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COMMENTARY

INTERACTION OF DRUGS WITH CALMODULIN

BIOCHEMICAL, PHARMACOLOGICAL AND CLINICAL IMPLICATIONS

BENJAMIN WEISS,* WALTER C. PROZIALECK† and THOMAS L. WALLACE‡
Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, 19129, U.S.A.
and
†Department of Physiology and Pharmacology, Philadelphia College of Osteopathic Medicine, Philadelphia, 19131, U.S.A.

A tenet of biochemical pharmacology is that the pharmacological effects of drugs are ultimately explainable by discrete biochemical actions. The caveat, of course, is that a particular biochemical action, no matter how well documented, may not necessarily be the cause of the pharmacological effect. In fact, rarely is the biochemical mechanism underlying the pharmacological effect of a drug known with certainty.

It is sometimes assumed that the many apparently unrelated effects of drugs are caused by several unrelated biochemical actions. This is not necessarily true, for if a drug affects a fundamental physiological or biochemical process, its pharmacological properties may be quite varied, but the initial event that led to these actions may be the same.

In this commentary, we wish to review some of the evidence that calmodulin, a calcium-binding protein that plays an important role in regulating many physiological processes, is one such fundamental biochemical regulator and may thereby provide an important site for pharmacological intervention. The identification of drugs that alter the activity of calmodulin may, therefore, provide a new approach to altering physiological or pathological processes. In fact, it is possible that several of the drugs currently in use may be acting by interfering with calmodulin.

In the mid-1970's, we reported [1–3] that certain phenothiazine derivatives, particularly those having antipsychotic activity, inhibited the calmodulin-induced activation of a calcium-sensitive form of phosphodiesterase. Subsequent studies have shown that the phenothiazine antipsychotics inhibit the actions of calmodulin on many enzymes (for reviews, see Refs. 4 and 5).

Although the phenothiazine antipsychotics were the first drugs demonstrated to be calmodulin inhibitors, several other types of antipsychotics and other classes of pharmacological agents have now been shown to inhibit the actions of calmodulin [4]. Since many types of drugs inhibit calmodulin, at first glance it may appear that this inhibition is not associated with any particular chemical or biological activity and that the interaction of drugs with calmodulin is structurally nonspecific and, therefore, irrelevant with regard to any particular pharmacological effect. However, there is evidence suggesting that the interaction of drugs with calmodulin actually may be quite selective. For example, the various classes of drugs differ greatly in their potencies as inhibitors of calmodulin. Furthermore, although the compounds having anti-calmodulin activity have been catalogued into different pharmacological classes, they share many common structural features and display considerable overlap in their pharmacological actions. In the sections that follow, we will discuss the physicochemical properties that enable a drug to inhibit calmodulin and consider the pharmacological and clinical implications of this interaction. In doing so, we will focus our attention on the interaction of antipsychotic drugs with calmodulin since they are among the most potent and widely studied group of calmodulin inhibitors.

Properties of calmodulin

Calmodulin is a widely distributed, acidic, heat-stable, Ca^{2+}-binding protein, having a molecular weight (M_n) of approximately 17,000 (for reviews, see Refs. 4 and 6–8). Discovered independently by Cheung [9] and Kakiuchi and Yamazaki [10] as an activator of phosphodiesterase and shortly thereafter as an activator of a specific molecular form of phosphodiesterase [11], calmodulin has been shown to activate many other Ca^{2+}-dependent enzymes including: adenylate cyclase, guanylate cyclase, $(Ca^{2+} + Mg^{2+})$-ATPase, phospholipase A_2, phosphorylase kinase, glycogen synthetase kinase, and myosin light chain kinase.

Calmodulin functions as an intracellular mediator of the effects of Ca^{2+}. Although some controversy exists, it appears that each calmodulin molecule can bind four Ca^{2+} ions, with dissociation constants in the range of 1–100 μM [12–14]. The binding of Ca^{2+} alters the conformation of calmodulin, increasing its helical content [13, 15, 16] and exposing hydrophobic regions [17]. In this conformation, the

* Author to whom correspondence should be addressed.
‡ Present address: Department of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

calmodulin–Ca^{2+} complex can bind to target enzymes [18] and, through an unknown mechanism, increase their activities.

