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Inhibition of the Activity of Phosphoinositide-Specific Phospholipase C Isozymes by Antipsychotics and Antidepressants

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Abstract—To elucidate the effect of antipsychotics and antidepressants on phosphoinositide(PI) second messenger system, we studied the dose-dependent inhibition of the phosphoinositide-specific phospholipase C(PLC) isozymes, β_1, γ_1 and δ_1, by fluphenazine and haloperidol as antipsychotics, and amitriptyline, maprotiline and mianserin as antidepressants. All the antipsychotics and antidepressants tested showed inhibition on at least one of the PLC isozymes with IC_{50} at the concentration between 25 and 250 μM. Maprotiline, mianserin and amitriptyline inhibited 80 to 90% of the activities of all three PLC isozymes at the concentration of 250 μM, while haloperidol and fluphenazine inhibited PLC β_1 and γ_1. But baclofen didn't inhibit any PLC isozyme. These results suggested that PLC isozymes are inhibited by antipsychotics and antidepessants even though the concentration is high, and these drugs may affect PI signal transduction system by direct inhibition of PLC isozymes.

Keywords □ phosphoinositide-specific phospholipase C (PLC), antipsychotics, antidepressants, enzyme activity inhibition.

Phosphoinositide-specific phospholipase C (PLC), which converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into two second messengers, inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), is a key enzyme in intracellular phosphoinositide signal transduction system (Berridge, 1984; Majerus *et al.*, 1986). PLC is known to have isozymes such as β_1, β_2, γ_1, γ_2, δ_1 and δ_2 (Rhee *et al.*, 1989; Kriz *et al.*, 1990). PLC β_1 is found predominantly in brain while γ_1 and δ_1 are found in other tissues as well as in brain. In brain, PLC β_1 and γ_1 are mainly distributed in the neurons, but PLC δ_1 is high in the glial cells. The differences in distribution of the isozymes are supposed to be related with the differences in the signal transduction system in various tissues (Gerfen *et al.*, 1988; Mizuguchi *et al.*, 1991; Suh *et al.*, 1988).

There are recent reports suggesting the relationship between the mechanism of action of antipsychotics or antidepressants and the metabolism of phosphoinositides (Leli *et al.*, 1989; Li *et al.*, 1991; Mori *et al.*, 1980; Pandey *et al.*, 1991). Long-term haloperidol treatment decreased carbachol and norepinephrine-induced inositol phosphate (IP) accumulation in rat frontal cortex and hippocampus (Li *et al.*, 1991). Various antipsychotics and antidepressants inhibited IP_3 formation in human platelets which suggests the possible inhibition of PLC activity (Pandey *et al.*, 1991). The accumulation of IP_3 by chlorpromazine in C_6 glial cell might be the result of direct increase of PLC activity not that of membrane receptor binding (Leli *et al.*, 1989). It has also been reported that chlorpromazine, amitriptyline, and imipramine can have indirect effects on PLC activity by influencing other enzymes in intracellular phospholipid metabolism (Abdel-Latif, 1983; Koul and Hauser, 1987; Leli *et al.*, 1989; Pelech and Vance, 1984; Wakatabe *et al.*, 1991; Zborowski and Brindley, 1983). The possibility of G-protein mediated effects of antipsychotics and/or antidepressants on the PLC has been suggested by the effect of these drugs on D_2 and/or 5-HT_2 receptor which is supposedly coupled with PLC

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(Fisher and Agranoff, 1987; Roth and Ciaranello, 1991; Vallar and Meldolesi, 1989).

We are studying the effects of antipsychotics and antidepressants on the intracellular phosphoinositide signal transduction system. Since most of the previous studies have been done with *in vivo* or *ex vivo* system, the direct effects of these drugs on PLC isozymes are not clarified yet. In this paper, we reported the inhibition of the activity of purified PLC isozymes, β_1, γ_1 and δ_1, by various antipsychotics and antidepressants.

