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Short communication

Effects of imipramine and sertraline on protein kinase activity in rat frontal cortex

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Abstract

Three-week administration of sertraline or imipramine to rats (10 mg/kg, intraperitoneally, twice a day) increased ex vivo cyclic AMP-dependent protein kinase activity in the soluble but not in the particulate fraction of the frontal cortex. However, cyclic AMP-dependent protein kinase activity was not affected in either fraction of the parietotemporal cortex and hippocampus. Neither antidepressant altered protein kinase C activity in the soluble and particulate fractions or Ca^{2+}/calmodulin-dependent protein kinase II activity in the frontal cortex. Therefore, sertraline and imipramine both selectively enhance cyclic AMP-dependent protein kinase activity in the frontal cortex. This enhancement might be involved in their biochemical mechanisms. © 1998 Elsevier Science B.V.

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1. Introduction

Although the clinical efficacy of antidepressants is generally accepted, the precise neurochemical mechanisms underlying their antidepressive effects are still unclear. Many investigators report that long-term administration of tricyclic antidepressants results in the down-regulation of β and 5-HT_2 receptors and desensitization of adenylate cyclase coupled to monoamine receptors in rat brain. However, long-term administration of selective serotonin reuptake inhibitors, such as fluoxetine, dose not produce these effects in rat brain (Goodnough and Baker, 1994). The results of our previous study also indicate that long-term administration of sertraline, a potent selective serotonin reuptake inhibitor, has little effect on monoamine receptors and adenylate cyclase activity in rat brain (Tadokoro et al., 1997). Therefore, it seems unlikely that such changes are primarily responsible for the clinical effects of antidepressants.

Recently, long-term administration of antidepressants has been reported to affect molecules, such as protein kinases, involved in the intracellular signal transduction system in neurons (Nestler et al., 1989; Perez et al., 1989; Racagni et al., 1992; Mann et al., 1995; Popoli et al., 1995; Shelton et al., 1996). Such findings suggest that changes in the phosphorylation of functional proteins by protein kinases in neurons are involved in the therapeutic mechanisms of antidepressants. In neurons, cyclic AMP-dependent protein kinase, protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II are important serine/threonine protein kinases that modulate a variety of functions. Perez et al. (1989) have reported that long-term desmethylimipramine administration increases the endogenous phosphorylation of MAP-2, a representative microtubule-associated protein, in the rat cerebral cortex. MAP-2 can be phosphorylated by cyclic AMP-dependent protein kinase, protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II in the brain (Fukunaga et al., 1992). The functions of cyclic AMP-dependent protein kinase and protein kinase C are known to be regulated by changes in the subcellular distribution of their activity. Therefore, in the present study we investigated the effects of the antidepressants imipramine and sertraline on the activities of cyclic AMP-dependent protein kinase and protein kinase C in the soluble and particulate fractions and on the total activity of Ca^{2+}/calmodulin-dependent protein kinase II in rat cerebral cortex. We also discuss the possible in-

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volvement of protein phosphorylation in the effects of antidepressants.

2. Materials and methods

2.1. Chemicals

[γ-^{32}P]ATP (tetra [triethylammonium] salt) (3000 Ci/mmol) was purchased from Dupont-NEN (Boston, MA). The following reagents were obtained from commercial sources: imipramine, leupeptin and protein kinase C (from rat brain; Sigma Chemical Co., St. Louis, MO), protein kinase C inhibitor peptide and cyclic AMP-dependent protein kinase inhibitor peptide (UBI, Lake Placid, NY), cyclic AMP-dependent protein kinase (Pierce, Rockford, IL) and syntide-2 (Bachem Fein Chemikaling, Bubendorf, Switzerland). Sertraline was kindly donated by Pfizer Pharmaceuticals (Groton, CT). All other chemicals were of analytical grade or of the highest grade commercially available.

2.2. Animals

Male Sprague–Dawley rats (6 weeks old) purchased from Sankyo Labo Service Co. (Tokyo, Japan) were housed in groups of six in stainless-steel cages. Room temperature and lighting were maintained at 24°C and there was a 12–12 h light–dark cycle. Food and water were freely available. All procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals and were approved by our Animal Care and Use Committee.

Sertraline (10 mg/kg), imipramine (10 mg/kg), or vehicle (1.5% Tween 80, control) was administered intraperitoneally in a volume of 2 ml/kg twice a day for 3 weeks. 24 h after the last injection, rats were decapitated and their brains were rapidly removed. According to the methods of Glowinski and Iversen (1966), the cerebral cortex and hippocampus were dissected and the cortex was divided into two parts by coronal section at the level of the chiasma opticum. The anterior (rostral) half was considered the frontal cortex and the posterior (caudal) half was considered the parietotemporal cortex.

