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MODULATION OF VASCULAR AND CARDIAC CONTRACTILE PROTEIN REGULATORY MECHANISMS BY CALMODULIN INHIBITORS AND RELATED COMPOUNDS

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Abstract—The abilities of several calmodulin antagonists and other compounds belonging to different pharmacological classes to modulate Ca^{2+}·calmodulin mediated arterial myosin light chain phosphorylation and Ca^{2+}–troponin C regulated cardiac myofibrillar ATPase activity have been quantitated in Triton X-100 purified preparations of bovine aortic actomyosin and canine ventricular myofibrils. At submaximal free Ca^{2+} concentrations, all calmodulin antagonists inhibited myosin phosphorylation; however, some (calmidazolium, trifluoperazine, chlorpromazine, pimozide) stimulated myofibrillar ATPase activity, some (compound 48/80, W-5) had no effect on activity, while others (W-7, haloperidol, mastoparan) inhibited ATPase activity. The relative order of potency for several agents in both preparations was the same, as IC_{50} values for inhibition of arterial myosin phosphorylation were: calmidazolium, 0.5 μM; trifluoperazine, 22 μM; perhexiline, 35 μM; and concentrations which stimulated cardiac myofibrillar ATPase activity by 50% were: calmidazolium, 9 μM; trifluoperazine, 45 μM; perhexiline, 90 μM. A common feature of stimulation of cardiac ATPase activity by these agents was a leftward shift in the pCa relationship, although different shape changes in the pCa curves were also apparent. Maximum ATPase activity was either not affected or inhibited (trifluoperazine). Several other agents belonging to diverse pharmacological classes also had differential effects on myosin phosphorylation and ATPase activity. These results show that structurally-distinct calmodulin antagonists and other compounds differentially affect cardiac myofibrillar ATPase activity. Moreover, several agents have been identified which inhibit arterial, and stimulate cardiac, contractile protein regulatory mechanisms. Thus, it may be possible to develop mechanistically novel cardiotonic/vasodilator agents, Ca^{2+} binding protein modulators, which function primarily by altering the Ca^{2+} sensitivity of contractile protein interactions.

Regulation of contractile protein interactions in both smooth and cardiac muscle involves Ca^{2+} binding to distinct, but structurally-related Ca^{2+} binding proteins. In cardiac muscle, the Ca^{2+}-receptive protein is troponin C, and Ca^{2+} binding to the troponin complex induces a conformational change which ultimately results in increased actin–myosin interactions [see Ref. 1 for review]. In smooth muscle, the primary Ca^{2+}-receptive protein is calmodulin, and Ca^{2+} binding leads to Ca^{2+}–calmodulin activation of myosin light chain kinase, increased myosin light chain phosphorylation, and stimulation of actin–myosin interactions [see Refs. 2–5 for some reviews].

Pharmacologically, several classes of agents with differing chemical structures have been identified as Ca^{2+}–calmodulin antagonists [6–10]. Primarily, most of these agents compete with the target enzyme (usually calmodulin-sensitive phosphodiesterase) for the Ca^{2+}–calmodulin complex. However, direct comparative studies have revealed differing potencies among some agents in different calmodulin-regulated systems, suggesting that other mechanisms may also be involved [11, 12].

Relatively fewer drugs which directly influence Ca^{2+}–troponin C interactions have been identified. Among these, some cardiotonic agents such as ARL 115BS (Vardax) and APP-201-533 have been reported [13–15] to increase cardiac myofibrillar ATPase activity primarily through an increase in Ca^{2+} sensitivity of Ca^{2+} binding to troponin C.

