BLOCKADE OF HEPATIC α-ADRENERGIC RECEPTORS AND RESPONSES BY CHLORPROMAZINE AND TRIFLUOPERAZINE

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1. Introduction

There are many physiological processes in which the calcium-dependent regulator protein calmodulin seems to play an important role [1]. Indeed the protein has been proposed to be 'a universal receptor of the Ca^{2+} signal' in cells. According to this scheme, Ca^{2+} first binds to calmodulin causing a conformational change. The active conformer of calmodulin then binds to certain enzymes with resultant changes in conformation and activity [1].

One experimental approach which has been used to show if calmodulin is involved in a particular metabolic process is to examine the effects of antipsychotic phenothiazine drugs such as chlorpromazine and trifluoperazine, which bind to calmodulin and prevent its activation by Ca^{2+} [2]. However, it has been known for a long time that these drugs also have local anesthetic ($>10^{-5}$ M), cholinergic blocking, and α-adrenergic blocking activities [3]. In particular, radioligand binding studies have shown that chlorpromazine and trifluoperazine displace [^{3}H]epinephrine, [^{3}H]norepinephrine, [^{3}H]clonidine and [^{3}H]WB-4101 from brain α-adrenergic receptors [4–6]. Hence, studies of the inhibitory effects of these phenothiazines on the actions of α-adrenergic agonists in tissues should not be interpreted as indicating the involvement of calmodulin in α-adrenergic action without additional information such as that presented below. However, three examples of such interpretation have appeared [7–9]. Another illustration of the need for caution in the use of pharmacological agents to elucidate hormone mechanisms relates to the calcium antagonists D-600 and verapamil. These have also been shown to inhibit the binding of α-agonists to their receptors [10–12].

Here, we report that chlorpromazine and trifluoperazine inhibit the actions of α-adrenergic agonists in hepatocytes, but not those of glucagon, vasopressin and A23187. Like α-agonists, the latter two agents have been demonstrated to be dependent on Ca^{2+} for their actions [13]. It is also shown that the two antipsychotic drugs inhibit the binding of [^{3}H]epinephrine to α-adrenergic sites in liver plasma membranes.

It is therefore proposed that the inhibition of α-adrenergic effects in the liver by chlorpromazine and trifluoperazine [7,8] is explicable in terms of an effect at the α-adrenergic receptor and that a role for calmodulin should not be inferred from experiments using these agents alone.

2. Materials and methods

Methods for the isolation and incubation of hepatocytes have been described [14]. Phosphorylase *a*, glycogen synthase and cellular calcium content were determined in 0.5 ml aliquots of cell suspension as detailed in [13,14]. Binding of [^{3}H]epinephrine to rat liver plasma membranes was measured as in [10,15]. Trifluoperazine and chlorpromazine were gifts from Smith, Kline and French.

3. Results and discussion

Effect of chlorpromazine on phenylephrine-induced activation of phosphorylase and calcium efflux

The results in fig.1 show that 10^{-5} M chlorpromazine completely inhibits 10^{-6} M phenylephrine

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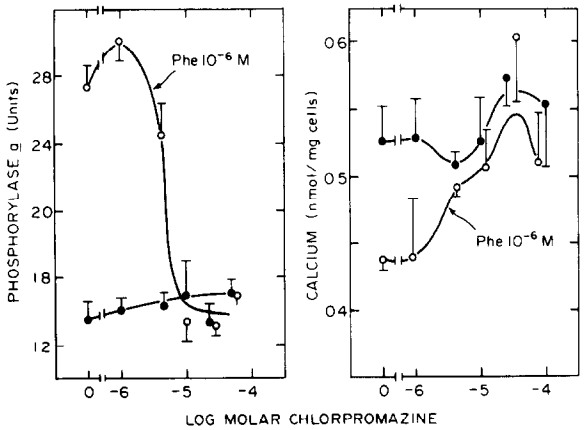

Fig.1. Effect of chlorpromazine on phosphorylase activation and calcium efflux induced by phenylephrine. Rat liver cells were incubated in the presence of various concentrations of chlorpromazine and in the presence of phenylephrine (Phe 10^{-6} M) or saline (control). After a 5 min incubation, aliquots of cells were taken for measurement of phosphorylase *a* and calcium content. Units of phosphorylase are defined as μmol [^{14}C]glucose-1-P incorporated into glycogen . min^{-1} . g wet wt $cells^{-1}$. Results are means of 3 expt performed in duplicate and are given ± SEM.

action on phosphorylase activation and calcium efflux in hepatocytes. A half-maximal effect of chlorpromazine is observed at ~5 X 10^{-6} M. This inhibitory action of chlorpromazine on phenylephrine effects is also observed at several concentrations of phenylephrine (fig.2).