Factors influencing the interaction of drugs with calmodulin

The mechanism by which the phenothiazine antipsychotics and related agents inhibit the actions of calmodulin is by binding directly to calmodulin in a Ca^{2+}-dependent manner [17, 19–21]. There are between one and three Ca^{2+}-dependent drug binding sites per calmodulin molecule, with dissociation constants for the most potent agents in the range of 1–10 μM. Significantly, antipsychotic drugs belonging to different chemical classes can compete for the same high affinity binding sites on calmodulin [5, 20].

This binding of antipsychotics apparently is relatively selective for calmodulin since the drugs show little or no calcium-dependent binding to a number of other proteins, including several calcium-binding proteins [20]. However, it should be emphasized that this selectivity is only relative since the drugs do bind to some extent to other calcium-binding proteins [20], and the drugs have also been reported to block the activation of phosphodiesterase induced by certain phospholipids [22].

Although some properties of the binding of phenothiazines to calmodulin are known, the specific structural factors influencing this interaction are poorly understood. Norman *et al.* [23] have shown that the abilities of various antipsychotic drugs to inhibit calmodulin are closely related to their partitioning between a lipid and an aqueous phase (octanol/water partition coefficients). Likewise, Landry *et al.* [24], have suggested a relationship between calmodulin inhibition and the abilities of drugs to stabilize membranes. These studies are consistent with those of LaPorte *et al.* [17] and Klevit *et al.* [25] which indicate that hydrophobic regions of calmodulin are involved in the binding of drugs to the protein. According to their proposed models, the binding of Ca^{2+} induces a conformational change in calmodulin, exposing a hydrophobic domain which can then participate in the binding of lipid-soluble drugs.

To examine more closely the role of hydrophobic bonding in the interaction of drugs with calmodulin, we have determined the octanol/water partition coefficients for a wide variety of compounds, using this coefficient as a measure of the relative hydrophobicities of the compounds. We found that, if a selected group of phenothiazines and structurally related antipsychotic drugs were considered, an excellent correlation existed ($r = -0.96$; $P < 0.001$) between calmodulin inhibition and hydrophobicity (W. C. Prozialeck and B. Weiss, unpublished results). If, on the other hand, other compounds were selected for comparison, a poor correlation existed. For example, Table 1 shows that certain drugs such as chlordiazepoxide, diazepam, reserpine and pentobarbital have octanol/water partition coefficients that are similar to, or greater than, that of chlorpromazine but are very weak inhibitors of calmodulin. Conversely, the antimalarial drug, quina-

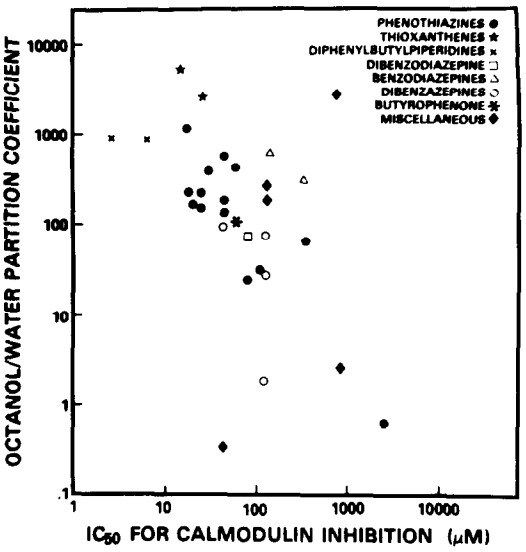


Fig. 1. Relationship between octanol/water partitioning and inhibition of calmodulin. Octanol/water partition coefficients were determined by a modification of the method of Leo *et al.* [26]. Twenty-five milliliters of buffer (pH 6.0), containing a 500 μM concentration of the drug under study, was shaken vigorously with 2.5 ml octanol. After the samples were centrifuged to separate the phases, the concentration of drug in each phase was determined by ultraviolet spectroscopy. The partition coefficient was the ratio of the concentration of drug in the octanol phase to that in the aqueous phase. The concentration of drug needed to inhibit 50% of the calmodulin-induced activation of the phosphodiesterase (IC_{50}) was determined by the method of Levin and Weiss [3]. The correlation coefficient was determined by linear regression analysis of the common logarithms of P and IC_{50}.

