Inhibition of Cerebral Protein Kinase C in Vitro by Cocaine and Methamphetamine

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ABSTRACT. Protein kinase C, which participates in cellular responses to various stimuli such as hormones, neurotransmitters and growth factors, is essential for cell proliferation and differentiation. Activation of the enzyme has been suggested to be important in neurotransmitter release, learning and memory, long-term potentiation, and cocaine-induced motor activity. Our previous study showed that monoamine uptake inhibitors imipramine and desipramine inhibited protein kinase C activity in a crude extract from the rat cerebral cortex. The present study examined the effect of cocaine and methamphetamine on activity of the soluble protein kinase C in a crude extract of the rat cerebral cortex. Cocaine and methamphetamine were found to inhibit protein kinase C in the soluble fraction at higher concentrations. It is, therefore, conceivable that the neural action of cocaine and methamphetamine may, at least in part, be associated with their inhibitory effect on protein kinase C.

Key words: cocaine — methamphetamine — protein kinase C

Protein kinase C plays an important role in cell proliferation and differentiation, and neural function by responding to hormones, growth factors and neurotransmitters.[1] A behavioral study showed that intracerebroventricular administration of protein kinase C activator phorbol esters improved Morris water maze performance in the rat.[2] Injection of a protein kinase C inhibitor, H7, into the A10 dopamine region dose dependently suppressed cocaine-induced motor activity.[3] Polymyxin B, another inhibitor of the enzyme, prevented the maintenance of synaptic long-term potentiation in hippocampal CA1 neurons.[4] These findings suggest involvement of protein kinase C in a variety of neural functions such as memory, long-term potentiation, and stimulant-induced motor activity.

A protein kinase C activator, phorbol 12-myristate 13-acetate, has been shown to increase both spontaneous and K^+-evoked [^{3}H] dopamine release from cultures of rat fetal mesencephalic cells.[5] Treatment with same activator also reduced the uptake of [^{3}H] dopamine in COS cells that expressed rat dopamine transporters.[6] Injection of H7 into the A10 region blocked a cocaine-induced increase in extracellular dopamine in the nucleus accumbens.[3] Furthermore, treatment with phorbol esters resulted in a concentration-dependent increase in glutamate transport in glial cells[7] or substantial reduction in the rate of serotonin transport in platelets.[8]

Recently, it has been reported that a number of monoamine uptake inhibitors such as imipramine and desipramine inhibit the activity of soluble

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protein kinase C including.[9,10] Both cocaine and methamphetamine inhibit the uptake of monoamines including dopamine.[11,12] Taken together, these findings suggest the possibility that cocaine and methamphetamine may influence protein kinase C activity in the brain. The present study examined the effects of cocaine and methamphetamine on the activity of soluble protein kinase C in a crude extract from the rat cerebral cortex.

METHODS

Protein kinase C was prepared according to a procedure described by Takai *et al*,[13] with slight modifications. The rat cerebral cortex was homogenized with a glass homogenizer in 3 volumes of 20 mM Tris-HCl buffer (pH 7.5) containing 10 mM benzamide, 50 μg/ml phenylmethylsulfonyl fluoride and 2 mM EDTA. The homogenate was centrifuged at 20,000 g for 40 min. Then supernatant was filtered through a glass wool filter to remove lipids. The filtrate served in the assay as the soluble fraction in the crude extract. All procedures were performed at 0-4℃. The concentraion of protein in samples was determined by the method of Lowry *et al* with bovine serum albumin as a standard.[14]

Activation of the soluble protein kinase C in the crude extract was assayed with the Protein kinase C Enzyme Assay System (Amersham) by measuring the amount of ^{32}P incorporated into an acceptor peptide.[10] The assay mixture (75 μl) contained 0.2 μCi/ml [γ-^{32}p] ATP, 45 mM magnesium acetate, 12 mM calcium acetate, 8 mol% L-α-phosphatidyl-L-serine, 24 μg/ml phorbol 12-myristate 13-acetate, 30 mM dithiothreitol, 0.05% (W/V) sodium azide, 900 μM acceptor peptide, and 5 μg protein kinase C in 50 mM Tris-HCl buffer, pH 7.5. Incubation was carried out at 25℃ for 15 min with or without either cocaine or methamphetamine at the various concentrations indicated. The reaction was terminated by the addition of 100 μl of a stop reagent. Subsequently, 125 μl of an aliquot of the reaction mixture was transferred onto a binding paper, and washed twice with 10 ml of 5% (V/V) acetic acid per paper. The radioactivity of ^{32}P was determined in 10 ml of a scintillation fluid using a liquid scintillation spectrometer (ALOKA, LSC-700). Statistical analysis of data was conducted using Student's t-test.

