Biochemical Pharmacology, Vol. 36, No. 12, pp. 1989–1996, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

COMPARATIVE EFFECTS OF CALMODULIN INHIBITORS ON CALMODULIN'S HYDROPHOBIC SITES AND ON THE ACTIVATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE BY CALMODULIN

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(*Received* 29 *July* 1986; *accepted* 31 *December* 1986)

Abstract—Experiments were designed to investigate the effect of inhibitors on calmodulin's hydrophobic sites and their consequences on the activation of a target enzyme, cyclic nucleotide phosphodiesterase. Two fluorescent probes, 2-(*p*-toluidinyl)-naphthalene-6-sulfonate (TNS) and 9-anthroylcholine (9AC) were used to study the interactions with calmodulin of inhibitors devoid of direct effect on the probes. Contrary to W-7, nicergoline, nicardipine and quercetin, which decreased the fluorescence of the two probes bound to calmodulin, bepridil only decreased 9AC fluorescence but increased the fluorescence intensity at the wavelength of the emission maximum of TNS. In spite of this difference, bepridil as well as W-7 and nicergoline competitively inhibited calmodulin activation of phosphodiesterase. In addition, nicergoline also inhibited phosphodiesterase activity competitively to cyclic GMP. These results show differences in the interactions of inhibitors with calmodulin; these differences are not detected in functional studies of the effect of inhibitors on phosphodiesterase activation.

The binding of Ca^{2+} to calmodulin induces a conformational change which allows the resulting calcium/calmodulin complex to activate a great number of enzymes (for review see [1]). The binding of calcium-activated calmodulin to enzymes is due to the exposure at the surface of calmodulin of hydrophobic regions which allow the interaction of the calcium/calmodulin complex with corresponding hydrophobic sites on enzymes [2, 3]. These hydrophobic enzyme-binding regions on calmodulin have been characterized by the fluorescent hydrophobic probes 2-(*p*-toluidinyl)-naphthalene-6-sulfonate (TNS)§ and 9-anthroylcholine (9AC) [2, 4], the fluorescence quantum yield of which increases when they bind to hydrophobic sites [2, 5]. Both TNS and 9AC inhibit the activation of cyclic nucleotide phosphodiesterase by calmodulin [2, 3]. They are used to detect the enzyme-binding sites of calmodulin, and the decrease of their fluorescence by drugs can be studied to assess the calmodulin inhibitory potency of the compounds [6]. However, TNS and 9AC have been shown to possess multiple binding sites on calmodulin, two having been demonstrated for TNS [7] and 4–6 sites for 9AC [2]. This suggests the possibility that the different hydrophobic sites may not necessarily all be involved in the activation of phosphodiesterase, and that the structurally heterogeneous calmodulin inhibitors may not all bind to the same sites on calmodulin. In this case, calmodulin inhibitors with different selectivity for the hydrophobic sites on calmodulin may exist, and the study of their inhibitory effect on phosphodiesterase activation by calmodulin could allow a better definition of the structural constraints which must be met by the hydrophobic sites of calmodulin involved in the activation of phosphodiesterase. In addition, the hydrophobic sites responsible for the activation of target enzymes different from phosphodiesterase may not all be identical. Selective inhibition of some enzymes by calmodulin inhibitors could thus be imagined.

In this paper, the interaction of calmodulin inhibitors with the hydrophobic sites labelled by the negatively-charged TNS and the positively-charged 9AC was therefore compared to their inhibitory effects on the activation by calmodulin of cyclic nucleotide phosphodiesterase. Inhibitors were chosen from different chemical classes and special emphasis was laid on some hydrophobic calcium antagonist drugs [8], the calmodulin inhibitory properties of which have only recently been discovered [9, 10] and have not yet been studied extensively, unlike the properties of other calcium antagonists [6, 11, 12]. Accordingly, the calcium antagonists bepridil, nicardipine and nicergoline were studied along with the reference calmodulin inhibitor W-7 [13]. Other drugs known to be calmodulin inhibitors, like quinacrine [14], flunarizine [10], calmidazolium [15] and 48/80 [16] could not be studied because of their intrinsic fluorescence (quinacrine), low solubility (flunarizine), or because they increased the fluorescence quantum

§ Abbreviations used: 9AC, 9-anthroylcholine; EGTA ethyleneglycol bis(β-aminoethylether)-*N,N,N',N'*-tetraacetic acid; TNS, 2-(*p*-toluidinyl)-naphthalene-6-sulfonate.