Table 1. Lack of correlation between octanol/water partitioning and inhibition of calmodulin*

Compound	Partition coefficient	Calmodulin inhibition IC_{50} (μM)
Quinacrine	0.36	43
Pentobarbital	115	> 10,000
Papavarine	186	130
Chlorpromazine	186	42
Chlordiazepoxide	302	320
Diazepam	625	140
Reserpine	2880	750

* Octanol/water partition coefficients and IC_{50} values were determined as described in the legend to Fig. 1.

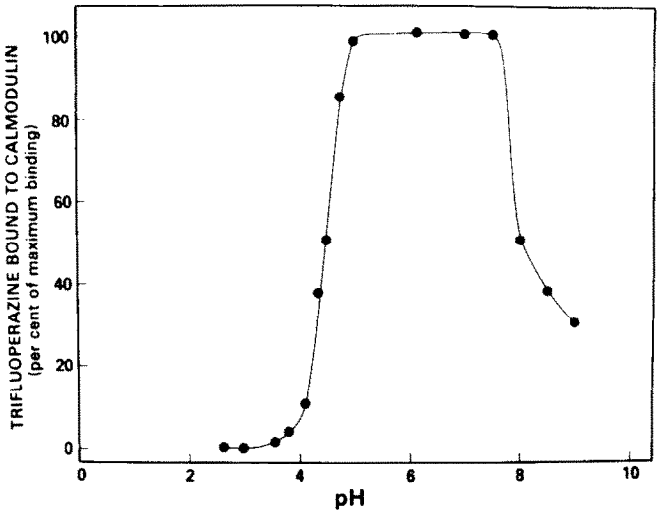


Fig. 2. Effect of pH on the binding of trifluoperazine to calmodulin. Calmodulin was dialyzed to equilibrium against [^{3}H]trifluoperazine (1 μM) in the presence of 100 μM calcium using either citric acid–phosphate buffer (pH 3–7) or Tris–HCl buffer (pH 5–9). The points represent the mean of duplicate samples. Taken from Weiss *et al.* [5].

crine, has a very low partition coefficient but is as potent as chlorpromazine in its anti-calmodulin activity. When all the data are combined as shown in Fig. 1, it can be seen that although there is a general correlation between the hydrophobicities of the various compounds and their potencies as inhibitors of calmodulin, since most of the compounds that are potent calmodulin inhibitors are highly lipophilic, the correlation is barely significant ($r = -0.375$; $P < 0.05$). Thus, it appears that, although lipid solubility may be one determinant of the ability of a drug to inhibit calmodulin, other factors, discussed below, such as the geometric structure of the drug or its ionic characteristics are also involved.

Studies on the pH dependence of the binding of trifluoperazine to calmodulin (Fig. 2) suggest that ionic forces play an important role in this interaction. Binding between the drug and calmodulin increased markedly above pH 4.0, reached a maximum at pH 5.0, and remained constant up to pH 8.0. Above pH 8.0, the binding decreased markedly. Since the isoelectric point of calmodulin is about 4 [6, 8] and the pK_a of trifluoperazine is 8.1 [27], these results suggest that there is an electrostatic interaction between the negatively charged calmodulin and the positively charged phenothiazine.

Recent studies on the effects of neuropeptides on calmodulin also show that the most potent inhibitors of calmodulin are those that are negatively charged at physiological pH [5, 28]. These include β-endorphin, adrenocorticotrophic hormone and dynorphin. However, several peptides such as neurotensin, substance P and alpha melanocyte stimulating hormone are negatively charged but are poor inhibitors of calmodulin [5, 28], indicating that other structural characteristics are also important.

Specific structural requirements for inhibiting calmodulin. An examination of the various compounds known to inhibit calmodulin shows that they have many structural similarities, suggesting that the geometric structure of a drug is important in determining its interaction with calmodulin. In many cases, slight modifications in chemical structure can greatly alter the ability of a compound to bind to calmodulin and inhibit its activity. Such structural specificity is suggestive of specific drug–receptor interactions and not simple hydrophobic binding. The structures of some representative compounds and their IC_{50} values for inhibiting calmodulin-sensitive phosphodiesterase are shown in Fig. 3. Although only a few compounds have been studied and definitive conclusions are difficult to draw, some general observations can be made.