2.3. Cyclic AMP-dependent protein kinase assay

The frontal cortex, parietotemporal cortex and hippocampus were each homogenized with eight strokes (800 rpm) in a glass/Teflon homogenizer in ice-cold 25 mM Tris–HCl (pH 7.2) buffer containing 1 mM EGTA, 10 mM NaF, 1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride and 10 mg/l leupeptin. The homogenate was then ultracentrifuged at 100,000 g for 10 min at 4°C. The supernatant was designated the soluble fraction. The pellet was resuspended in homogenization buffer and designated the particulate fraction. The cyclic AMP-dependent protein kinase activity in both subcellular fractions was assayed with a colorimetric assay kit (SpinZyme Format, Pierce), following the manufacturer's instructions. Specific activity was defined as the difference between total activity and activity in the presence of 30 μM cyclic AMP-dependent protein kinase inhibitor peptide.

2.4. Protein kinase C assay

The frontal cortex was homogenized in ice-cold 50 mM Tris–HCl (pH 7.5) buffer containing 5 mM EDTA, 10 mM EGTA, 50 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride and 50 mg/l leupeptin with eight strokes (800 rpm) in a glass/Teflon homogenizer. The homogenate was then centrifuged at 100 000 $\times$ g for 30 min at 4°C. The resulting supernatant was designated the soluble fraction. The pellet was suspended in homogenization buffer containing 0.5% Triton X-100 and incubated for 45 min on ice. The supernatant after recentrifugation at 100 000 $\times$ g for 30 min at 4°C was designated the particulate fraction. Protein kinase C activity in subcellular tissue fractions was measured with a MESACUP protein kinase assay kit (Medical and Biological Laboratories, Nagoya, Japan), following the manufacturer's instructions. Specific activity was defined as the difference between the total activity and activity in the presence of 20 μM protein kinase C inhibitor peptide.

2.5. Ca^{2+}/calmodulin-dependent protein kinase II assay

The frontal cortex was homogenized in ice-cold 50 mM HEPES (pH 7.4) buffer containing 0.1% Triton X-100, 25 mM NaF, 15 mM $Na_4P_2O_7$, 100 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, 75 μM pepstatin, 100 nM okadaic acid and 100 nM calyculin A. After three 5 s sonications (Sonifier 200, Branson, Danbury, CT), the homogenate was centrifuged at 15,000 g for 5 min at 4°C. The supernatant was used in the kinase assay. The Ca^{2+}/calmodulin-dependent protein kinase II assay contained 50 mM HEPES (pH 7.5), 1 mM $CaCl_2$, 10 mM magnesium acetate, 0.1 mM [γ-^{32}P]ATP, 1 mg/ml bovine serum albumin, 3 μM calmodulin and 40 μM syntide-2 in a final volume of 25 μl. After incubation at 30°C for 3 min, a 15 μl aliquot was spotted on phosphocellulose paper. The paper was washed four times in 75 mM phosphoric acid. The radioactivity remaining on the filter was determined with a Tri-Carb Liquid Scintillation Counter (Packard, Meriden, CT) in Aquasol-2. In the preliminary experiment, the kinase activity was checked and found linear for 3 min.

The protein concentration was determined according to the method of Markwell et al. (1978), using bovine serum albumin as standard.

2.6. Statistics

Data are expressed as means ± S.E.M. for six rats. Statistical analysis was performed with a Student's *t*-test. A probability level (*P*-value) of < 0.05 was considered to indicate statistical significance.

3. Results

Long-term administration of imipramine and sertraline slightly but significantly increased the ex vivo activity of cyclic AMP-dependent protein kinase compared with that of control in the soluble fraction (8.0 and 12.3% greater than control values, respectively) but not in particulate fraction of frontal cortex (Fig. 1). However, neither imipramine nor sertraline significantly affected cyclic AMP-dependent protein kinase activity in either fraction of the parietotemporal cortex or hippocampus.