In earlier studies, we reported that the lipophilic, class III (as classified by Spedding [16]) Ca^{2+} antagonist agents perhexiline and cinnarizine functioned as Ca^{2+}–calmodulin antagonists by directly inhibiting myosin light chain phosphorylation and actin–myosin interactions in Triton X-100 purified native arterial actomyosin [15]. Moreover, differential effects were apparent in the troponin C regulated cardiac myofibrillar preparation, as perhexiline was stimulatory, cinnarizine was without effect, and W-7 was inhibitory, suggesting that calmodulin antagonists might differentially affect troponin C regulated myofibrillar ATPase activity. In the present study, we extended our original findings and further examined other calmodulin antagonists and structurally-related agents for the ability to modulate the Ca^{2+} sensitivity of calmodulin and troponin C regulated contractile protein interactions.

MATERIALS AND METHODS

Materials. Drugs/compounds were obtained from the following sources: trifluoperazine (Boehringer Mannheim GmbH, West Germany); calmidazolium

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(Boehringer Mannheim Biochemicals, Indianapolis, IN); buproprion (Burroughs-Welcome Research, Triangle Park, NC); ouabain (Calbiochem, San Diego, CA); maprotiline (Ciba-Geigy Pharmaceuticals, Summit, NJ); ethmozin (DuPont Pharmaceuticals, Wilmington, DE); aprindine and fluoxetine (Eli Lilly, Indianapolis, IN); sodium azide (Fisher Scientific, Pittsburgh, PA); lorcainide and pimozide (Janssen Pharmaceuticals, Piscataway, NJ); bepridil and haloperidol (McNeil Pharmaceuticals, Ft. Washington, PA); zimelidine (Merck Sharp & Dohme, West Point, PA); desmethylimipramine (Merrell-Dow Research Center, Cincinnati, OH); mianserin (Organon, Inc., West Orange, NJ); mastoparan (Ocean Biologics, Inc., Edmunds, WA); fleckainide (Riker Laboratories, Inc., Northridge, CA); APP-201-533 and clozapine (Sandoz, Ltd., East Hanover, NJ, and Basel, Switzerland); disopyramide (Searle Laboratories, Inc., Chicago, IL); W-5 (Seikagaku America, Inc., St. Petersburgh, FL); compound 48/80, imipramine, perhexiline, procainamide and W-7 (Sigma Chemical Co., St. Louis, MO); chlorpromazine (Smith Kline & French, Laboratories, Philadelphia, PA); and iprindole (Wyeth Laboratories, Inc.). Bay K 8644 and Vardax were supplied by Mr. H. Fletcher (Wyeth Laboratories, Inc.).

Contractile protein preparations. Triton-purified native actomyosin from bovine aortic muscularis and Triton-purified myofibrils from canine ventricles were prepared as previously described [15, 17–19]. Bovine aortas from freshly slaughtered steer were obtained from a local abbatoir and packed immediately in ice for transport to the laboratory. Upon arrival at the laboratory, the muscularis was cleaned of connective and endothelial tissue and then subsequently homogenized in 2.5 vol (wet weight/volume) of a solution containing (mm) MOPS*, 20 (pH 7.0); KCl, 80; $MgCl_2$, 4; DTT, 1; EGTA, 4; and disodium ATP, 4. This and subsequent procedures were performed at 0–4°. Crude actomyosin was extracted by stirring (4 hr) and precipitated from a supernatant fraction (30,000 *g*, 30 min) by dialysis against 5 liters of a 4 mM MOPS (pH 7.0), 125 mM KCl, 0.2 mM EGTA and 1 mM DTT solution. After dialysis, the crude actomyosin was collected by centrifugation (10,000 *g*, 20 min) and purified by sequential washing/centrifugation in 2.5 pellet volumes of a solution (pH 7.0) containing 50 mM KCl, 1 mM $MgCl_2$, 1 mM DTT in first the presence and then the absence of 1% Triton X-100.