The potent calmodulin inhibitors are amphipathic amines that contain large hydrophobic regions and carry a positive charge at neutral pH. Both the hydrophobic region and the charged amino group appear to be important for inhibiting calmodulin activity. The most potent inhibitors, the diphenylbutylpiperidines (e.g. pimozide), contain two aromatic rings connected by a single carbon atom. The phenothiazines (e.g. chlorpromazine) are also potent inhibitors of calmodulin and contain two aromatic rings joined at two positions by a sulfur and a substituted nitrogen. Compounds that contain only one aromatic ring such as the butyrophenones (e.g. haloperidol) are less potent.

Substituents that increase the lipid solubility of the ring, such as the chlorine group on chlorpromazine or the fluorine groups on the diphenylbutylpiperdines, increase the potency in antagonizing calmodulin. By contrast, substituents that reduce the hydrophobicity of the ring, such as hydroxyl or sulfoxide, tend to reduce the potency of the compounds.

The position of the side-chain amino group also seems to be important in determining the ability of a drug to inhibit calmodulin. The most potent inhibitors of calmodulin, the diphenylbutylpiperidine and phenothiazine antipsychotics, have an amino group three-carbons removed from the bridge atom between the aromatic rings. Compounds in which this chain is shorter (i.e. prenylamine and prometh-

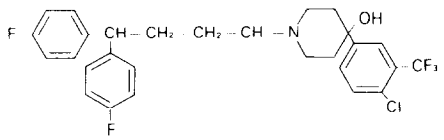
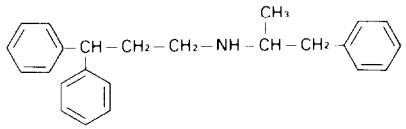
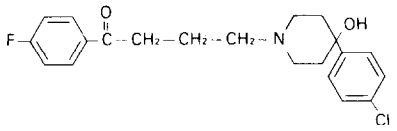
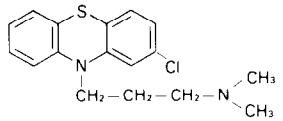
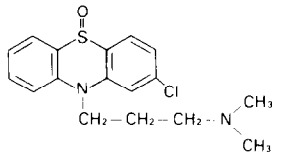
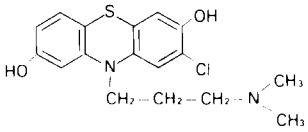
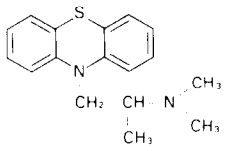
CLASS	COMPOUND	STRUCTURE	CALMODULIN INHIBITION IC_{50} (μM)
diphenylbutylpiperidine	Penfluridol		2.5
diphenylpropylamine	Prenylamine		18
butyrophenone	Haloperidol		60
phenothiazine	Chlorpromazine		42
phenothiazine	Chlorpromazine sulfoxide		2,500
phenothiazine	3,8-dihydroxy chlorpromazine		165
phenothiazine	Promethazine		340

Fig. 3. Structure–activity relationships of calmodulin inhibitors.

azine) appear to be less potent. Although this side-chain amino group must be charged for drugs to be potent inhibitors of calmodulin, not enough data currently are available to draw any firm conclusion concerning the importance of the substituents on the amino group, although it does appear that the piperazine phenothiazines (e.g trifluoperazine) may be more potent than the propylamines (e.g. chlorpromazine). Again, it should be noted that these are general observations based on a limited number of compounds. Obviously, more systematic and thorough structure–activity studies are needed to verify these preliminary conclusions.

Thus, the interactions of drugs with calmodulin appear to involve two kinds of attachments. One is a hydrophobic interaction between lipophilic portions of the drug and non-polar regions of calmodulin. At the other point of attachment, an electrostatic interaction occurs between a positively charged amino group on the drug and a negatively charged

acidic residue on calmodulin. Further, the amino group and the hydrophobic region of the drug should be properly oriented to each other.