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yield of TNS in the absence of calmodulin (calmidazolium, 48/80).

MATERIALS AND METHODS

Preparation of calmodulin and cyclic nucleotide phosphodiesterase. Calmodulin was purified from bovine brain by the method of Isobe *et al.* [17] modified as in [7]. Calmodulin-dependent cyclic nucleotide phosphodiesterase was prepared from bovine aorta as previously described [18].

Fluorescence assays. Calmodulin was incubated with fluorescence probes and drugs in a buffer containing 100 mM Tris–HCl (pH 7.5), 0.5 mM $CaCl_2$. Adding 3 mM $MgCl_2$ and 120 mM KCl to the incubation buffer did not affect the results. Calmodulin concentrations were determined spectrophotometrically at 277 nm, using an extinction coefficient of $3.3 \cdot 10^3\ M^{-1}\ cm^{-1}$. Fluorescence spectra were recorded on a FICA 55 absolute spectrofluorometer, using an excitation wavelength of 365 ± 5 nm for TNS and 360 ± 5 nm for 9AC. Concentration-effect curves for displacement of hydrophobic probes from calmodulin were obtained by determining the fluorescence intensity at the emission maximum of the hydrophobic probe bound to calmodulin at different drug concentrations. Data were fitted by a nonlinear least-squares fit to the sigmoidal equation $F = F_0/(1 + (i/K_{0.5})^n)$ where F = fluorescence intensity of the probe, F_0 = maximal fluorescence intensity in the absence of drug, i = drug concentration, $K_{0.5}$ = concentration of drug producing a half maximal fluorescence decrease and n = Hill coefficient of drug binding to calmodulin. Marquardt's method as described by Bevington [19] was used for nonlinear least-squares regression.

Fluorescence life times were measured with the single photoelectron technique [20] and analysed statistically using the residual distribution form [21, 22].

Phosphodiesterase assays. Phosphodiesterase assays were conducted as previously described [23, 24]. Phosphodiesterase (50 µl of diluted enzyme which hydrolysed about 2 pmol cGMP/min at 1 µM substrate concentration) was incubated in a final volume of 250 µl for 20 min at 30° with drug and calmodulin in the following buffer: 40 mM Tris–HCl (pH 7.5), 2 mM magnesium acetate, 1 µM cGMP, 0.25 g/l bovine serum albumin, 0.1 mM EGTA and $CaCl_2$ to a free calcium concentration of 100 µM. The order of addition of the reagents was the following: phosphodiesterase, drug or vehicle, calmodulin, Ca^{2+}, Mg^{2+}. Reaction was initiated by the addition of the substrate cGMP to a final concentration of 1 µM. All experimental points were obtained in duplicate. Data were fitted by a nonlinear least-squares fit to the equation for competitive inhibition of phosphodiesterase activation adapted for drug binding to calmodulin according to the Hill equation: $A = 100/((K/CaM)(1 + (i/K_{0.5})^n) + 1)$ where A = percent activation of phosphodiesterase, K = apparent activation constant of calmodulin for phosphodiesterase, i = drug concentration, CaM = calmodulin concentration, $K_{0.5}$ = constant of half-maximal drug binding to calmodulin, n = Hill coefficient for drug binding to calmodulin. Standard errors for parameters determined from nonlinear least-squares fits (i.e. K, $K_{0.5}$ and n) were calculated as described by Cleland [25].