As outlined in section 1, chlorpromazine not only inhibits calmodulin-mediated actions, but also has effects at the brain α-adrenergic receptor. Thus it can not be concluded from these results (fig.1,2) where

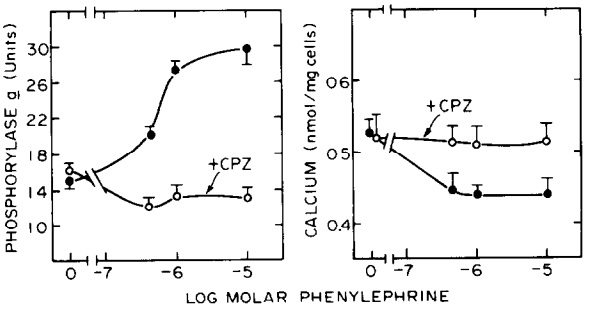

Fig.2. Effect of chlorpromazine on the dose–response of phenylephrine-induced changes in phosphorylase and calcium content. Liver cells were incubated in the presence of various concentrations of phenylephrine in the presence and absence of chlorpromazine (CPZ 10^{-5} M). After a 5 min incubation, aliquots of cells were removed for measurement of phosphorylase *a* and calcium. Results are means of 3 expt performed in duplicate and are given ± SFM.

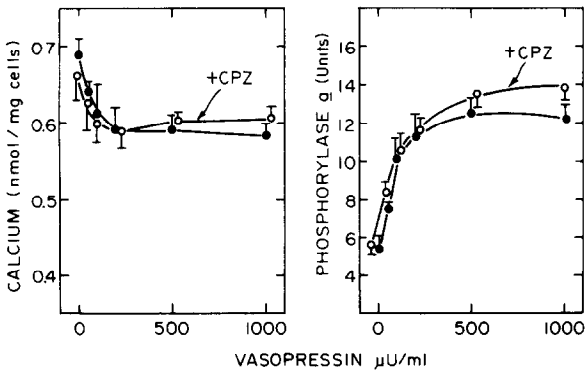

Fig.3. Effect of chlorpromazine on the dose response of vasopressin on phosphorylase activation and calcium content. Liver cells were incubated in the presence of various concentrations of vasopressin in the presence and absence of chlorpromazine (10^{-5} M). See legend of fig.2 for other details.

chlorpromazine is acting. One additional experimental approach is to use agents which elicit the same responses as α-adrenergic agonists in a Ca^{2+}-dependent manner, but do not act via the α-receptor. Two such agents are vasopressin and the divalent cationophore A23187 [13,16].

The results in fig.3,4 show that 10^{-5} M chlorpromazine does not inhibit the effects of vasopressin and A23187 on calcium fluxes and phosphorylase activation in hepatocytes. These findings indicate that it is invalid to attribute the inhibitory effect of the phenothiazine on α-adrenergic responses to an interaction with calmodulin [7,8]. Chlorpromazine also did not modify phosphorylase activation by glucagon, an agent which acts in a Ca^{2+}-independent manner in hepatocytes [17] (not shown). In confir-

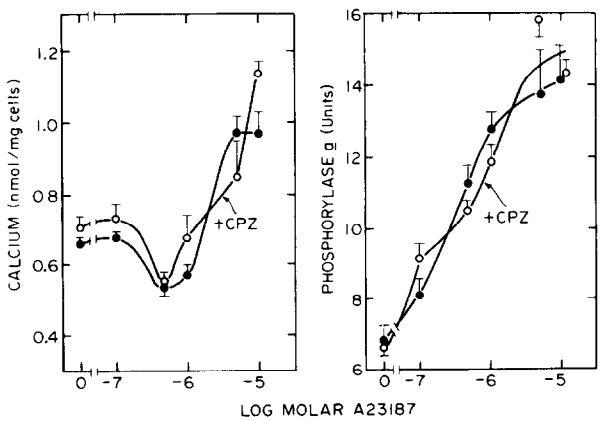

Fig.4. Effect of chlorpromazine on the dose response of A23187 on phosphorylase activation and calcium content. See legend of fig.2 for other details.

mation of earlier results, glucagon (10^{-9} M) had minimal effects on Ca^{2+} efflux from liver cells (not shown) [10,13,16–18] and chlorpromazine did not have any effect by itself or in combination with glucagon [7,8]. In summary, the above studies suggest that the unique inhibitory action of chlorpromazine on the actions of α-adrenergic agonists can be explained in terms of inhibition of binding at the α-adrenergic receptor [4–6]. To establish that this is indeed the case, competitive binding experiments were performed using partially purified rat liver plasma membranes. The results in fig.5 show that trifluoperazine inhibits specific binding of [^{3}H]epinephrine to α-adrenergic receptors. Epinephrine binding to isolated plasma membranes has been demonstrated to be correlated directly with phosphorylase activation and Ca^{2+} efflux in isolated hepatocytes [15]. The results in fig.5 also show a good correlation between trifluoperazine inhibition of epinephrine activation of phosphorylase and epinephrine binding. Half-maximal inhibition of both binding and phosphorylase activation is seen at ~3 X