From the limited number of compounds studied, our preliminary assessment is that the necessary structural requirements for a compound to be a potent inhibitor of calmodulin are those shown in Fig. 4. The essential features of the structure are a very large hydrophobic region, consisting of two aromatic rings, joined at either one or two positions, and a side-chain amino group that is at least four atoms removed from the aromatic ring structure. This structure is remarkably similar to that proposed by Kaufman and Koski [29] in describing the structural requirements for antipsychotic activity. It should be noted, however, that this general structure is not unique to the antipsychotics but is also found in a wide variety of other pharmacological classes of drugs, including antimalarials, α-adrenergic blocking agents, antispasmodics, antihistamines and anticholinergic drugs. Interestingly, many agents in these pharmacological classes have now been shown to have anti-calmodulin activity. This is discussed more fully in a subsequent section.

Recently, Hidaka *et al.* [30, 31] reported that certain vascular relaxing agents are potent inhibitors of calmodulin. These compounds include prenylamine, a diphenylpropylamine which is similar to the diphenylbutylpiperidines, and the compounds [*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) and N^2-dansyl-L-arginine-4-t-butylpiperidine amide (No. 233)] which are naphthalenesulfonamides. Although the latter two compounds, at first glance, appear to be quite different from our proposed structure, they may, in fact, be quite similar. Both contain a large hydrophobic region (naphthalene ring) and a side-chain amino group several atoms removed from the aromatic ring. Space-filling models show that compounds W-7 and 233 can assume conformations in which the hydrophobic region and the amino group are oriented as in our proposed structure. Further, compounds W-7, 233 and chlorpromazine all compete for the same binding site on calmodulin, suggesting that they may have similar three-dimensional shapes [31].

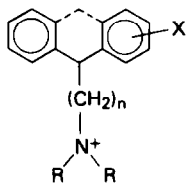


Fig. 4. Generalized structural characteristics of calmodulin inhibitors. This generalized structure depicts a positively-charged group (N^+) attached to two hydrophobic groups. The hydrophobic groups need not necessarily be attached to each other at the ring. "X" represents a moiety that increases the hydrophobicity of the ring. For high potency, "n" should be at least 3 carbons long. More details are provided in the text.

Pharmacological and clinical implications of inhibiting calmodulin

Since calmodulin influences such a variety of important biological events, the identification of drugs that alter its activity should have profound clinical applications. Several groups of pharmacological agents have now been reported to inhibit calmodulin. In the following sections, we will consider the effects and possible clinical relevance of inhibiting calmodulin with various drugs. We will concentrate on the antipsychotics since this group of compounds has been studied in the greatest detail.

Inhibition of calmodulin activity by antipsychotic drugs. Based on our observations that (a) certain phenothiazine antipsychotic drugs inhibit the calmodulin-induced activation of a particular form of phosphodiesterase [1, 3], (b) the mechanism by which these drugs acted was by binding directly to calmodulin in a reversible, calcium-dependent manner [19], and (c) of a series of psychoactive compounds, the most potent inhibitors of calmodulin were the clinically effective antipsychotics, we suggested that the inhibition of calmodulin may provide a common mechanism for explaining some of the diverse biochemical actions of these drugs [3–5, 21]. This was an intriguing possibility to us since the antipsychotics are well known to produce multiple biochemical effects. The findings that phenothiazine antipsychotics inhibit not only calmodulin-dependent phosphodiesterase activity but also a wide variety of other calmodulin-dependent events and processes (see Table 2) lent support to our hypothesis that several of the apparently unrelated biochemical effects of these drugs may be explained by a common mechanism of binding to, and inhibiting, calmodulin.

The obvious questions that stemmed from this hypothesis concerned the specificity of this interaction and whether it might also account for some of the pharmacological and clinical effects of the antipsychotics. The pharmacological actions of phenothiazines on the central nervous system are quite varied; these include antipsychotic, sedative and extrapyramidal effects. These drugs also influence the neuroendocrine, gastrointestinal and cardiovascular systems, particularly when used in high concentrations. Whether some of these pharmacological actions can be explained by the inhibition of calmodulin has been addressed earlier [4]. Only the actions on the central nervous system will be considered below.