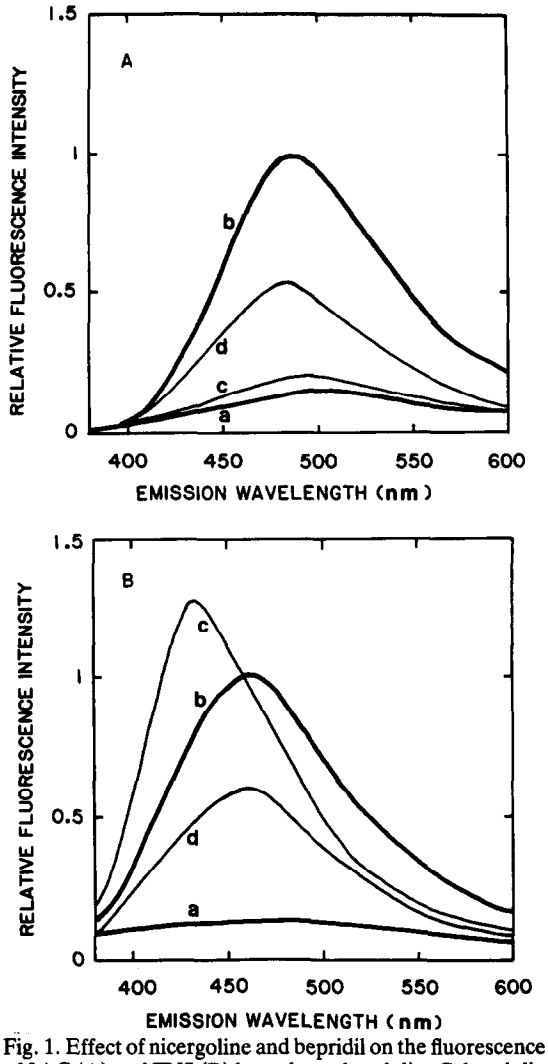

Fig. 1. Effect of nicergoline and bepridil on the fluorescence of 9AC (A) and TNS (B) bound to calmodulin. Calmodulin (4 µM) was incubated with 10 µM 9AC (A) or TNS (B) in 100 mM Tris–HCl (pH 7.5), 0.5 mM $CaCl_2$. Fluorescence spectra were recorded from 380 to 600 nm, excitation wavelength being 360 and 365 nm for 9AC and TNS respectively: (a) fluorescence spectra of TNS or 9AC alone, (b) TNS or 9AC in the presence of calmodulin, (c) fluorescent probe, calmodulin and 30 µM of bepridil, (d) fluorescent probe, calmodulin, and 30 µM of nicergoline.

Drugs. Bepridil was from R.L.-C.E.R.M. (Riom, France), nicergoline was from Specia (France), nicardipine was from Sandoz, W-7, quercetin and TNS were from Sigma, and 9AC was from Molecular Probes Inc. All other reagents were analytical grade. Solutions were prepared daily.

RESULTS

1. *Effect of drugs on fluorescent probes bound to calmodulin*

Figure 1 illustrates the interactions of drugs with 9AC (A) and TNS (B) bound to calmodulin. Comparison of curves a and b in Fig. 1A shows that the

Table 1. Decrease of the maximal fluorescence intensity of the hydrophobic probes TNS and 9AC bound to calmodulin

	TNS		9AC	
	$K_{0.5}$ (μM)	n	$K_{0.5}$ (μM)	n
Nicergoline	18 ± 3	0.85 ± 0.01	22 ± 4	1.01 ± 0.05
Nicardipine	5 ± 1.3	1.4 ± 0.1	5 ± 0.5	1.5 ± 0.15
Quercetin	12 ± 1	1.6 ± 0.2	10.5 ± 0.7	1.47 ± 0.02
W-7	11 ± 2	1.24 ± 0.05	36 ± 6	0.81 ± 0.04
Bepridil	15 ± 5*	1.6 ± 0.2	4.9 ± 0.2	1.54 ± 0.04

* Coefficient of half-maximal fluorescence intensity increase at 433 nm.

Calmodulin (2 μM) was incubated in 100 mM Tris–HCl (pH 7.5), 0.5 mM $CaCl_2$ with 10 μM of TNS or 9AC at room temperature. Fluorescence spectrum was recorded and maximal fluorescence intensity determined at increasing concentrations of drug. Data were fitted to the sigmoidal curve and the coefficient of half-maximal fluorescence decrease ($K_{0.5}$) and the Hill coefficient (n) determined. Results are given as mean ± SE from two to four determinations.

addition of calmodulin in the presence of calcium resulted in an important increase of the fluorescence intensity together with a shift towards lower wavelengths of the emission maximum of the probe, which results from its binding to hydrophobic regions of calmodulin. Both nicergoline (curve d) and bepridil (curve c) depressed 9AC fluorescence (Fig. 1A). In Fig. 1B, the addition of calcium-bound calmodulin to TNS induced a similar effect as in the case of 9AC. Nicergoline depressed TNS fluorescence (Fig. 1B, curve d). By contrast, bepridil increased the fluorescence intensity at the wavelength of the emission maximum (λ_{max}) of TNS bound to calmodulin, and it shifted the λ_{max} from 465 to 433 nm.

