Bifunctional Action of Imipramine on Protein Kinase C from Rabbit Platelets *in Vitro*

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ABSTRACT. Protein kinase C from rabbit platelets in vitro is inhibited by 1.0 mM imipramine, which is a tricyclic antidepressant. However, 0.2 mM imipramine activated protein kinase C activity from rabbit platelets. Imipramine had a bifunctional action to protein kinase C from platelets in vitro. It is reported that activation of protein kinase C is associated with inhibition of monoamine reuptake in platelets. It is supposed that inhibitory effect of 5-HT uptake of imipramine may, at least in part, be associated with its stimulatory effect on protein kinase C.

Key words : imipramine — tricyclic antidepressants — protein kinase C — signal transduction — platelet

Protein kinase C(PKC), which takes part in cellular responses to various stimuli such as neurotransmitters, hormones and growth factors, plays an important role in cell proliferation and differentiation.[1] Several lines of evidence indicate that neurotransmitter uptake is linked to PKC activation. Treatment of human platelets with phorbol ester, which is an activator of PKC, has resulted in substantial reduction in the rate of platelet serotonin(5-HT) uptake.[2] It has also been reported that the activity of the presynaptic GABA transporter is suppressed by activation of the PKC.[3] Incubation of glial cells with phorbol ester has increased glutamate transport.[4] Na^+/phosphate cotransport in an epithelial cell line of renal origin was increased by phorbol ester.[5] These findings suggest that the uptake of neurotransmitters is modulated by PKC.

A number of monoamine uptake inhibitors such as imipramine have been revealed to inhibit the activity of PKC in rat brain.[6,7] However, PKC activation induced inhibition of the uptake of 5-HT in platelets.[2] Therefore, we intended to examine the effect of imipramine on PKC from platelets.

METHODS

Platelet PKC was prepared from rabbit blood by the methods of Fukamachi *et al*[8] and Kikkawa *et al*.[9] The platelets were subjected to a Branson Sonifer, model 250, for 45 sec. The concentration of protein in samples which were employed for an enzyme assay was determined by the method of Lowry *et al*.[10] All procedures were performed at 0-4℃.

Activation of PKC from the platelets was assayed by the Protein Kinase C

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Enzyme Assay System (Amersham) by measuring the amount of ^{32}p incorporated into an acceptor peptide.[7] 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 protein kinase C in 50 mM Tris-HCl buffer, pH 7.5. Incubation was carried out at 25℃ for 15 min with or without imipramine at the various concentrations indicated in Fig 2. The reaction was terminated by adding 100 μl of a stop reagent. Subsequently, 125 μl of aliquots of the reaction mixture was transferred onto binding paper and washed twice with 10 ml of 5%(v/v) acetic acid per sheet. The radioactivity of ^{32}p was determined in 10 ml of a scintillation fluid using a liquid scintillation spectrometer(ALOKA, LSC-700). The radioactivity was expressed as bequerel per mg protein per incubation time(Bq/mg protein/min). Statistical analyses of the data were conducted using the Student's t-test.

RESULTS

Fig 1 illustrates the optimal condition of PKC protein concentrations. Thirty μg protein was optimum. Twenty μg protein was added to this assay system, because of this amount was maximum dose in this incubation mixture.

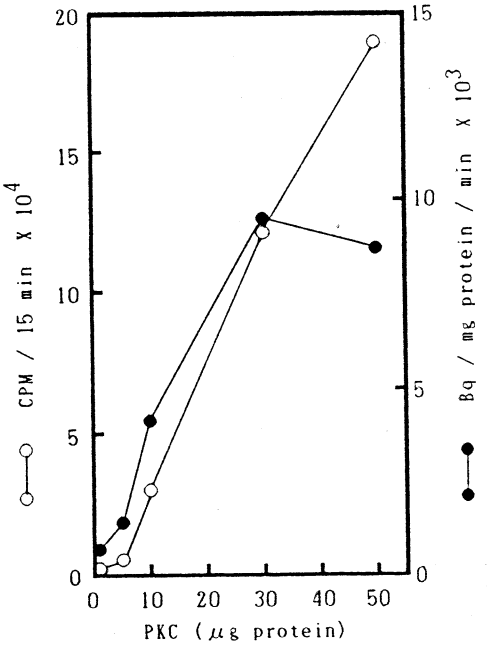

Fig 1. Optimum concentration of protein kinase C

Fig 2 illustrates the effect of imipramine on PKC activity from rabbit platelets. The platelets incubated with imipramine showed a biphasic dose-response change in the activity of PKC. At 0.2 mM, imipramine significantly increased the activity of PKC(from 5365.9±160.4 Bq/mg protein/

min to 6379.7±64.0 Bq/mg protein/min, n=5, p<0.01). At 1.0 mM, however, imipramine had the opposite effect of decreasing the activity of PKC(to 2091.3±67.0 Bq/mg protein/min, n=5, p<0.01).

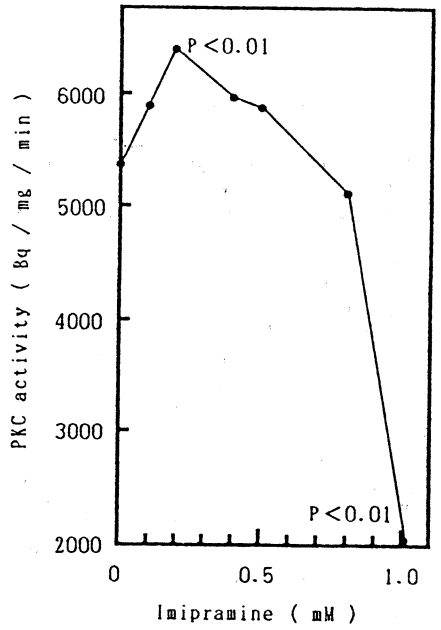

Fig 2. Effect of imipramine on protein kinase C

DISCUSSION

Imipramine as a tricyclic antidepressant inhibits the neuronal uptake of 5-HT at nerve terminals and also inhibits the uptake of 5-HT in platelets.[11] The neurotransmitters, including 5-HT and norepinephrine, uptake across neurotransmitter transporters on the plasma membrane of cells. Several lines of evidence indicate that neurotransmitter transporters are linked to PKC activation. Anderson and Horne[2] reported that PKC activators decrease 5-HT transport into human blood platelets. The present study revealed that PKC was increased by 0.2 mM of imipramine from platelets in vitro. To our knowledge, this is the first report demonstrating that imipramine increases PKC. We have also previously reported that desipramine, which is also a tricyclic antidepressant, increased PKC activity in platelets in vitro.[12]

However, imipramine has also been reported to inhibit PKC[6] and we also reported the same finding.[7] But these studies showed the data in the rat brain. Recently, many isoforms of the PKC family have been identified in many tissues. It is likely that the uptake and release of neurotransmitters are modulated by each PKC isoform.[13] We have no data on the effect of imipramine on each PKC isoform. It is also unknown what relationship there is between 5-HT uptake and PKC activity in the brain. The effect of imipramine on PKC in the brain may be different from that in platelets, or it may be that each PKC isoform has different action. However, the present study

supposes that the inhibitory effect of 5-HT uptake of imipramine may, at least in part, be associated with its stimulatory effect on PKC.

ACKNOWLEDGMENT

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