The Direct Effect of Neuroleptics on C-kinase and M-kinase in Rat Brain

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ABSTRACT. Phospholipid-sensitive Ca^{2+}-dependent protein kinase (C-kinase) and its catalytic fragment (M-kinase) were extracted from the rat cerebral cortex. Chlorpromazine and mosapramine inhibited the activity of C-kinase and M-kinase. Haloperidol inhibited the activity of M-kinase. Sulpiride have no effect on C-kinase and M-kinase. These neuroleptics have different effects on clinical psychiatric symptoms. It is possible that the differential effect of neuroleptics may be related to the second messenger level.

Key words: neuroleptics — C-kinase — M-kinase — antipsychotic effect

Protein kinase C (C-kinase), which is believed to phosphorylate a number of proteins, is one of the agents in the signal transduction pathways. First identified in 1977,[1,2] it is a calcium and phospholipid dependent protein serine and threonine specific kinase. It is now known that sn-1, 2-diacylglycerol (DG), generated by receptor mediated hydrolysis of membrane phospholipids, particularly phosphatidylinositol 4, 5-bisphosphate (PIP_2), activates this enzyme by increasing its affinity for calcium ions.

Many drugs and compounds such as tranquilizers have been shown to interact with membrane phospholipid and then affect a variety of neuronal as well as non-neuronal cellular activities.[3] In this study, we investigated the effects of several classes of neuroleptics on C-kinase and its catalytic fragment (M-kinase) in the rat cerebral cortex in vitro.

MATERIALS AND METHODS

C-kinase and M-kinase were extracted from rat brain and enzyme activity was assayed as previously described.[4]

Source of enzyme and crude enzyme preparations

Wistar rats were decapitated and the cerebral cortex was quickly removed and homogenized in a homogenizer with 3 volumes of 20 mM Tris-HCl at pH 7.5 containing 2 mM EDTA, 10 mM benzamide and 0.05 mg/ml PMSF. The homogenate was centrifuged for 40 min at 20000 X g. The supernatant was filtered through glass wool to remove lipids. The filtrate was employed as the crude enzyme. Proteins concentrations was determined by the method of Lowry using bovine serum albumin as the standard.

Enzyme assay

C-kinase activity in the crude enzyme was assayed in tripricate using the Protein Kinase C Enzyme Assay System (Amersham), and 0.005 mg of protein per assay. 1 mM of each neuroleptics was added directly to the assay system. M-kinase activity in the crude enzyme was assayed in tripricate using the protein kinase C Enzyme Assay System without Ca^{2+}, phospholipid and phorbol 12-myristate 13-acetate.

RESULTS

The results are shown in Fig 1.

1) 1 mM of chlorpromazine inhibited the enzyme activity of C-kinase and M-kinase.
2) 1 mM of haloperidol had no effect on the enzyme activity of C-kinase, but inhibited the enzyme activity of M-kinase.
3) 1 mM of mosapramine inhibited the enzyme activity of C-kinase and M-kinase.
4) 1 mM of sulpiride had no effect on the enzyme activity of C-kinase and M-kinase.

DISCUSSION

Since the introduction of chlorpromazine for the treatment of the schizophrenia in the 1950s, the efficacy of the neuroleptics had been clear and dramatic. Since then, a large number of structural analogs of chlorpromazine and other more novel compounds have been prepared, and they may be categorized into several classes: the phenothiazines (for example, chlorpromazine), the butyrophenones (for example, haloperidol), the iminodibenzyles (for example, mosapramine), the benzamides (for example, sulpiride), the thioxanthenes and the indole derivatives. The prevailing theory regarding the mechanism of action of neuroleptics is based on the observation that all of the currently available neuroleptics have a similar action on the dopamine system: They block binding of dopamine to the postsynaptic dopamine receptor in the brain.[5] The dopamine-2 (D-2) receptor, which is not linked to adenylate cyclase, is believed to be responsible for the action of this class of drugs.[6] However, each class of the neuroleptics has a different effect. For example, the phenothiazines are the most sedative, and the butyrophenones have powerful antipsychotic effects but have relatively little sedative effect. Those effects cannot be explained by only the blockage of the activity of dopamine. The mechanism of the action of neuroleptics is far more complex than solely action.