Concentration-dependence of the effects of the two drugs were compared with those of other calmodulin inhibitors on the two fluorescent probes. Like nicergoline, W-7, nicardipine and quercetin decreased both 9AC and TNS fluorescence; at high concentration, they could all abolish fluorescence of the two probes (not shown).

Results in Table 1 show that concentrations of drug producing half-maximal decrease of fluorescence were identical on the two probes in the cases of nicergoline, nicardipine and quercetin. W-7 acted at a three times lower concentration on TNS than on 9AC, whereas bepridil was three times more potent to decrease 9AC fluorescence than to increase TNS fluorescence intensity at the λ_{max}. With the exception of the decrease of 9AC fluorescence by nicergoline, the Hill coefficients were different from unity, suggesting the existence of complex cooperative interactions.

Bepridil did not alter the fluorescence spectrum of TNS in the absence of calmodulin, even in hydrophobic solvents. This shows that the effect of bepridil on the fluorescence of TNS bound to calmodulin is not due to non-specific interactions of bepridil and TNS favored by the hydrophobic surface of calmodulin. Moreover, the effect of bepridil on TNS fluorescence was concentration-dependent, and W-7 was able to abolish the fluorescence of TNS bound to calmodulin in the presence of 30 μM of bepridil (not shown): W-7 decreased TNS fluorescence in the presence of bepridil with $K_{0.5}$ = 15 ± 0.2 μM and Hill coefficient 1.3 ± 0.15.

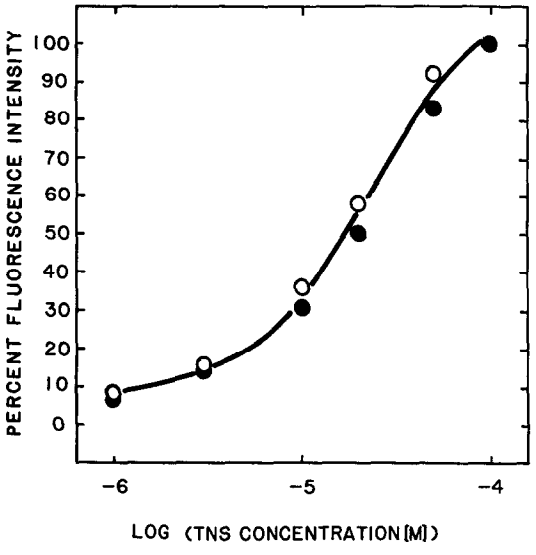

Fig. 2. Fluorescence of TNS bound to calmodulin at increasing TNS concentrations in the absence and presence of bepridil. Calmodulin (4 μM) was incubated with increasing concentrations of TNS in 100 mM Tris–HCl (pH 7.5), 0.5 mM $CaCl_2$ in the presence (●) or absence (○) of 50 μM bepridil. Fluorescence spectra were recorded at each concentration of TNS and maximal fluorescence intensity determined at 465 or 433 nm in the absence and presence of bepridil, respectively. Results were corrected for the basal fluorescence of TNS. Results for each curve are expressed as percent of the respective maximal increase. The maximal increase of fluorescence intensity in the presence of bepridil was 1.4 times higher than in the absence of bepridil.

In order to further investigate the mechanism by which bepridil affected the fluorescence emission of TNS, the fluorescence intensity at the λ_{max} of TNS bound to calmodulin was studied at increasing concentrations of TNS and in the absence and presence of 50 μM of bepridil. As shown by Fig. 2, bepridil

did not increase the apparent affinity of TNS for calmodulin, because it did not shift the curve of TNS fluorescence intensity to lower concentrations, but identically increased the fluorescence intensity at the λ_{max} at each concentration of TNS. This result suggests that the effect of bepridil on the fluorescence emission of TNS bound to calmodulin is not due to an allosteric increase in TNS binding to calmodulin. Furthermore, the finding that bepridil shifted the λ_{max} of the emission of TNS bound to calmodulin to a lower wavelength, shows that the binding of bepridil to calmodulin affected the environment of at least one TNS bound to calmodulin and thus changed its fluorescence properties. Indeed, a shift to lower wavelengths of the TNS emission λ_{max}, due to the passage of the probe from a less hydrophobic to a more hydrophobic environment, should normally be accompanied by an increase in its quantum yield [4]. However, in this case, it was impossible to appreciate the bound TNS quantum yield due to the presence of free and bound molecules in solution. The total fluorescence intensity (which corresponds to the area under the TNS fluorescence spectrum) was therefore measured: very similar values were found either in the presence or absence of bepridil. In order to explain this result, the fluorescence lifetime of TNS bound to calmodulin was measured in the absence and presence of bepridil: the addition of 30 μM of bepridil changed the fluorescence lifetime of TNS from 7.4 ± 0.07 nsec to 8.8 ± 0.4 nsec, confirming that bepridil binding to calmodulin affected the environment of the bound TNS molecules.

