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PRELIMINARY COMMUNICATIONS

CAN CALMODULIN INHIBITORS BE USED TO PROBE CALMODULIN EFFECTS ?

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As a result of recent works on calcium-binding proteins, and in particular on calmodulin, the role of calcium ions in cellular regulation is beginning to be understood at the molecular level. Many of the Ca^{2+} effects - including contraction, secretion, and most metabolic pathways - appear to be exerted through calmodulin-activated enzymes (for review, see ref. 1 and 2). The inhibition of calmodulin can be observed *in vitro* using various drugs, the more potent being phenothiazines and butyrophenones (3,4). Consequently, calmodulin inhibitors are currently being used to probe calmodulin effects, i.e. to suggest that calmodulin plays a role in various phenomena. The dissociation constant (K_d) of the drugs for calmodulin is about 10^{-6}M in the presence of calcium (4). Here, it can be pointed out that their K_d for dopamine and 5-hydroxytryptamine receptors are about 1000-fold smaller, and it was recently shown that the inhibition of calmodulin by these neuroleptics is unrelated to their clinical effectiveness (5). The molecules used as calmodulin inhibitors, are highly hydrophobic. The octanol:water partition coefficient of these compounds, used as an index of liposolubility, correlate very well with the concentrations necessary to cause half-maximal inhibition of calmodulin activity. Therefore, it was suggested that these drugs inhibit calmodulin through non-stereospecific hydrophobic interactions (5,6). The present paper shows a correlation between the two properties of phenothiazines to inhibit calmodulin and to stabilize membranes, and consequently questioned their use as probes for calmodulin effects.

The potency of drugs as calmodulin inhibitors was determined by measuring the activity of a cyclic nucleotide phosphodiesterase purified from rabbit aorta (7) in the presence of Ca^{2+}, calmodulin, and various drug concentrations. The results obtained were in accordance with previous determinations using brain enzyme and calmodulin (3,4). The membrane stabilization values were those reported by Seeman et al (8,9), measuring the protection provided by drugs against the lysis induced by hypotonic medium of human red blood cells. Fig. 1 shows the correlation between the inhibition of calmodulin by drugs and the membrane stabilization property of the same compounds. Both phenomena were observed within the same range of drug concentrations, the better calmodulin inhibitors being at the same time the better membrane stabilizers. The correlation coefficient is 0.801 indicating that both properties of drugs are closely related. Consequently, when these drugs are applied to biological systems, the effects observed may result either from calmodulin inhibition or from membrane stabilization.

Membrane stabilization involved impairment of ionic fluxes. As calcium-dependent phenomena are regulated through the fluxes of Ca^{2+} at the level of the plasma or organelles membranes, the impairment of the fluxes by drugs is sufficient to observe an effect, independently of calmodulin. Alternatively, it can be suggested that membrane stabilization is partly linked to calmodulin inhibition. One of the various enzymes stimulated by calmodulin is phospholipase A_2 (10) which hydrolyzed membranous phospholipids, thus increasing membrane permeability (11). Consequently, the decrease of the activity of this enzyme by calmodulin inhibitors could contribute to the stabilization effect. These different possibilities should be taken into account when interpreting data obtained by means of "calmodulin inhibitors".

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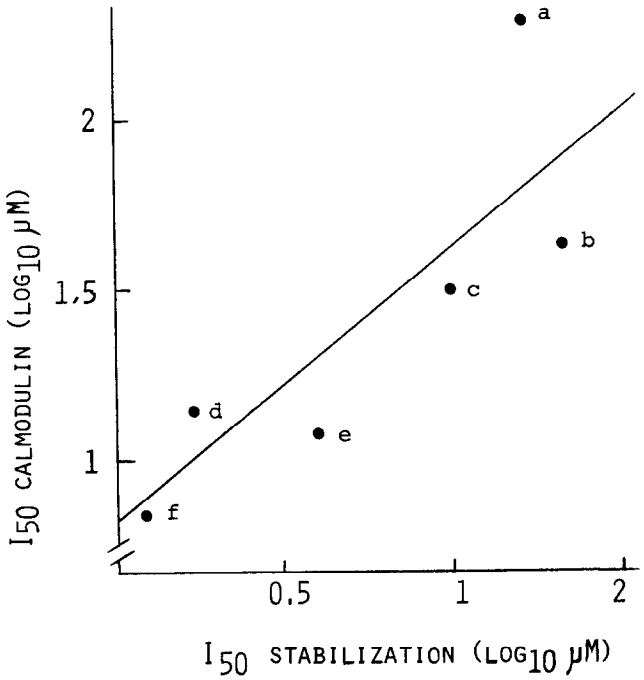

Figure 1 : Correlation between the two properties of drugs to inhibit calmodulin and to stabilize membranes. Drugs were (a) promethazine, (b) haloperidol, (c) chlorpromazine, (d) prochlorperazine, (e) fluphenazine, and (f) trifluoperazine.
Logarithm of micromolar concentrations leading to 50% inhibition were calculated from experimental values obtained as previously described in the case of calmodulin (7) and from results by Seeman (8,9) in the case of membrane stabilization.

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