Inhibitory Action of Drugs on Calcium-activated, Phospholipid-dependent Protein Kinase*

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Ca2+-activated, phospholipid-dependent protein kinase recently found in mammalian tissues (Takai, Kinase* 1978, Nishizuka, Kishimoto, et al. 1979, Yoshimi et al., 1979) is inhibited by various phospholipid-interacting drugs such as chlorpromazine, imipramine, phenolamine, dibucaine, verapamil, and tetracaine. This effect is attributed to the inhibition of the activation process but not to the interaction with the active site of enzyme. This is supported by the fact that a catalytic fragment of this enzyme, which is obtained by limited proteolysis with Ca2+-dependent neutral protease, is fully active without Ca2+ and phospholipid and is not susceptible to any of these drugs. Kinetic analysis suggests that these drugs cause such inhibition competitively with phospholipid. None of these drugs appears to compete with Ca2+ or to counteract the unique effect of unsaturated diacylglycerol. Unsaturated diacylglycerol has been shown previously to increase markedly the affinity of enzyme for Ca2+ as well as for phospholipid and thereby serves as an initiator for the activation of this protein kinase. Nor cyclic AMP-dependent nor cyclic GMP-dependent protein kinase is susceptible to these phospholipid-interacting drugs.

Many drugs and compounds such as local anesthetics and tranquillizers have been shown to interact with membrane phospholipid and then affect a variety of neuronal as well as non-neuronal cellular activities (1, 2). For instance, several local anesthetics and other phospholipid-interacting com-

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RESULTS AND DISCUSSION

Among a large number of local anesthetics, tranquilizers, and other drugs which are known as phospholipid-interacting compounds, the following six drugs were chosen simply because these were often used to modify various cellular processes (1, 2): chlorpromazine, imipramine, phentolamine, dibucaine, verapamil, and tetracaine. Fig. 1 shows that protein kinase C was inhibited to variable extents by these drugs when the enzyme was assayed in the presence of Ca"", phospholipid, and diolein as an unsaturated diacylglycerol. Chlorpromazine appeared to be the most potent inhibitor. In preceding reports from this laboratory (19, 30), protein kinase C has been shown to be alternatively activated in an irreversible manner by limited proteolysis with Ca""-dependent neutral protease. In this process, a catalytically fully active fragment is produced which is entirely independent of Ca", phospholipid, and unsaturated diacylglycerol. It was noted that such a catalytic fragment was not susceptible to any of these drugs, as shown in Table 1. It is unlikely, therefore, that these drugs interact with the active center of enzyme or with the substrate protein and thereby inhibit the enzymatic reaction. In addition, Ca""-dependent neutral protease was inhibited by none of these drugs. Thus, these drugs specifically blocked the reversible activation process which may be elicited by association with membrane phospholipid but did not interfere with the irreversible activation process which may be mediated by Ca""-dependent neutral protease. Inversely, the irreversible proteolytic activation process was inhibited in purified system by various thiold protease inhibitors such as leupeptin and E-64. These protease inhibitors did not affect the reversible activation process nor the already proteolytically activated protein kinase C. It was also noted that protein kinases A and G were not susceptible to any of the phospholipid-interacting drugs nor to protease inhibitors.

Next, experiments were performed to explore the mode of inhibitory action of these drugs. Since the reversible activation of protein kinase C requires the simultaneous presence of Ca"", phospholipid, and unsaturated diacylglycerol, each of the three factors was varied in the presence and absence of the phospholipid-interacting drugs mentioned above. As shown in Fig. 2, these drugs appeared to slightly modify the $K_v$ value for Ca"", the concentration needed for the half-maximum velocity, but the results seemed to be difficult to

![Fig. 1. Inhibition of protein kinase C by various phospholipid-interacting drugs.](image1)

![Fig. 2. Effects of various phospholipid-interacting drugs on reaction velocity of protein kinase C at various concentrations of CaCl2.](image2)

**Table 1**

Effects of various phospholipid-interacting drugs on protein kinase C and its catalytic fragment

| Drug added  | Protein kinase C | Catalytic fragment |
|-------------|-----------------|-------------------|
| None        | 19,000          | 19,180            |
| Chlorpromazine | 1,380 (93)     | 19,000 (0)        |
| Imipramine  | 1,190 (94)      | 15,900 (20)       |
| Phentolamine| 1,580 (92)      | 15,150 (20)       |
| Dibucaine   | 1,600 (92)      | 18,990 (0)        |
| Verapamil   | 2,970 (85)      | 26,300 (15)       |
| Tetracaine  | 7,720 (61)      | 17,260 (10)       |
protein kinase C may not be necessarily the sole target of these drug actions, several drugs mentioned above may serve as a useful tool for differentiating roles of the three sets of closely similar enzyme systems, namely protein kinases A, G, and C.

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