Cardiac myofibrils were prepared using a modification of the method of Solaro *et al.* [19]. Canine ventricular muscle was obtained from animals anesthetized with nembutol (35 mg/kg). Fat and connective tissue were trimmed away, and the tissue was ground and subsequently homogenized in 4 volumes (wet weight/volume) of a standard buffer solution which contained 20 mM MOPS (pH 7.0), 60 mM KCl, 2 mM $MgCl_2$ and 1 mM DTT. This and subsequent procedures were performed at 0–4°. The homogenate was centrifuged at 17,300 *g* for 20 min, and the loose portion of the pellet was resuspended to the original homogenate volume with standard buffer solution. This suspension was homogenized and centrifuged at 750 *g* for 15 min. This resultant pellet was resuspended, filtered through cheesecloth, and centrifuged at 750 *g* for 15 min for a total of four times. The resulting pellet, which was light brown in color, was sequentially washed and centrifuged (750 *g*, 15 min) in standard buffer solution containing 2 mM EGTA (one time), standard buffer solution plus 1% Triton X-100 (two times) and standard buffer solution only (four times). The last six washes were done with eight pellet volumes of buffer. Purified myofibrils were resuspended at a final concentration of 8 mg/ml in standard buffer solution. As with vascular actomyosin, the protein concentration of the myofibrils was determined by the method of Bradford [20].

Vascular myosin light chain phosphorylation. Effects of agents on Ca^{2+}-dependent phosphorylation of the 20,000 dalton myosin light chain were adjudged in assays performed within 24 hr following final purification of actomyosin and 84 hr following initial processing of the tissue. Reaction mixtures (1 ml) containing 1.0 mg actomyosin, 20 mM MOPS (pH 7.0), 50 mM KCl, and 3 mM EGTA were incubated for 5 min at 25° prior to assay. Sufficient $CaCl_2$ was added to yield 3.8 μM free Ca^{2+} [21], and the reaction was initiated by the addition of $MgCl_2$-ATP (pH 7.0) to final concentrations of 10 and 2 mM respectively. All drugs were freshly dissolved in 95% ethanol or 100% dimethylacetamide at a stock concentration of 10^{-2} M just prior to assay. Appropriate precautions with light-sensitive compounds were followed. Compounds were incubated in the reaction mixtures (-$CaCl_2$) at the indicated concentrations for 5 min at 25° prior to assay. Reactions were terminated 6 min after the addition of $MgCl_2$-ATP, which was shown previously to present maximal, steady-state myosin light chain phosphorylation [17]. Reactions were halted by denaturation of 500 μl of reactant in an equal volume of a solution containing 8 M urea, 15 mM β-mercaptoethanol, 2% Triton X-100, and 2% ampholines (1.6% pH 4–6 LKB Instruments Inc., Rockville, MD; 0.4% pH 3–10 Bio-Rad Laboratories, Richmond, CA).

The extent of myosin light chain phosphorylation in the reaction mixtures was determined by isoelectric focusing on polyacrylamide gels [17, 18, 22]. Samples (100 μg total protein) were electrofocused on polyacrylamide tube gels (75 × 5 mm) for 4.5 hr at a constant voltage setting of 400 V. Tube gels consisted of 8 M urea, 7.5% polyacrylamide, 1% Triton X-100, and 2% ampholines (same distribution as in the denaturation buffer). The top electrophoresis buffer (cathode solution) was 20 mM NaOH, while the bottom buffer (anode solution) was 80 mM H_3PO_4. During electrofocusing, the bottom buffer was cooled by a circulating water bath maintained at 12°. Following electrofocusing, the gels were fixed in 15% trichloroacetic acid and washed in 15% methanol/10% acetic acid. Gels were stained with Coomassie Blue R-250 (Sigma) and subsequently destained in methanol/acetic acid. Following destaining, the extent of myosin light chain phosphorylation was quantitated by densitometric

* Abbreviations: MOPS, morpholinopropane sulfonic acid; EGTA, ethylene glycol bis (β-aminoethyl ether)-*N,N'*-tetraacetic acid; and DTT, dithiothreitol.

scanning and determination of the peak heights for the nonphosphorylated and phosphorylated forms of the light chain. Phosphate content of the light chain was calculated (on a mole phosphate/mole of light chain basis) by expressing the phosphorylated form as a percentage of the total light chain. The effects of antagonists were compared to vehicle control responses (either 0.95% ethanol or 0.3% dimethylacetamide, final concentration) sampled in each preparation. Neither vehicle affected the extent of myosin light chain phosphorylation.