RESULTS

Fig 1 illustrates the effects of cocaine and methamphetamine on the soluble protein kinase C activity in the crude extract from cerebral cortex. The results are presented as percent radioactivity of ^{32}P incorporated into the acceptor peptide in the drug-treated sample as compared with that in the control sample. Although neither drug had any effect on the activity of protein kinase C at concentrations ranging from 0.001 nM to 100 μM. At 10 mM, radioactivity was significantly decreased in both the cocaine-treated sample (1957.57 $\pm$ 381.33 Bq/mg/min, $p<0.01$, $n=6$) and the methamphetamine-treated sample (2105.62 $\pm$ 226.46 Bq/mg/min, $p<0.01$, $n=6$) as compared with the control sample (2741.73 $\pm$ 381.33 Bq/mg/min, $n=6$).

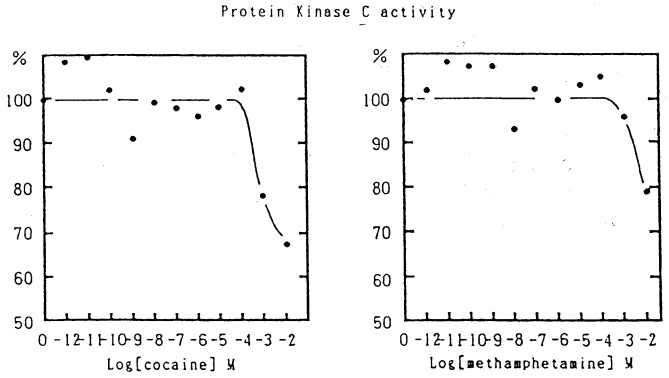

Fig 1. Effect of cocaine and methamphetamine on activity of the soluble protein kinase C in the crude extract from rat cerebral cortex

DISCUSSION

The present study revealed that both cocaine and methamphetamine inhibited protein kinase C in a crude extract from the rat cerebral cortex. The inhibitory effect of cocaine is in line with a report that the enzyme was inhibited by the local anesthetic dibucaine.[9] Local anesthetics have been shown to affect a variety of neural activities through interaction with membrane phospholipids.[15,16] Inositol phospholipid, one of the membrane phospholipids, is hydrolyzed to diacylglycerol, which activates cerebral protein kinase C.[1] Based on these factors, Mori *et al* hypothesized that the local anesthetics dibucaine and tetracaine inhibit protein kinase C activity by their interaction with membrane phospholipids.[9] Since cocaine has local anesthetic properties, it is possible that the inhibitory effect of cocaine on protein kinase C may be, at least in part, due to the interaction of cocaine with membrane phospholipids including inositol phospholipid.

Studies conducted by Mori *et al* and the authors have found that various antidepressants including imipramine and desipramine inhibit protein kinase C in vitro.[9,10] In the previous study we reported that methamphetamine did not affect to protein kinase C from 0.001 mM to 1.0 mM.[10] But in this study we found methamphetamine inhibited protein kinase C at 10 mM like antidepressants. Like those antidepressants, both cocaine and methamphetamine inhibit the uptake of monoamines in neurons.[11,12] The similarity in the effect on monoamine uptake between the drugs used in the present study and the antidepressants suggests the alternative possibility that the inhibitory effect of cocaine and methamphetamine may be associated with the inhibitory mechanism on monoamine uptake. This possibility seems to be supported by the findings that activation or inhibition of protein kinase C modulates the release or uptake of monoamines and glutamate.[3–6,8]

Recently, Giambalvo found that rats and synaptosomes treated with a small dose of amphetamine showed an increase in soluble and a decrease in the particulate protein kinase C activity, while a large dose had the opposite effect, decreasing the soluble and increasing the particulate activity of the enzyme.[17,18] Because the total activity in the soluble and particulate fractions was unchanged, the author suggested a translocation of the enzyme between the two

compartments. The present study measured protein kinase C activity in the soluble fraction from the rat cerebral cortex, and found that cocaine and methamphetamine significantly inhibited the activity of the enzyme at higher concentrations. It cannot be ruled out that the inhibitory effect of the two drugs our recent studies have indicated that the effects of antidepressants and carbamazapine are, at least in part, mediated via inhibiting action on protein kinase C.[10,19] Therefore, it is conceivable that cocaine and methamphetamine may produce neural action, at least in part, through the inhibitory effect on protein kinase C.

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