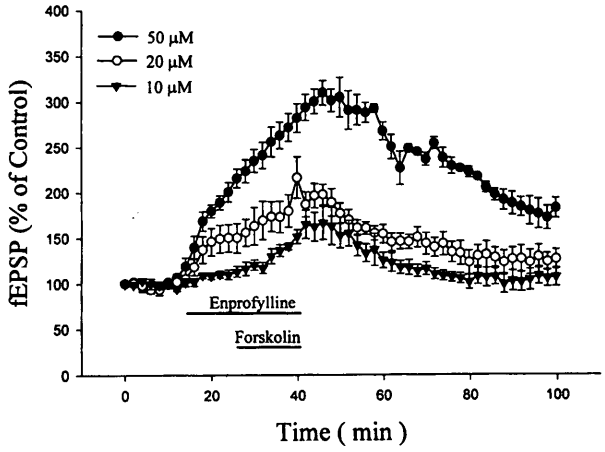

Fig. 3. Concentration-dependent effect of enprofylline on the fEPSP. Application of enprofylline of increasing concentrations enhanced the fEPSP slope and promoted the forskolin-induced LTP.

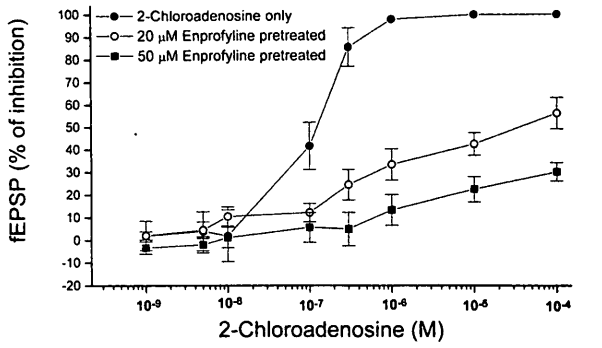

Fig. 4. Antagonism of 2-CA-mediated inhibition of fEPSP by enprofylline. The percent inhibition of fEPSP was plotted against the concentrations of 2-CA in the absence and the presence of enprofylline.

agonist caused an inhibition of fEPSPs. The effect reached a steady state within 5 min and readily reversed when the 2-CA was washed from the tissue. The inhibition of fEPSPs was concentration-dependent and a 50% inhibition (EC_{50}) was about 100 nM. Figure 4 shows that the concentration-response curve for the inhibitory effect of 2-CA was shifed to the right by enprofylline. The fEPSP slope was reduced by 98.4±1.5% (n=6) in the presence of 1 μM 2-CA. Same concentration of 2-CA only reduced the fEPSP by 33.5±7.3% (n=6) and 13.3±6.8% when slices were pretreated with 20 and 50 μM of enprofylline respectively (Fig. 4). There is a significant difference ($p<0.01$) between control and those enprofylline-pretreated slices.

CGS-15943, 9-chloro-2-(furyl)[1,2,4]triazolo[1, 5-c]quinazolin-5-amine, is a novel nonxanthine adenosine antagonist without exhibiting inhibitory activity on the phosphodiesterases (6, 7). Figure 5 is a summary of 6-7 experiments showing that superfusion of 5, 50 and 100 μM of CGS-15943 increased the slope of fEPSPs by 6.1±5.4, 11.6±4.5 and 54.5±3.6% respectively. Furthermore, in the presence of CGS-15943 (100 μM), forskolin induced LTP. The fEPSP slope was 128±3% (n=7, $p<0.001$) of control 50 min after washout of the drugs (Fig. 5B).

To investigate whether forskolin+caffeine-induced LTP is mediated through activation of cAMP-dependent protein kinase (PKA), we performed experiments with the specific PKA regulatory site antagonist, Rp-cyclic adenosine 3',5'-monophosphothioate (Rp-cAMPS). Slices were presoaked initially in 100 μM solution of Rp-cAMPS in the incubation beaker and then transferred into the

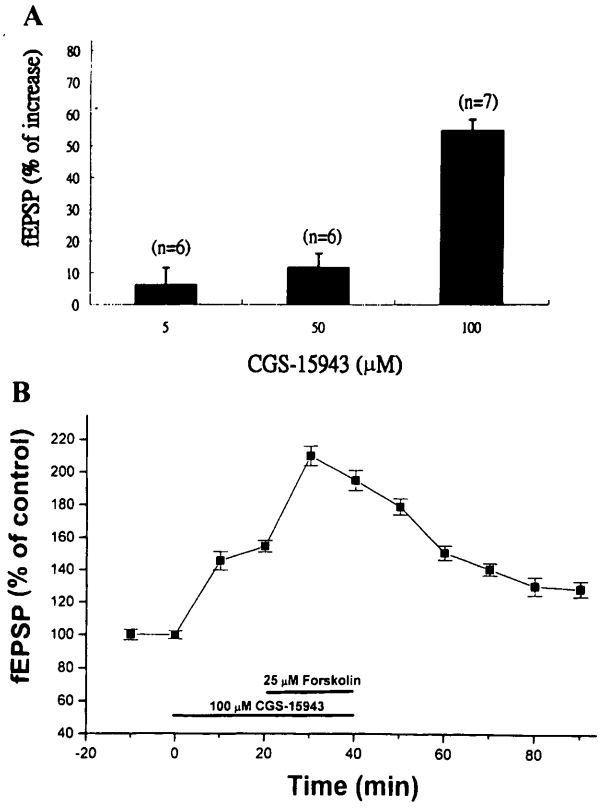

Fig. 5. Pretreatment with nonxanthine antagonist CGS-15943 promotes forskolin-induced LTP. A, Concentration-dependent enhancement of fEPSP by CGS-15943. B, Effects of CGS-15943 on the fEPSP and forskolin-induced LTP.