Cardiac myofibrillar ATPase activity. Calcium-dependent myofibrillar ATPase activity was quantitated by measuring the rate of release of inorganic phosphate. As with vascular actomyosin, all assays were performed within 24 hr of final purification. Reaction mixtures (8 ml) containing 1.0 mg/ml cardiac myofibrils, 20 mM MOPS (pH 7.0), 65 mM KCl, 3 mM EGTA, the indicated concentration of drug or appropriate vehicle, and the desired amount of free Ca^{2+} [21] were incubated for 5 min at 25° prior to assay. All drugs were dissolved in 95% ethanol or 100% dimethylacetamide; the effects of each compound were compared with the appropriate vehicle controls. Reactions were initiated by the addition of $MgCl_2$-ATP (pH 7.0) to final concentrations of 10 and 1 mM respectively. Aliquots (1 ml) were withdrawn from the reaction mixtures at 0, 2, 4, and 6 min; reactions were halted with an equal volume of ice-cold 10% trichloroacetic acid. Inorganic phosphate in supernatant fractions (2000 *g*, 15 min) was measured using the method of Rockstein and Herron [23], and the specific activity of the ATPase (nmoles phosphate released/mg protein/min) was calculated by quantifying the linear rate of release of phosphate. Phosphate release was linear for all compounds and vehicles tested.

The vehicles used (either 0.95% ethanol or 0.3% dimethylacetamide, final concentration) produced no significant effect on ATPase activity measured at 2 μM free Ca^{2+} (95–99 nmoles phosphate/mg protein/min). The absence of contaminating, membrane-associated ATPase activity as a result of Triton purification [19] was confirmed as neither 1 mM