2. *Effect of drugs on the activation of phosphodiesterase by calmodulin*

The functional consequences of the binding of inhibitors to calmodulin were assessed by studying the effect of the inhibitors on the activation of phosphodiesterase by calmodulin. However, the effects of nicardipine and quercetin on phosphodiesterase were not studied, because these drugs have been shown to inhibit phosphodiesterase activity in the absence of calmodulin [26, 27].

Calmodulin (0.3–300 nM) activated cyclic nucleotide phosphodiesterase in a concentration-dependent manner, with an apparent activation constant of 5 ± 0.5 nM and a Hill coefficient of 1.0 ± 0.1 (Fig. 3). Bepridil (3–30 μM) (Fig. 3B) and W-7 (10–50 μM) (Fig. 3A) inhibited calmodulin activation of phosphodiesterase in an apparently competitive manner by shifting the activation curve to higher calmodulin concentrations.

In order to quantify the effects of bepridil and W-7, it was attempted to determine their K_i by Dixon plots. However, it was found that the data for phosphodiesterase inhibition by bepridil and W-7 could not be analysed by this method, because curvilinear instead of linear Dixon plots were thus obtained (not shown), in agreement with similar observations on the inhibition by bepridil of the activation of erythrocyte Ca^{2+}-ATPase by calmodulin [28]. The data for the activation of phosphodiesterase by calmodulin and its inhibition by bepridil and W-7 were therefore fitted to the Hill equation applied to competitive inhibition of phosphodiesterase activation by calmodulin. As shown in Fig.