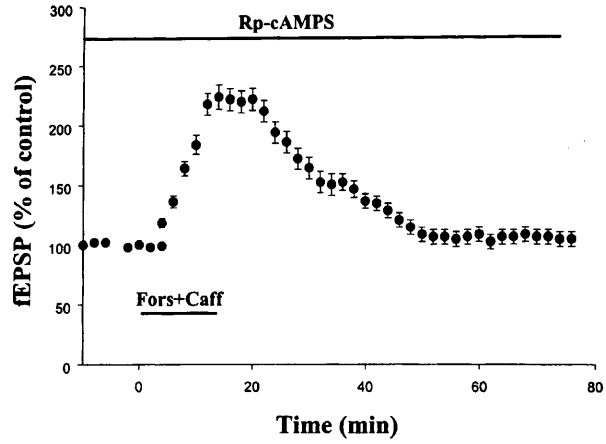

Fig. 6. Promotion of forskolin-induced LTP by caffeine is blocked by Rp-cAMPS. The percent change of fEPSP was plotted as a function of time. Bars denote the application of Rp-cAMPS (25 μM) and forskolin (25 μM)+caffeine (100 μM).

recording chamber where the concentration was maintained at 25 μM. As shown in figure 6, forskolin+caffeine-induced LTP was blocked (106±6% of control, n=6, $p<0.01$ unpaired t-test).

Discussion

Pharmacologically, it is well known that xanthine-like compounds have several profound central effects: proconvulsant, anxiogenic, antidepressant and CNS stimulatory actions. The results of this study add an additional, long-term effect of caffeine in enhancing the cognitive performance, provided that LTP represents a mechanism for learning and memory (5). At the cellular level, caffeine has three distinct effects: inhibition of phosphodiesterase (23), blockade of adenosine receptors (8, 14) and release of Ca^{++} from intracellular stores (16, 18). Since caffeine requires concentrations in the millimolar ranges (1-10 mM) for significant Ca^{++} release (16, 18), it is unlikely that induction of Ca^{++} release is the mechanism behind caffeine's enhancement of synaptic transmission and promotion of forskolin-induced LTP.

To differentiate between phosphodiesterase inhibition and adenosine antagonism, we employed enprofylline which has been shown to exhibit differed pharmacological profiles from those of classical methylxanthines owing to its low potency as an adenosine A_1 antagonist (19). Unexpectedly, we found that enprofylline on its own enhanced the fEPSP and shifted the dose-response curve of 2-CA-mediated inhibition to the right. This result indicates that enprofylline does possess adenosine A_1 antagonistic property which increased fEPSP slope by removing tonic inhibition exerted by the endogenous adenosine in this region of the brain. The parallel increase of fEPSP and promotion of LTP observed with enprofylline suggests that adenosine A_1 antagonism is the primary mechanism behind caffeine's promotion of LTP. Consistently, it has been shown that rolipram and Ro20-1724, specific phosphodiesterase inhibitors (3), had no effect on the basal synaptic transmission (2, 17, 21). Finally, this conclusion is further strengthened by the finding that promotion of forskolin-induced LTP is mimicked by the nonxanthine adenosine receptor antagonist CGS 15943. However, we could not rule out the possible involvement of phosphodiesterase inhibitory effect for caffeine to promote LTP because a reagent, which inhibits phosphodiesterase without antagonizing A_1 receptor, was not used in the present study.

Convergent pharmacological and genetic evidence has implicated cAMP and cAMP-dependent protein kinase A (PKA) in the late phase of LTP (L-LTP) in Schaffer collateral-CA1 synapses (1,12,15). In the present study, forskolin at the concentration used (25 μM) did not produce long-term effect on the synaptic transmission (20). Only in the presence of caffeine did forskolin induce LTP suggesting a role played by the adenosine. It is likely that activation of adenylyl cyclase by forskolin resulted in a large

increase in cAMP which left the cell (13, 17, 21, 22) and acted on adenosine receptors to curtail forskolin-induced LTP.

In summary, it is well established that adenosine exerts an inhibitory tone in the mammalian brain, primarily by depressing the release of neurotransmitter (8, 10, 14). Anticipatedly, adenosine antagonists like caffeine enhance transmitter release and exhibit CNS stimulatory, proconvulsant, anxiogenic and antidepressant activities. The results of this study add an additional, long-term effect of caffeine in enhancing the cognitive performance, provided that LTP represents a cellular mechanism for learning and memory (5).

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