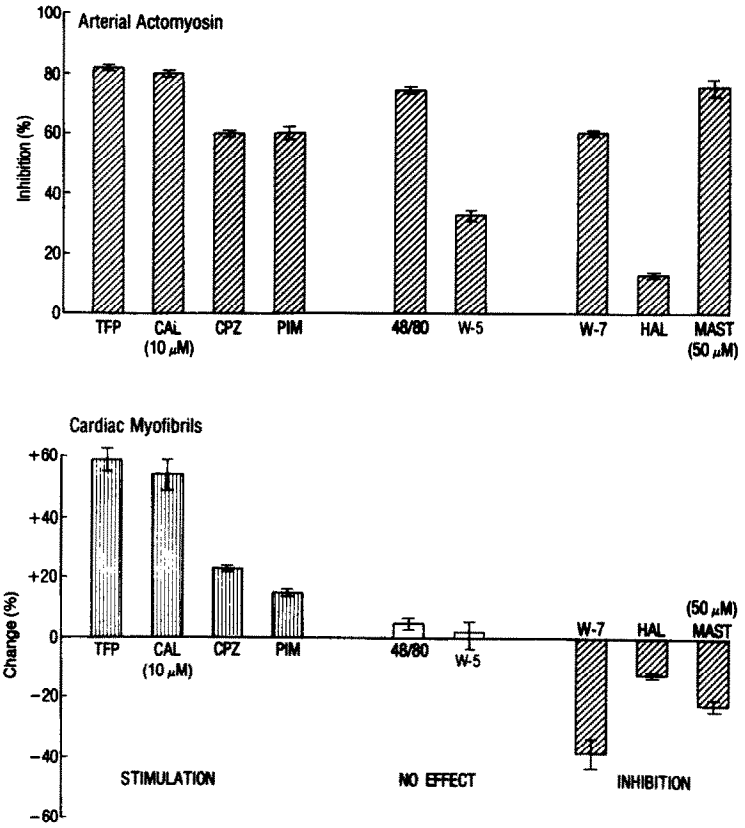

Fig. 1. Effects of calmodulin antagonists on arterial actomyosin P-light chain phosphorylation (top panel) and cardiac myofibrillar ATPase activity (bottom panel). Agents were tested at a final concentration of 100 μM unless otherwise indicated. The free Ca^{2+} concentration in each preparation was either 3.8 μM (actomyosin) or 2 μM (myofibrils); each was submaximal in the respective preparation. Results are the mean ± S.E. for four to six preparations and are expressed as either percent inhibition (actomyosin) or percent change (myofibrils). The extent of control (0.95% ethanol vehicle) myosin light chain phosphorylation was 0.69 ± 0.02 mole phosphate/mole light chain, and control (0.95% ethanol vehicle) myofibrillar ATPase activity was 99 ± 4 nmoles phosphate released/mg protein/min. Abbreviations: TFP, trifluoperazine; CAL, calmidazolium; CPZ, chlorpromazine; PIM, pimozide; 48/80, compound 48/80; HAL, haloperidol; and MAST, mastoparan.

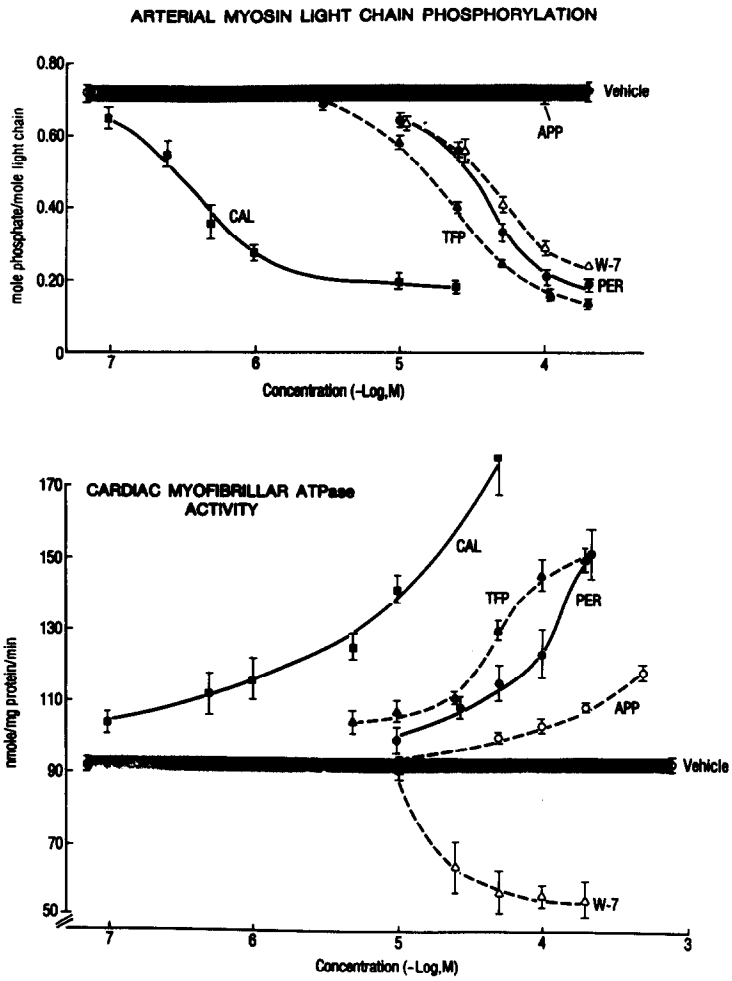

Fig. 2. Concentration–response for inhibition of arterial actomyosin P-light chain phosphorylation (top panel) and stimulation/inhibition of cardiar myofibrillar ATPase activity (bottom panel). Results are the mean ± S.E. for five to seven contractile protein preparations and are expressed either as mole phosphate/mole light chain (actomyosin) or nmole phosphate released/mg protein/min (myofibrils). Assays were performed at the indicated drug concentrations as described in the text. Calmidazolium was insoluble at final concentrations greater than 25 μM. Vehicles were either 0.95% ethanol or 0.3% dimethylacetamide. Abbreviations: CAL, calmidazolium; TFP, trifluoperazine; PER, perhexiline; and APP, APP-201-533.

sodium azide (102 ± 2 nmoles/mg/min) nor 100 μM ouabain (100 ± 2 nmoles/mg/min) significantly affected myofibrillar ATPase activity.