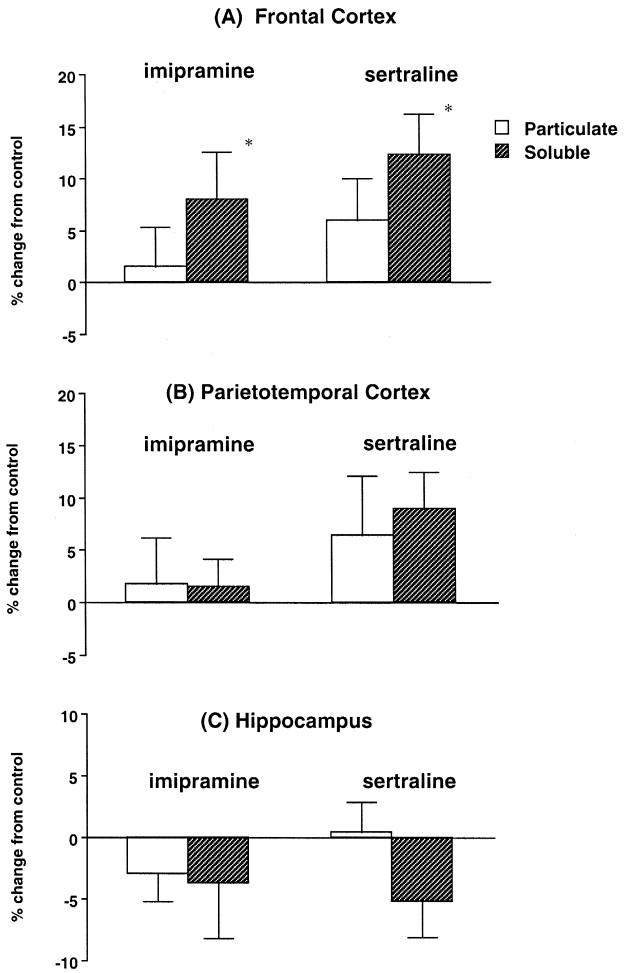

Fig. 1. Changes in cyclic AMP-dependent protein kinase activity in rat frontal cortex (A), parietotemporal cortex (B) and hippocampus (C) after administration of imipramine or sertraline (10 mg/kg, intraperitoneally, twice a day for 21 days). Data are percent changes from control and represent the means ± S.E.M. for six animals in each treatment group. The cyclic AMP-dependent protein kinase activity in soluble and particulate fractions of the frontal cortex of control rats was 10.39 ± 0.30 and 12.96 ± 0.39 U/ng protein, respectively. * $P < 0.05$ versus control.

We also determined the in vitro cyclic AMP-dependent protein kinase activity in the soluble and particulate fractions of the frontal cortex after incubation with imipramine and sertraline (1, 10 and 100 μM) at 37°C for 5 min. However, we did not observe a significant change in the kinase activity in either fraction with either antidepressant (data not shown).

Protein kinase C activity in the frontal cortex after vehicle (control), imipramine and sertraline treatment was 63.6 ± 3.1 (100.0%), 63.5 ± 3.4 (99.9%) and 71.7 ± 4.2 (112.7%) U/ng protein in the soluble fractions and 43.9 ± 1.9 (100.0%), 44.6 ± 2.6 (101.6%) and 47.4 ± 2.0 (107.9%) U/ng protein in the particulate fractions, respectively. Total Ca^{2+}/calmodulin-dependent protein kinase II activity in the frontal cortex after treatment with vehicle, imipramine and sertraline was 419.8 ± 12.5 (100.0%), 409.0 ± 7.7 (97.4%) and 428.8 ± 11.9 (102.1%) fmol/mg protein/min, respectively. Thus, long-term administration of imipramine or sertraline did not significantly affect either protein kinase C activity in the soluble and particulate fractions or total Ca^{2+}/calmodulin-dependent protein kinase II activity in the frontal cortex.

4. Discussion

Cyclic AMP-dependent protein kinase consists of a catalytic subunit and a regulatory subunit, which suppresses its catalytic activity. When the kinase is activated, the catalytic subunit is translocated from the cytosol to the nucleus. Therefore, in the present study we used subcellular fractionation to assess the change in the translocation after long-term administration of antidepressants. We found that long-term administration of either imipramine or sertraline increased ex vivo cyclic AMP-dependent protein kinase activity in the soluble fraction of the frontal cortex but not in the parietotemporal cortex or the hippocampus. This regional increase in the kinase activity might result from either increased protein expression or increased affinity of the enzyme for substrate in the soluble fraction. Because an excessive amount of cyclic AMP (100 μM) was added to the incubation medium, the measured cyclic AMP-dependent protein kinase activity might reflect the content of the kinase protein. However, determining changes in translocation of the enzyme on the basis of these findings might be difficult. Further studies, especially those measuring protein levels of cyclic AMP-dependent protein kinase are necessary to elucidate the precise mechanism of the increased activity. However, the increased activity was unlikely to be due to acute and direct stimulation of enzyme activity, since in vitro experiments showed that cyclic AMP-dependent protein kinase activity was not directly enhanced when either antidepressant is administered.

Few studies have examined the effects of long-term administration of antidepressants on cyclic AMP-dependent protein kinase in the rat brain. Nestler et al. (1989) have reported that imipramine and tranylcypromine may stimulate the translocation of the kinase in rat frontal cortex. Racagni et al. (1992) have demonstrated that long-term administration of desipramine increases the regulatory subunit of cyclic AMP-dependent protein kinase in the soluble fraction of rat cortex; however, the functional significance of this increase remains uncertain. Patients with untreated depression have significantly lower cyclic AMP-dependent protein kinase activity in skin fibroblasts than do normal subjects (Shelton et al., 1996). Although comparison of data from humans with the present findings for rats is difficult, our results suggest that the increase in cyclic AMP-dependent protein kinase activity in the frontal cortex may be involved in the neurochemical mechanism of action of antidepressants.