In support of the notion that calmodulin inhibition may explain some of the central pharmacological actions of the antipsychotic drugs is the evidence that, of a series of drugs that affect the central nervous system, the most potent inhibitors of the calmodulin-induced activation of phosphodiesterase were clinically-effective antipsychotics and that this action was shared by neuroleptics of different chemical classes including the phenothiazines, diphenylbutylpiperidines and thioxanthenes [3]. Further studies of a series of phenothiazine derivatives demonstrated a positive correlation between clinical potency and calmodulin inhibitory activity [81] (Fig. 3). However, there are important questions remaining and certain inconsistencies regarding the clinical significance of these findings. For although there

Table 2. Calmodulin-dependent enzymes and processes that are inhibited by phenothiazine antipsychotics

	References
Enzymes	
Adenylate cyclase	32–35
$(Ca^{2+} + Mg^{2+})$-ATPase	36–40
15-Hydroxyprostaglandin dehydrogenase*	41
Myosin light chain kinase	31, 42
NAD kinase	43
Phosphodiesterase	1, 3, 44, 45
Phospholipase A_2	46
Phosphorylase b kinase	47
Protein kinase	48–52
Tryptophan hydroxylase	53
Processes	
ADH-mediated water transport	54
α-Adrenergic responses	55–57
Calcium uptake	58
Catecholaminergic function	59
Chloride secretion in intestine	60
DNA synthesis	61
Endocytosis	62–64
Exocytosis	65
Insulin release	66–69
Leukocyte function	70–72
Neuromuscular transmission	73
Neurotransmitter release	74, 75
Phospholipid methylation	76
Platelet function	77
Release of trichocyst in paramecium	78
Smooth muscle contraction	30, 79, 80

* Calmodulin inhibits 15-hydroxyprostaglandin dehydrogenase.

appears to be a general correlation between the ability of a drug to inhibit calmodulin and its antipsychotic activity, not all types of neuroleptic agents are potent calmodulin inhibitors. Results from our [3, 20] and other [23, 38] laboratories show that the butyrophenone antipsychotic drugs (e.g. haloperidol), which are very potent clinically, are relatively poor inhibitors of calmodulin and that the benzamide derivative sulpiride is an effective antipsychotic [82] but is practically devoid of anti-calmodulin activity [23]. There is also little evidence of stereoselectivity in the interaction of isomers of butaclamol and flupenthixol with calmodulin [5, 23, 81] despite the fact that these agents showed stereospecificity in other biochemical systems [32, 83]. Each of these issues will be considered in more detail below.

Raess and Vincenzi [38] reported that, although the phenothiazine antipsychotics, trifluoperazine and chlorpromazine, inhibited the calmodulin-induced activation of the $(Ca^{2+} + Mg^{2+})$-ATPase of erythrocyte membranes, the non-phenothiazine antipsychotics, haloperidol and butaclamol, showed little or no inhibition even at concentrations as high as 100 μM. At first glance, these results appear to be in conflict with those from our [3, 81] and other [23] laboratories showing that haloperidol and butaclamol do inhibit the activation of phosphodiesterase by calmodulin, albeit at substantially higher concentrations than those of the phenothiazine antipsychotics. The reason for this apparent discrepancy may be explained by the high concentration of calmodulin used in their studies. Substantially higher concentrations of haloperidol and butaclamol would have had to be used in order to effect a greater inhibition of calmodulin. This may also explain the results of Roufogalis [84] who found chlorpromazine to be about one-fourth less potent an inhibitor of calmodulin than did Levin and Weiss [3]. In any case, their data are in general agreement with ours in that haloperidol and butaclamol are substantially less potent than are the phenothiazine antipsychotics in inhibiting calmodulin.