RESULTS

Effects of calmodulin antagonists. The effects of structurally-diverse calmodulin antagonists on both arterial myosin light chain phosphorylation and cardiac myofibrillar ATPase activity are shown in Fig. 1. As expected, all agents inhibited myosin light chain phosphorylation. However, differential effects (either stimulation, inhibition, or no effect) were apparent in cardiac myofibrils.

In further concentration-related experiments, two of the more effective ATPase stimulants (calmidazolium and trifluoperazine) were compared with perhexiline (a lipophilic Ca^{2+} blocker/calmodulin antagonist), the cardiotonic ATPase stimulant APP-201-533 [14], and an ATPase inhibitor, W-7 (Fig. 2). In arterial actomyosin, the relative order of potency and concentrations which produced half-maximal (IC_{50}) inhibition were: calmidazolium, 0.5 μM; trifluoperazine, 22 μM; perhexiline, 35 μM; and W-7, 40 μM. APP-201-533 was ineffective at a concentration as high as 100 μM in this preparation. Interestingly, the same relative order of potency for stimulation of cardiac myofibrillar ATPase activity (expressed as the concentration which increased maximal activity by 50%) was apparent for calmidazolium (9 μM), trifluoperazine (45 μM) and perhexiline (90 μM). Inhibition of ATPase activity by W-7 occurred over roughly the same concentration range (IC_{50} = 26 μM) as inhibition of arterial myosin phosphorylation.

Effects of antiarrhythmic, antidepressant, and anti-

Table 1. Effects of various agents on arterial myosin phosphorylation and cardiac ATPase activity

Class of agent	Effect on arterial myosin light chain phosphorylation	Effect on cardiac myofibrillar ATPase activity
Anti-arrhythmic		
Aprindine	−(40%)	+(23%)
Disopyramide	0	+(13%)
Ethomozin	0	0
Fleckainide	0	+(13%)
Lorcainide	0	+(16%)
Procainamide	0	0
Anti-depressant		
Bupropion	0	0
Maprotiline	0	+(23%)
Mianserin	0	+(17%)
Fluoxetine	−(80%)	0
Desmethylimipramine	−(48%)	0
Imipramine	−(50%)	0
Iprindole	−(60%)	+(29%)
Zimelidine	−(40%)	+(15%)
Anti-psychotic		
Clozapine	0	+(31%)
Haloperidol	−(15%)	−(13%)
Trifluoperazine	−(80%)	+(47%)
Chlorpromazine	−(60%)	+(23%)
Calcium agonist/antagonist		
Bay K 8644	0	0
Bepridil	−(59%)	+(61%)
Cinnarizine	−(58%)	0
Perhexiline	−(73%)	+(37%)

Values are the mean for three to nine preparations of actomyosin or myofibrils; standard errors of the mean ranged from 1 to 5%. The concentration of all agents was 100 μM, and comparisons were made to vehicle-control (0.95% ethanol) assays as described in the text. Key (−) = inhibitory, (+) = stimulatory, and (0) = not active (≤ 10% change).

psychotic agents. The relative effects at a final concentration of 100 μM of several agents belonging to different pharmacological classes on both arterial myosin light chain phosphorylation and cardiac myofibrillar ATPase activity are shown in Table 1. As with some of the calmodulin antagonists, some agents, such as fluoxetine, imipramine and desmethylimipramine, significantly affected myosin light chain phosphorylation but did not affect ATPase activity. Conversely, several agents, such as disopyramide, fleckainide, lorcainide, maprotiline, mianserine, and clozapine, were ineffective in arterial actomyosin but did significantly stimulate myofibrillar ATPase activity. Agents which markedly affected both variables were aprindine, iprindole and bepridil. Inhibition of calmodulin-regulated systems by these three agents has been reported previously [24–27].