C-kinase was one of the signal transduction pathway components. C-kinase is ubiquitous in tissues and organs and is normally recovered from the soluble fraction. When cells are stimulated, an apparent translocation to the membrane occurs in a calcium dependent manner.[7] Once bound to the membrane, C-kinase is believed to phosphorylate a number of proteins. It is converted to M-kinase upon limited proteolysis with membrane-bound, trypsin-like protease.[8] M-kinase is entirely independent of Ca^{2+}, phospholipid, and DG. This M-kinase could phosphorylate protein more rapidly than C-kinase.[9]

Chlorpromazine, which is one of the neuroleptics, has inhibited the

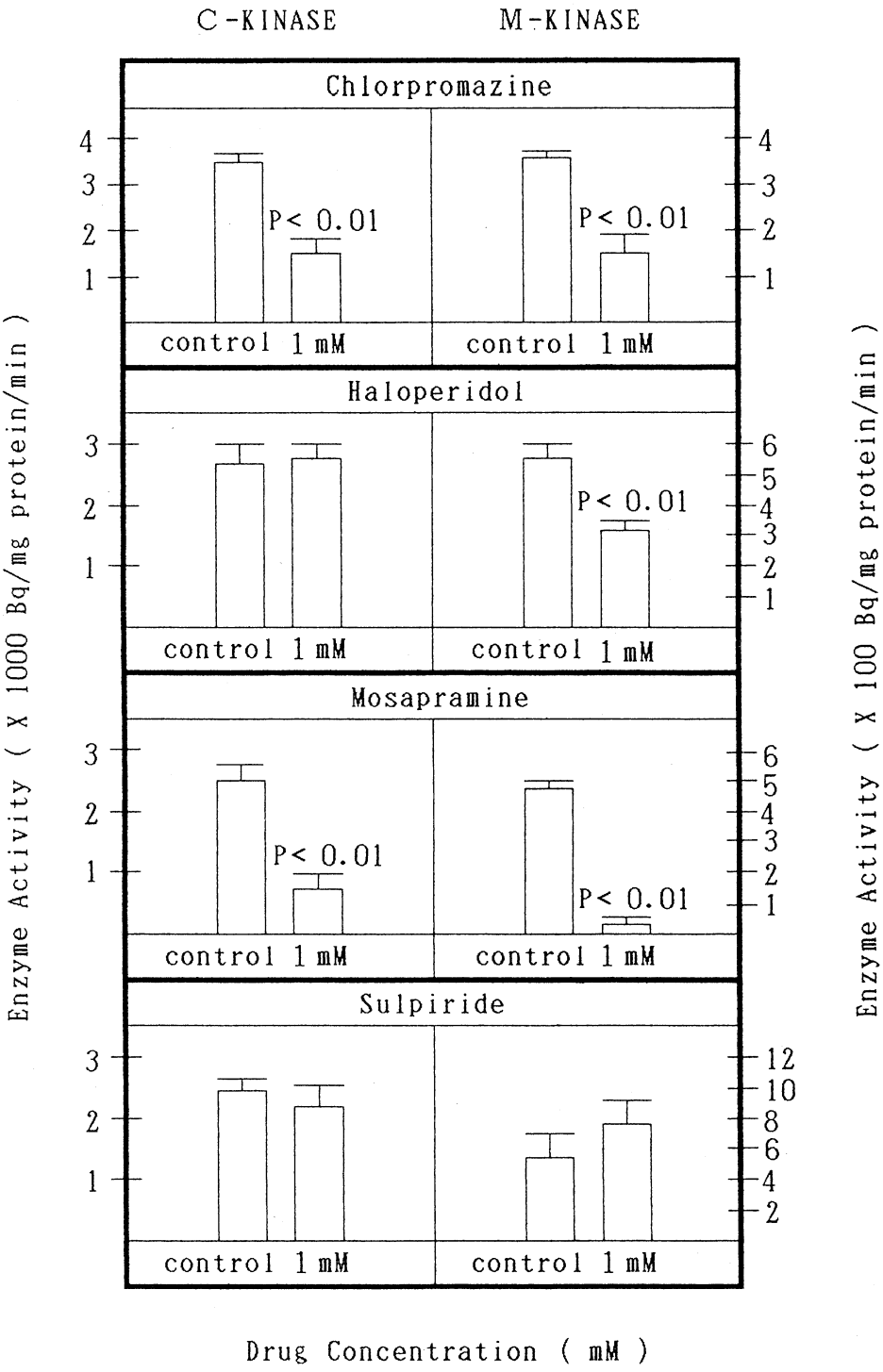

Fig 1. Effect of the neuroleptics (1 mM) on C-kinase and M-kinase
control : without drugs n=3

activity of C-kinase in rat brain.[10] Giambalvo[11] reported that C-kinase was related to dopamine transport. This evidence suggests that the second messengers, including C-kinase and M-kinase, are related to the mechanism of schizophrenia.

In the present study, four classes of neuroleptics (the phenothazines, butyrophenones, iminodibenzyles and benzamides) were studied. These drugs have different power against clinical psychotic symptoms. However, these mechanisms cannot be explained by only the blockage of the activity of dopamine. These neuroleptics affect the inhibition of the activity of C-kinase and M-kinase differently. Thus, it is possible that the differential effect of the neuroleptics may be related to the second messenger level.

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