Roufogalis [84] examined the abilities of analogues of chlorpromazine, differing in the position of the chlorine substituent in the aromatic ring, to inhibit the calmodulin-induced activation of $(Ca^{2+} + Mg^{2+})$-ATPase of erythrocyte ghosts. He reported that all the derivatives were approximately equal in their abilities to inhibit calmodulin activity. Since several of these compounds were not potent cataleptic agents in mice [85], he concluded that inhibition of calmodulin is not related to antipsychotic activity but is merely due to nonspecific hydrophobic interactions. Although we do not disagree with his findings, we question whether catalepsy alone is an unequivocal determinant of antipsychotic activity since some clinically effective antipsychotics produce low degrees of catalepsy [86]. Antipsychotic activity is usually presumed only after a wide variety of tests, that allow reasonable predictions about clinical efficacy [87], have been conducted.

Insofar as the question of stereospecificity is concerned, work from this laboratory has shown that inhibition of calmodulin-activated phosphodiesterase or displacement of trifluoperazine from purified

calmodulin by antipsychotic agents is not stereospecific *in vitro* [4, 5, 81]. Of the isomers examined, (+) and (−)butaclamol showed some differential effects, but the *cis* and *trans* isomers of thiothixene and flupenthixol were approximately equipotent in their interactions with calmodulin [4, 5, 81]. This lack of stereospecificity for calmodulin *in vitro* contrasts with several other *in vitro* effects of these agents which do show stereospecificity: these include the inhibition of dopamine and haloperidol binding to brain membranes [88] and inhibition of catecholamine-sensitive adenylate cyclase in brain [32, 83].

Since it is commonly believed that specific antipsychotic drug receptors should exhibit drug stereospecificity, the question has been raised as to why purified calmodulin does not exhibit stereospecificity for these agents [81]. This is an important issue which has yet to be resolved, and one must consider the possible explanations for the apparent lack of stereospecificity *in vitro*. Perhaps, during purification, calmodulin undergoes conformational changes that cause a loss of stereospecificity; purification of many hormone receptors has resulted in an alteration of their binding characteristics [89–91]. Stereospecificity may also be conferred on calmodulin *in vivo* by adjacent molecules such as lipids, which are known to modulate ligand-binding properties of receptor proteins [92]. For example, stereospecific binding of certain drugs to opiate receptors is augmented by phosphatidylserine [93, 94]. Furthermore, there may exist, *in vivo*, stereospecific transport mechanisms as has been shown for certain amino acids and sugars [95]. We are currently exploring the possibility that calmodulin may show stereospecificity for antipsychotic drugs when this protein is found in a more physiological state.

Several investigators have put forth the argument that, because calmodulin does not display stereospecificity for antipsychotic drugs *in vitro*, inhibition of calmodulin activity is unrelated to clinical antipsychotic activity [23, 38]. The basis of this argument lies in observations that many behavioral effects of antipsychotic drugs in animals are stereospecific and in the supposition that clinical antipsychotic effects also show stereospecificity. However, the evidence supporting the clinical stereospecificity of thioxanthene antipsychotic drugs is weak [96–98], and studies supporting the clinical stereospecificity of other antipsychotics, have not been published, to our knowledge. For example, although the + and − isomers of butaclamol have been shown to differentially displace drugs from putative dopamine receptors *in vitro* [99, 100], we have not found any published studies indicating that these isomers have different clinical antipsychotic effects. Studies to date have focused on either the + isomer or the racemic mixture without actually comparing the clinical activities of the + and − isomers separately [101–103].

It should be noted that rarely has a direct correlation been found between any *in vitro* action and clinical antipsychotic potency. Many factors, besides interaction with receptor sites, determine the action of a drug; drugs acting at the same molecular sites might produce quite different pharmacological effects if their pharmacokinetic properties (i.e. absorption, distribution and metabolism) are different. Furthermore, human behavior (psychotic or otherwise) involves an extremely complex series of chemical events, and there is no definitive evidence that all antipsychotic drugs produce their effects at the same biochemical site; different classes of drugs might act at different sites to produce similar effects.

Table 3. Pharmacological classes of drugs that inhibit calmodulin

Class	References
α-Adrenergic blocker	57
Antidepressant	3
Antihistaminic	3
Antimalarial	104
Antipsychotic	3, 4, 38, 39, 81
Cancer chemotherapeutic	105, 106
Local anesthetic	104
Neuropeptide	5, 28
Smooth muscle relaxant	30, 31

In conclusion, the ability of compounds to inhibit the actions of calmodulin is not a property unique to the phenothiazine antipsychotic drugs but is shared by butyrophenones, benzocycloheptapyridisoquinolines, diphenylbutypiperidines and thioxanthenes [5, 23, 39, 53, 81]. The possibility that at least some of the therapeutic effects or side effects of these drugs might be due to inhibition of calmodulin certainly warrants further attention.