Effect of ATPase stimulants on myofibrillar Ca^{2+} *sensitivity, maximum activity and cardiac myosin light chain phosphorylation.* The effects of calmidazolium (25 μM), trifluoperazine (100 μM), and perhexiline (100 μM) on the Ca^{2+} dependence of cardiac myofibrillar ATPase activity were determined (Fig. 3). All three stimulants produced a leftward shift in the pCa-ATPase relationship. pCa is defined as the negative log of the molar free Ca^{2+} concentration. However, changes in the shape of the pCa curves were also evident, suggesting other more complex effects. Moreover, differential effects on maximum ATPase activity were apparent as trifluoperazine inhibited maximum activity while perhexiline and calmidazolium were without effect. APP-201-533 (250 μM) increased both Ca^{2+} sensitivity and maximum ATPase activity.

The possibility of selective modulation of cardiac myosin light chain phosphorylation in the myofibrillar preparation by some of these agents was also examined. However, none of the agents listed in Fig. 1 or Table 1 significantly affected the extent of cardiac myosin light chain phosphorylation over the time course of the ATPase assay (data not shown).

DISCUSSION

This report shows that structurally diverse agents with differing pharmacological profiles can modulate either vascular or cardiac contractile protein regulatory mechanisms. Within the diverse group of agents previously shown to antagonize calmodulin-regulated enzymes [6–10], differential effects on cardiac myofibrillar ATPase activity were evident (Fig.