Both protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II are important protein kinases in the regulation of receptor function, neurotransmitter release, and neuronal plasticity. Because we detected changes in cyclic AMP-dependent protein kinase activity in the frontal cortex, we also examined whether the activities of protein kinase C and Ca^{2+}/calmodulin-dependent protein kinase II are also affected in the same regions of the rat brain. However, we did not observe changes in either kinase activity in the frontal cortex after long-term administration of antidepressants. These findings suggest that, of the three major protein kinases in rat frontal cortex, only cyclic AMP-dependent protein kinase exhibits increased activity in the soluble fraction after treatment with imipramine and sertraline. However, whether translocation is involved in this change is unknown.

Mann et al. (1995) have reported that administration of fluoxetine and desipramine inhibits ex vivo protein kinase C activity in the rat brain. Although their dosage protocol and assay procedure were different from ours, the reason for the discrepancy is unclear. Our findings for Ca^{2+}/calmodulin-dependent protein kinase II activity seem to be in accordance with those of Popoli et al. (1995), who found that long-term administration of paroxetine, fluvoxamine and venlafaxine did not affect Ca^{2+}-dependent autophospho-rylation of Ca^{2+}/calmodulin-dependent protein kinase II in the total cortex.

Our present findings suggest that cyclic AMP-dependent protein kinase in the frontal cortex might represent an intracellular target of antidepressants beyond the level of receptors. Cyclic AMP-dependent protein kinase can phosphorylate MAP-2 and lead to alteration of microtubule function in neurons (Perez et al., 1989). Furthermore, activation of cyclic AMP-dependent protein kinase might result in the phosphorylation of specific nuclear proteins, such as cyclic AMP response element binding protein. Therefore, antidepressant-induced increases in cyclic AMP-dependent protein kinase activity might result in alterations in cytoskeleton function and gene expression and lead to changes in neuronal plasticity in the brain, which might be involved in the biochemical mechanisms of action of antidepressants.

References

Fukunaga, K., Soderling, T.R., Miyamoto, E., 1992. Activation of Ca^{2+}/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. J. Biol. Chem. 267, 22527–22533.

Glowinski, J., Iversen, L.L., 1966. Regional studies of catecholamines in the rat brain-1. The disposition of ^{3}H-nor-epinephrine, ^{3}H-dopamine and ^{3}H-dopa-in various regions of the brain. J. Neurochem. 13, 655–669.

Goodnough, D.B., Baker, G.B., 1994. 5-Hydroxytryptamine 2 and β-adrenergic receptor regulation in rat brain following chronic treatment with desipramine and fluoxetine alone and in combination. J. Neurochem. 62, 2262–2268.

Mann, C.D., Bich, V.T., Hirda, P.D., 1995. Protein kinase C in rat brain cortex and hippocampus: Effect of repeated administration of fluoxetine and desipramine. Br. J. Pharmacol. 115, 595–600.

Markwell, M.A.K., Haas, S.M., Bieber, L.L., Tolbert, N.E., 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. 87, 206–210.

Nestler, E.J., Terwilliger, R.Z., Duman, R.S., 1989. Chronic antidepressant administration alters the subcellular distribution of cyclic AMP-dependent protein kinase in rat frontal cortex. J. Neurochem. 53, 1644–1647.

Perez, J., Tinelli, D., Brunello, N., Racagni, G., 1989. cAMP-Dependent phosphorylation of soluble and crude microtubule fractions of rat cerebral cortex after prolonged desmethylimipramine treatment. Eur. J. Pharmacol. 172, 305–316.

Popoli, M., Vocaturo, C., Perez, J., Smeraldi, E., Racagni, G., 1995. Presynaptic Ca^{2+}/calmodulin-dependent protein kinase II: Autophosphorylation and activity increase in the hippocampus after long-term blockade of serotonin reuptake. Mol. Pharmacol. 48, 623–629.

Racagni, G., Brunello, N., Tinelli, D., Perez, J., 1992. New biochemical hypotheses on the mechanism of action of antidepressant drugs: cAMP-Dependent phosphorylation system. Pharmacopsychiatry 25, 51–55.

Shelton, R.C., Manier, D.H., Sulser, F., 1996. cAMP-Dependent protein kinase activity in major depression. Am. J. Psychiatry 153, 1037–1042.

Tadokoro, C., Kiuchi, Y., Yamazaki, Y., Nara, K., Oguchi, K., Kamijima, K., 1997. Behavioral stimulation without alteration of β and 5-HT receptors and adenylate cyclase activity in rat brain after chronic sertraline administration. Psychopharmacology 130, 124–130.