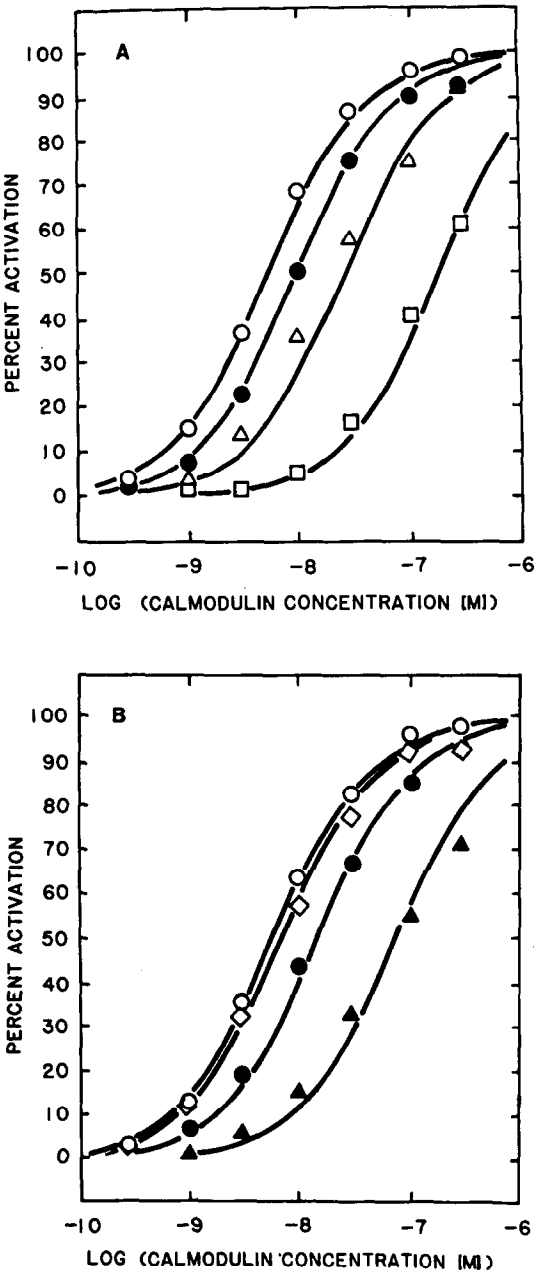

Fig. 3. Effects of W-7 (A) and bepridil (B) on the activation of phosphodiesterase by calmodulin. Concentrations-activation curves for calmodulin activation of phosphodiesterase were obtained at different concentrations of drugs and 1 μM of cGMP as substrate of phosphodiesterase. Results are expressed as percent maximal activation in the absence of drug, maximal activation corresponded to a phosphodiesterase activity 12 times higher than in the absence of calmodulin. Lines were drawn according to the results of a nonlinear least-squares fit to the equation of competitive inhibition according to the Hill equation for drug binding to calmodulin. The highest concentration of bepridil and W-7 inhibited phosphodiesterase activity in the absence of calmodulin by 16 and 20% respectively. Drug concentrations were: vehicle (○), 3 μM (◇), 10 μM (●), 20 μM (△), 30 μM (▲), 50 μM (□). Points represent the mean of three experiments, no error bars are shown as SE were less than 5% of the mean.

3, a good agreement could be obtained between the calculated curves and the experimental points for bepridil with $K_{0.5} = 8 \pm 1$ μM and Hill coefficient $n = 1.9 \pm 0.1$, and for W-7 with $K_{0.5} = 11 \pm 4$ μM and $n = 2.4 \pm 0.1$. This suggests that bepridil and W-7 are indeed acting in a competitive manner, but that more than one site of action and positive cooperativity are involved in their inhibitory effect on the activation of phosphodiesterase by calmodulin. However, these Hill coefficients for phosphodiesterase inhibition by bepridil and W-7 are higher than for displacement of hydrophobic probes from calmodulin. This could be explained by the fact that the highest concentrations of bepridil and W-7 directly inhibited cGMP hydrolysis by phosphodiesterase independently of its activation by calmodulin, or by the possibility that binding of drugs to some sites on calmodulin may not result in fluorescent probe displacement.

Nicergoline inhibited calmodulin activation of phosphodiesterase in an apparently non-competitive manner (Fig. 4A), suggesting that nicergoline inhibited phosphodiesterase directly, independently of calmodulin. To test this hypothesis the inhibitory action of nicergoline was assessed at constant high calmodulin concentrations (100 nM) and variable concentrations of the substrate of phosphodiesterase, cGMP. Under those conditions, the effect of a pure calmodulin inhibitor would not depend on the concentration of cGMP, whereas the effect of a competitive inhibitor of the hydrolysis of cGMP would be less at higher cGMP concentrations. In fact, it was found that nicergoline acted competitively to cGMP on phosphodiesterase thus activated by 100 nM of calmodulin, with an apparent K_i of about 60 μM (Fig. 4B) (two experiments with different cGMP concentrations gave K_i values of 67 μM and 49 μM respectively).

This direct inhibition is independent of calmodulin, and results in a depression of the maximal effect of calmodulin that can be corrected by expressing each activation curve relative to its maximum, and calculating the K_i for competitive inhibition from these corrected curves. When data were thus recalculated, an apparently competitive component of nicergoline's action could indeed be observed on Dixon plots (Fig. 4C), and a K_i value of 45 μM could thus be calculated. Nicergoline thus seems to have two mechanisms of action as inhibitor of calmodulin-activated phosphodiesterase: it inhibits cGMP hydrolysis directly with a K_i of about 60 μM, and inhibits the activation of phosphodiesterase by calmodulin with a K_i of about 45 μM. The activity of nicergoline was also assessed on two other forms of phosphodiesterase recently described [18]: at 100 μM nicergoline inhibited calmodulin-independent cGMP phosphodiesterase by 12% and cAMP phosphodiesterase by 34%.

DISCUSSION

This paper shows different effects of calmodulin inhibitors on the fluorescence emission of hydrophobic probes bound to calmodulin. Whereas nicardipine, quercetin, nicergoline and W-7 all decreased the fluorescence intensity of TNS and 9AC bound to calmodulin, bepridil was the only drug studied to differentially affect the two hydrophobic probes: it decreased 9AC fluorescence, but increased the fluorescence intensity of TNS bound to calmodulin at its λ_{max}. The decrease of 9AC and TNS fluorescence by the drugs is probably due to probe displacement from calmodulin since the λ_{max} of the fluorescence spectra were not affected by the drugs, showing that the drugs did not change the environment of the molecules of 9AC or TNS still bound to calmodulin. The effect of bepridil on the fluorescence emission of TNS was more complex. On the one hand, the shift in λ_{max} associated with the increase in the fluorescence lifetime shows that bepridil induces a change in TNS environment. On the other hand, the absence of any modification of total fluorescence intensity could be explained if the effect of bepridil on the fluorescence quantum yield of the TNS molecules still bound to calmodulin was compensated by displacement of some TNS molecules from calmodulin. This is in accord with the existence of two TNS binding sites on calmodulin [7] and with the fact that bepridil actually decreased the fluorescence intensity of TNS bound to calmodulin at emission wavelengths higher than 460 nm (Fig. 1, curve c). Thus one TNS molecule may be displaced by bepridil towards the aqueous medium, whereas the other still remains bound to calmodulin in a more hydrophobic environment.