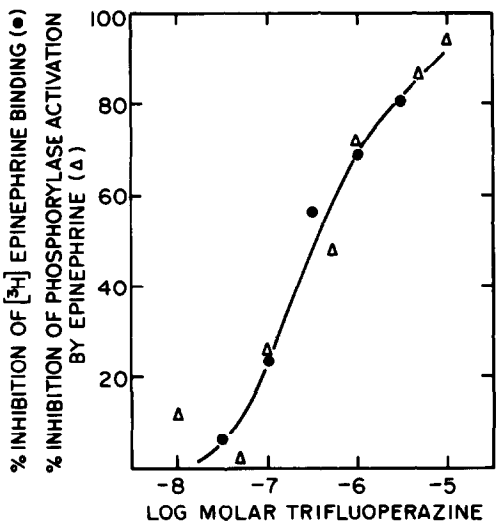

Fig.5. Displacement of (±)-[^{3}H]epinephrine bound to plasma membranes and inhibition of epinephrine induced activation of phosphorylase by trifluoperazine. Incubations for the displacement of 50 nM (±)-[^{3}H]epinephrine by trifluoperazine were carried out in duplicate and each incubation yielded 2 determinations. Inhibition of 100% is that produced by 10 μM phenoxybenzamine. Data shown are from representative experiments, each experiment was carried out at least twice. The inhibition by trifluoperazine of phosphorylase activation caused by 50 nM epinephrine was measured after 5 min incubation. Control and epinephrine-stimulated phosphorylase *a* levels were 15 and 22 units, respectively. Each point is the mean of 6 expt performed in duplicate.

10^{-7} M trifluoperazine. Chlorpromazine has similar inhibitory effects on epinephrine binding and phosphorylase activation (not shown).

Liver contains a calmodulin-dependent glycogen synthase kinase [19]. The following experiments were designed to show whether such kinases were involved in hormonal control of glycogen synthase in liver. No effect on the inactivation of glycogen synthase by A23187 (10^{-6} M) and vasopressin (250 μU/ml) was observed with chlorpromazine, trifluoperazine and fluphenazine at 10^{-6}–10^{-5} M (not shown). When chlorpromazine (10^{-5} M) was incubated with varying concentrations of vasopressin and A23187 there was also no inhibition of the inactivation of glycogen synthase (fig.6). Varying the time of preincubation with the phenothiazines from 1–10 min did not alter the results (not shown).

Trifluoperazine at 10^{-5} M has been shown to completely inactivate the calmodulin-dependent synthase kinase in vitro [19], hence if this kinase were responsible for the inactivation of glycogen synthase then one might have expected to see an effect. The uptake of chlorpromazine by liver cells does not appear to be a rate-limiting step for its action since hepatocytes have been shown to both absorb and adsorb this agent by unsaturable processes [21]. However since the phenothiazines are strong cationic surfactants they may adsorb onto intracellular cell membranes and hence the free intracellular concentration may be lower than expected. In addition, they are readily metabolized in the liver [22].

These results show that phenothiazines act as

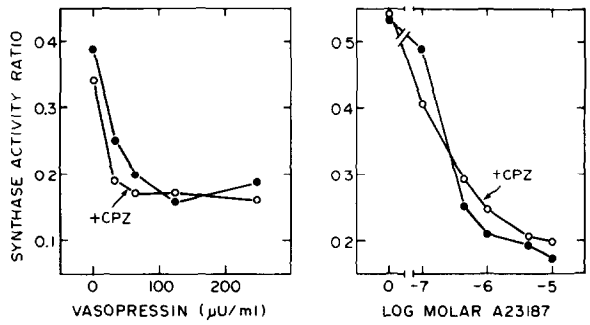

Fig.6. Effect of chlorpromazine on the dose–response of vasopressin and A23187 inactivation of glycogen synthase. Cells were preincubated for 7 min with 30 mM glucose and chlorpromazine (10^{-5} M), then either A23187 or vasopressin was added and the incubation continued for a further 5 min. Aliquots of cells (1 ml) were removed and rapidly frozen in liquid N_2 and assayed as in [14,20]. Each point is the mean of 2 expt performed in duplicate.

α-adrenergic antagonists and that they have little or no effect on the ability of A23187, vasopressin and glucagon to activate phosphorylase, inactivate glycogen synthase or elicit Ca^{2+} efflux in hepatocytes. Although the plasma membrane does not appear to limit the penetration of phenothiazines, the intracellular concentration of these agents may not reach a level that will inhibit calmodulin-dependent processes due to metabolism or binding to membranes. Furthermore, they may not interact as readily with calmodulin when this is the subunit of an enzyme [23]. In the same context, the binding of 50 nM (±)-[^{3}H]epinephrine to isolated plasma membranes is not modified by 0.1 mM EGTA or by 0.1 mM Ca^{2+} or 10^{-5} M calmodulin prepared according to [24] added either singly or in combination (not shown). Taking all of these points into consideration it would appear that an examination of the effects of phenothiazines in intact cells may not provide valid information concerning the role of calmodulin in certain responses [7,8]. In particular, the fact that these drugs interact with α-adrenergic receptors renders invalid their use in studies exploring the role of calmodulin in α-adrenergic responses. Until more specific inhibitors of calmodulin in action are found the question remains unanswered regarding the involvement of calmodulin in these and other hepatic responses. This study stresses the point we made in [10] that caution should be used when pharmacological agents with properties characterized for one system are used in studies or other systems.

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