Inhibition of calmodulin activity by non-antipsychotic drugs. Table 3 shows that, in addition to the antipsychotics, there are several other pharmacological classes of drugs that inhibit calmodulin. As can be seen, this list is varied and extensive, suggesting that calmodulin inhibitors have the potential for altering several physiological or pathological processes. As pointed out earlier, there are structural similarities among these agents that may help explain not only why they all inhibit calmodulin but also why there is an overlap in pharmacological activity of some of these drugs. For example, it is well known that some antispasmodics, antihistaminics, anticholinergics, and alpha-1 adrenergic blocking agents have similar structures and share certain common profiles of pharmacological activity. It might be noted here that phenothiazine antipsychotics also produce many of these same effects; that is, they are known to have antihistaminic, smooth muscle relaxant and α-adrenergic blocking properties. Whether any of these effects are caused by an action on calmodulin remains to be determined.

Unfortunately, this commonality of action also implies that calmodulin inhibitors would act nonspecifically. The possibility of developing specific pharmacological agents based on the ability to inhibit calmodulin is discussed briefly below.

Development of new agents for modifying calmodulin activity. Obviously, in using calmodulin inhibitors to modify a particular function, it would be desirable to have a high degree of specificity. However, since calmodulin regulates such a variety of biological processes, agents that inhibit calmodulin would be expected to exhibit a broad spectrum

of pharmacological activities. How might it be possible then for a drug that inhibits calmodulin to have a specific action? First, the specificity with which calmodulin inhibitors act would depend on some of the same factors that influence the specificity of any drug; these include drug disposition, localization, binding and transport across specialized membranes. In fact, preliminary results from our laboratory (C. Earl, W. C. Prozialeck and B. Weiss, unpublished results) show that chlorpromazine injected *in vivo* into rats produced markedly different degrees of inhibition of calmodulin in the various areas of the brain. Drug specificity might also depend on whether the small structural differences that exist in calmodulin [107–109] could be exploited with specific drugs. Finally, perhaps different concentrations of calmodulin are required to activate the different calcium-dependent enzymes. If this were true, a calmodulin inhibitor might exert a greater effect on one calmodulin-dependent process than another, even though in each case it was acting by inhibiting calmodulin. The question of whether new classes of pharmacological agents will be developed that act by inhibiting calmodulin will have to await further investigations.

Summary

Calmodulin is a widely distributed, highly active, calcium-binding protein that influences a number of important biological events. Accordingly, agents that inhibit the activity of calmodulin should have profound pharmacological effects. Within the past few years, a number of compounds have been identified that inhibit calmodulin. The most potent of these described so far include certain antipsychotic drugs, smooth muscle relaxants, α-adrenergic blocking agents and neuropeptides.

Studies of the physicochemical and structural properties of a variety of calmodulin inhibitors have shown that there are ionic and hydrophobic interactions between the drug and calmodulin. From the limited studies conducted so far, we conclude that, for a compound to inhibit calmodulin, it should carry a positive charge at physiological pH, presumably to interact with negative charges on the highly acidic calmodulin, and have hydrophobic groups, presumably to interact with lipophilic regions on calmodulin. But these two factors are not the only ones that are involved in inhibiting calmodulin, for many highly charged and highly hydrophobic agents have relatively little effect on calmodulin activity. The structural relationships between these ionic and hydrophobic regions and other, as yet identified, factors are also important.

Many of the biochemical actions of the phenothiazine antipsychotic agents can be explained by the common mechanism of their binding to, and inhibiting, calmodulin. The question of whether these biochemical actions can explain their pharmacological and clinical effects is still unclear.

The fundamental role calmodulin plays in biology suggests that this calcium binding protein may provide a new site for the pharmacological manipulation of biological activity. The calmodulin inhibitors described thus far hardly scratch the surface of this fertile area of research.

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