Bepridil seems to be unique in affecting calmodulin's hydrophobic sites in this manner. Although other drugs (verapamil, prenylamine) have been shown to increase the fluorescence emission of hydrophobic probes (*N*-phenyl-1-naphthylamine, TNS) bound to calmodulin, the λ_{max} of the probes was not affected by these drugs [6, 29]. This suggests that these drugs increased the fluorescence emission of the probes by increasing their binding to calmodulin. It has also been shown that prenylamine and calmidazolium increase the fluorescence of felodipine bound to calmodulin, by allosterically increasing the affinity of calmodulin for felodipine [30, 31].

Fluorescence studies thus show that two drugs with similar structural features (both are hydrophobic and possess a protonated nitrogen atom) like bepridil and W-7 may interact differently with calmodulin. This is consistent with the finding that bepridil binds to more sites on calmodulin than the other calmodulin inhibitors (five sites for bepridil versus one to three sites for other inhibitors) [32].

Despite the differences between bepridil's and W-7's effects on calmodulin hydrophobic sites, phosphodiesterase activation by calmodulin was inhibited by both bepridil and W-7 in a competitive manner. Furthermore, similar $K_{0.5}$ values were obtained for phosphodiesterase inhibition and displacement or stimulation of TNS and 9AC fluorescence by the two drugs. The finding that bepridil and W-7 inhibited calmodulin activation with positive cooperativity suggests that several hydrophobic sites are involved in the inhibitory action of bepridil and W-7. These results are in accord with the results of Reynolds and Claxton [33], who showed that calmodulin possesses two binding sites for tricyclic drugs, binding to one site being sufficient to abolish