We used haloperidol and fluphenazine as antipsychotics, and amitriptyline, maprotiline and mianserin as antidepressants. Since most of these drugs have cationic amphiphilic nature (Abdel-Latif, 1983; Kodavanti and Mehendale, 1990), baclofen, the charge of which is neutral in the experimental condition, was included (Ahuja, 1985).

All drugs except haloperidol were dissolved in distilled water. Haloperidol was dissolved in 0.1 N HCl. The activities of PLC isozymes were determined at various concentrations of each drugs, 2.5, 25, 250 and 500 μM. The PLC isozymes used in this study were purified from bovine brain by the procedure described (Rhee *et al.*, 1991). For the assay of the enzyme activity, 50 μl of enzyme fraction was added to 150 μl of reaction mixture which consists of phsophatidylinositol(300 μM, 20,000 cpm ^{3}H-PI, NEN), 1 mM ethyleneglycol bis(β-aminoethyl ether)tetraacetic acid (EGTA), 3 mM $CaCl_2$, 0.1% sodium deoxycholate and 50 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), pH 7.0. Each drug was added to the enzyme solution at various concentration and incubated at 4℃ for 30 min prior to start the reaction. The reaction mixture was incubated for 10 minutes at 37℃ and was suspended by adding 1 ml of chloroform/methanol (2 : 1). 0.3 ml of 5 mM EGTA, 1 N HCl solution was added to each reaction tube and thoroughly mixed. Half ml of aqueous layer was taken out, and the radioactivities were measured (Rhee *et al.*, 1991). All the experiments were repeated three times. The mean enzyme activities of three experiments with various concentrations of drugs were expressed as the percent activity compared to those without drugs.

Among the antipsychotics and antidepressants tested, fluphenazine, maprotiline and mianserin inhibited about 90 % of the PLC β_1 activity at the concentration of 250 μM, while haloperidol and amitriptyline reduced the enzyme activity by 70%. Baclofen did not inhibit any PLC activiity significantly at the same concentration. Among the drugs which showed inhibitory effect on the PLC β_1 activity at 250 μM, only maprotiline showed 50% inhibition of the enzyme activitity at 25 μM concentration while other drugs showed only 20 to 30% inhibition at the same concentration (Fig. 1A).

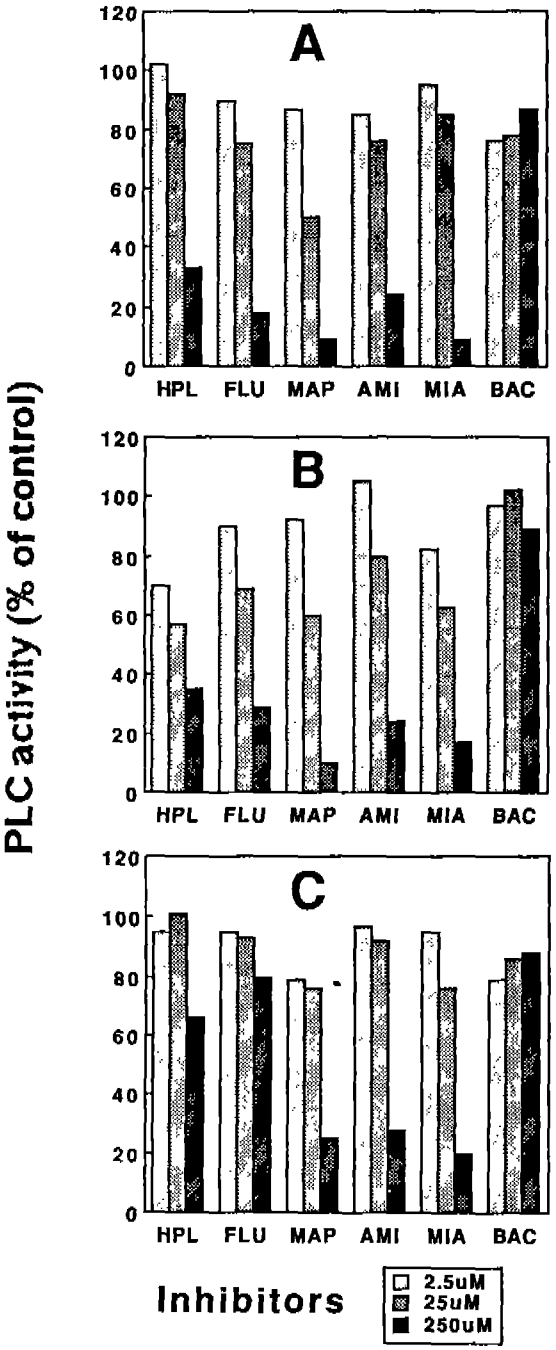

Fig. 1. Effect of antipsychotics and antidepressants on the PLC β_1 (A), PLC γ_1 (B) or PLC δ_1 (C) activities. The PLC acitivities are measured in the presence of designated concentrations of drugs and are expressed as percentage relative to the control (no inhibitor). Abbreviations used : Haloperidol; HPL, Fluphenazine; FLU, Maprotiline; MAP, Amitriptyline; AMI, Mianserin; MIA, Baclofen; BAC.

The inhibition of PLCγ_1 activity by the antipsychotics

and antidepressants were almost same as that of PLC β_1 activity. But haloperidol and mianserin showed about 20% more inhibition at the concentration of 25 μM compared to that of PLC β_1 (Fig. 1B).

Maprotiline, mianserin and amitriptyline also inhibited PLC δ_1 activity at the concentration of 250 μM, while haloperidol and fluphenazine did not show any significant reduction of the enzyme activity. At the concetration of 25 μM, any of the drug showed significant inhibition of the PLC δ_1 acivity. As of PLC β_1 and γ_1, baclofen did not inhibit PLC δ_1 (Fig. 1C). At the concentration of 2.5 and 0.25 μM, no drug showed inhibitory effect on the activity of any PLC isozymes.

These results indicate that antipsychotics and antidepressants tested in this experiment, except baclofen, showed inhibition of at least one of the PLC isozymes. But the inhibitory effects on each isozyme were somewhat different from drugs to drugs, while there was no difference between antipsychotics and antidepressants. Maprotiline, mianserin and amitriptyline inhibited 80 to 90% of the activities of all the three PLC isozymes at the concentration of 250 μM, while fluphenazine inhibited PLC β_1 and γ_1 at the same concentration. Haloperidol also inhibited PLC β_1 and γ_1, but the inhibition was weaker than other drugs. Baclofen, which is neutral in experimental condition didn't inhibit any PLC isozyme.

The inhibition of PLC isozymes by various antipsychotics and antidepressants may explain the reported effects of these drugs on phsophoinositide signal transduction system, such as the decrease of carbachol-stimulated and norepinephrine-sensitive IP accumulation in rat brain by haloperidol (Li *et al.*, 1991) and the inhibition of IP_3 formation in huamn platelet by various antipsychotics and antidepressants (Pandey *et al.*, 1991). Since most of these drugs inhibited PLC isozymes at relatively high concentration, it is uncertain that they really inhibit PLC isozymes in vivo at the therapeutic concentration. Usually the therapeutic plasma levels of these drugs are micromolar or lower (Baldessarini, 1985). But there is a possibility that cellular concentration of these drugs may be higher than that of plasma. The *in vitro* results may not reflect *in vivo* state because of many factors affecting the enzyme activity assay system *in vitro* and various unknown factors affecting PLC activity *in vivo* (Hofmann and Majerus, 1982).

The antipsychotics and antidepressants which exerted inhibition on PLC isozymes are cationic amphiphilic drugs (Abdel-Latif, 1983; Kodavanti and Mehendale, 1990), therefore these drugs may inhibit the activity of PLC isozymes by direct electrostatic interaction to the active site of the enzyme. Another possible mechanism is the reduction of available substrate by forming drug-phospholipid complex (Lullmann *et al.*, 1978), which is suggested by the detergent effects of cationic amphiphilic drugs (Ogiso et al, 1976; Yamaguchi *et al.*, 1985).

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