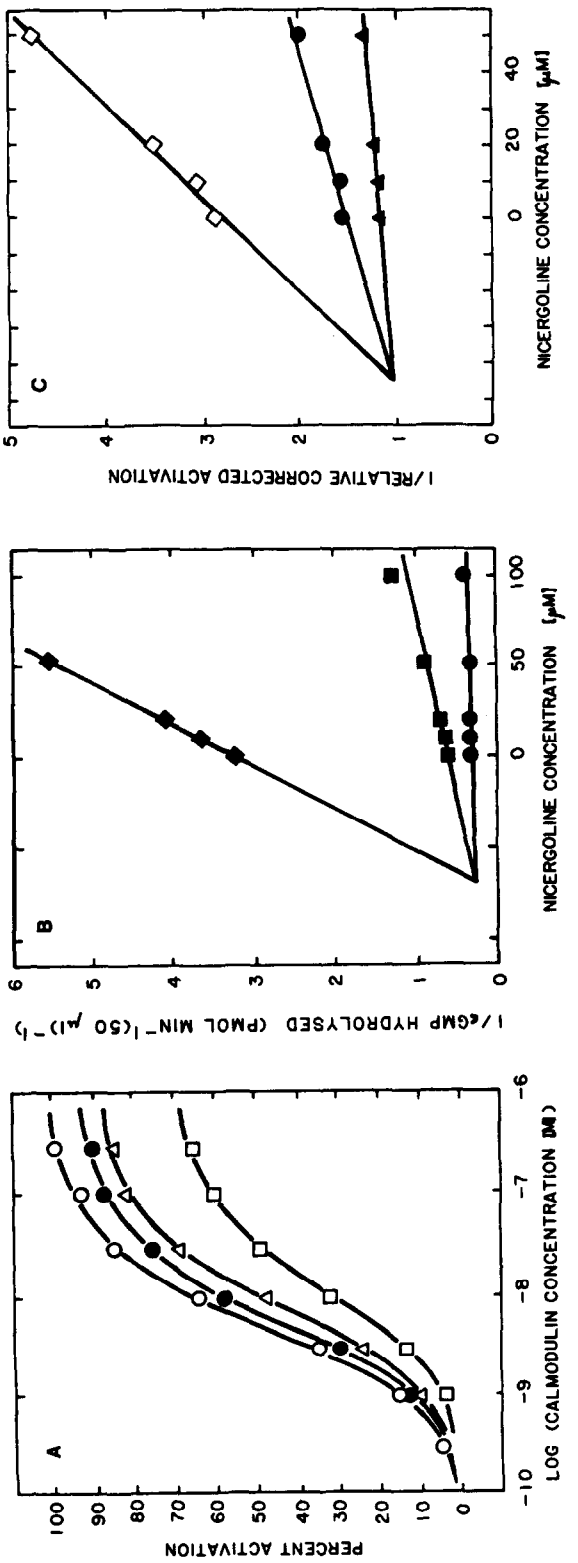

Fig. 4. Effects of nicergoline on calmodulin-dependent phosphodiesterase. (A) Effect of nicergoline on the activation of phosphodiesterase by calmodulin. Experimental conditions were the same as in Fig. 3. Nicergoline concentrations were vehicle (○), 10 μM (●), 20 μM (△), 50 μM (□). (B) Dixon plot of phosphodiesterase inhibition at different concentrations of cGMP. Calmodulin (100 nM) activated phosphodiesterase was incubated with increasing concentrations of nicergoline at different concentrations of cGMP: 0.1 μM (◆), 1 μM (■) and 10 μM (●). A K_i of 67 μM was obtained for the data shown. (C) Dixon plot of some of the data from Fig. 4A after correction for the depression of the maximal activation. Calmodulin concentrations used were 3 μM (◇), 10 μM (●), 30 μM (▲). A K_i of 45 μM was calculated from the plot.

the activation of phosphodiesterase. These results are also complementary to those of Giedroc *et al.* [34] and Newton *et al.* [35, 36], who showed that covalent binding of either one molecule of β-endorphin [34] or one molecule of phenothiazine derivative [35, 36] to calmodulin was sufficient to prevent the resulting complex from activating phosphodiesterase, whereas the covalent adduct between a phenothiazine and calmodulin was still able to activate calcineurin and phosphorylase kinase [36].

Phosphodiesterase was thus identically affected by bepridil and W-7 which had different effects on calmodulin's hydrophobic sites. This finding is consistent with the observation of others that prenylamine and trifluoperazine, which displaced [^{3}H]W-7 from calmodulin in a different manner (prenylamine acting non-competitively and trifluoperazine acting competitively, respectively), similarly inhibited calmodulin activation of phosphodiesterase [29]. This may not be the case for other calmodulin-dependent enzymes, however. Cardiac sarcolemmal Ca^{2+}-ATPase for instance has been reported to be differentially affected by bepridil, felodipine and prenylamine [37]. Whereas felodipine inhibited this enzyme at high Ca^{2+} concentrations, prenylamine was ineffective and bepridil had a stimulatory effect. It is tempting to speculate that this stimulatory effect of bepridil may be related to the effect of bepridil on TNS fluorescence described in this paper, but more detailed studies with cardiac sarcolemmal Ca^{2+}-ATPase will be necessary to demonstrate this point.

Nicergoline is an alpha-adrenoceptor-antagonist with additional calcium-antagonistic properties [38]. As shown here, nicergoline is also a calmodulin inhibitor, but unlike bepridil and W-7, nicergoline interacted directly with cGMP hydrolysis on phosphodiesterase at concentrations only slightly higher than those necessary for its binding to calmodulin. But nicergoline also inhibited phosphodiesterase activation by calmodulin in an apparently competitive manner, like bepridil and W-7. This calmodulin-competitive component of nicergoline's inhibitory activity on phosphodiesterase was well correlated with the displacement of TNS and 9AC from calmodulin by nicergoline. Nicergoline is thus yet another representative of the class of calcium antagonists with calmodulin inhibitory properties.

This paper demonstrates different effects of bepridil and other calmodulin inhibitors on the hydrophobic sites of calmodulin. These drugs do not all affect the fluorescence of hydrophobic probes bound to calmodulin in the same manner. However, these differences between inhibitors are not found on phosphodiesterase activation by calmodulin. Further studies with other enzymes may be necessary to establish the functional importance of these differences.

Acknowledgements—The authors are indebted to Dr Busch (R.L.-C.E.R.M., Riom) and Dr Meilhac (Specia) for their support and constant interest, as well as to Dr M. C. Kilhoffer for reading the manuscript.

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