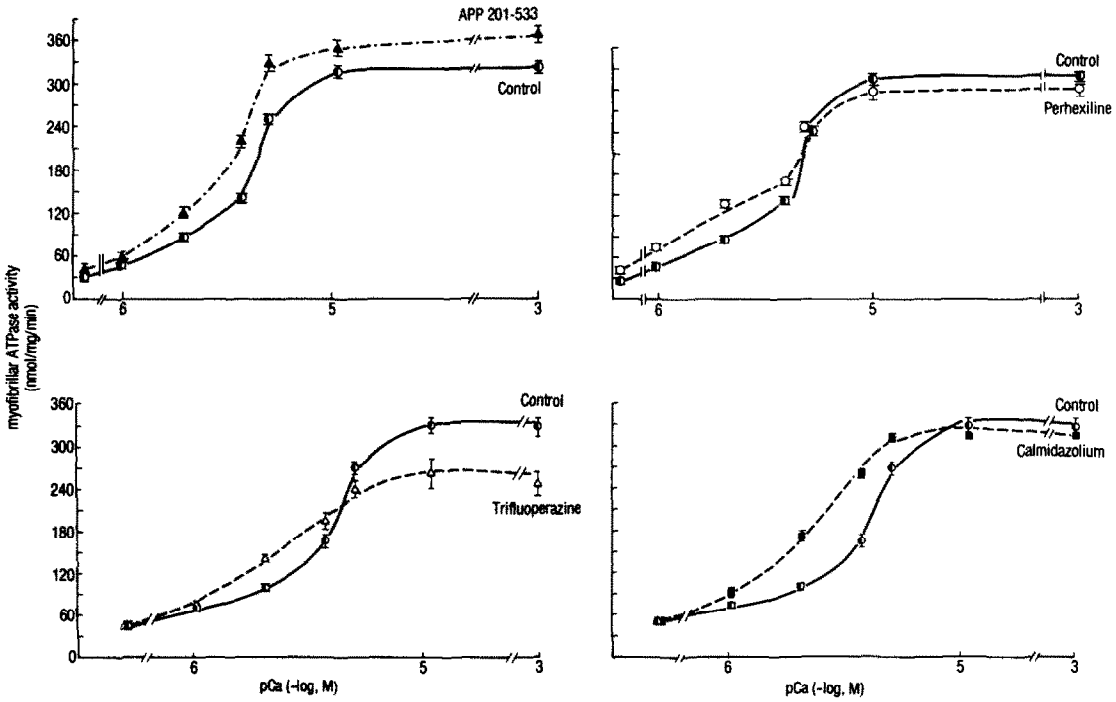

Fig. 3. Comparative effects of APP-201-533, perhexiline, trifluoperazine, and calmidazolium on Ca^{2+} sensitivity (expressed as pCa) and maximum activity of canine cardiac myofibrillar ATPase activity. Values are the mean ± S.E. for five myofibrillar preparations; assays measuring phosphate release were performed at the indicated concentrations of free Ca^{2+} as described in the text. All drugs were tested in the same preparations and compared to vehicle-treated controls. Dimethylacetamide (0.3%) was the vehicle for APP-201-533, whereas ethanol (0.95%) was the vehicle for perhexiline, trifluoperazine, and calmidazolium. Values to the left of the broken line were obtained in the absence of added Ca^{2+} ($<10^{-8}$ M). (●) control; (▲) 250 μM APP-201-533; (○) 100 μM perhexiline; (△) 100 μM trifluoperazine; and (■) 25 μM calmidazolium.

1, Table 1). Interestingly, the naphthalene sulfonamide, W-7, inhibited activity whereas the structurally-similar sulfonamide, W-5, was without effect. Similarly, although the structurally-similar tricyclic antidepressants iprindole and imipramine both inhibited arterial myosin phosphorylation, only iprindole significantly stimulated myofibrillar ATPase activity (Table 1). Among the agents tested in Table 1, the results obtained with haloperidol and clozapine were also of interest. Neither agent markedly affected arterial myosin light chain phosphorylation at a concentration of 100 μM, although both have been reported to inhibit calmodulin-regulated phosphodiesterase activity, with IC_{50} values of 19 μM (clozapine) and 41 μM (haloperidol) [28]. Haloperidol produced slight inhibition of cardiac myofibrillar ATPase activity while clozapine was one of the more effective ATPase stimulants. Thus, it appears that certain agents may affect different $Ca^{2+} \cdot Ca^{2+}$ binding protein-regulated systems via unique ways, possibly by mechanisms in addition to simple competition for the $Ca^{2+} \cdot Ca^{2+}$ binding protein complex [9, 11, 12, 29, 30].

Among the more effective ATPase-stimulating agents, all were more potent than the previously described cardiotonic APP-201-533 [14]. In this regard, calmidazolium was the most potent of any of the agents tested. It will be of further interest to examine this agent in more physiological models of contractility, such as force generation in skinned cardiac fibers.

Mechanistically, the more effective ATPase-stimulating agents that were tested all produced a leftward shift in the pCa-ATPase relationship (Fig. 3), which is consistent with an effect on Ca^{2+} binding to troponin C [13] or an enhancement of Ca^{2+}–troponin C binding to the thin-filament. Consistent with these possibilities are previous studies which have shown that calmodulin inhibitors like trifluoperazine can bind to troponin C [31, 32]. The cardiotonic agent APP-201-533 increased both Ca^{2+} sensitivity and maximum ATPase activity, calmidazolium and perhexiline did not affect maximum ATPase activity, while trifluoperazine inhibited maximum activity. Thus, both the concentration (Fig. 2) and free Ca^{2+} concentrations (Fig. 3) are important determinants in assessing the relative effects of agents on cardiac myofibrillar ATPase activity. A previous study [33] with porcine skeletal muscle myofibrils showed inhibition of maximal ATPase activity with 100 μM trifluoperazine, similar to our results with canine cardiac myofibrils (Fig. 3). However, effects of the drug on Ca^{2+} activation of porcine skeletal muscle myofibrils were not reported.

The general structural requirements for calmodulin inhibitors have been reported previously to

include a hydrophophic region and charged amino group separated by an alkyl chain at least 3 atoms long [7, 9]. In the present study, a common structural feature, which was shared by all ATPase modifiers producing at least a 15% increase in activity at 100 μM, was a nitrogen atom in, or in close proximity to, a cyclo-alkane moiety. However, other features, such as the presence or absence of hydrophophic regions, may also modify activity. While further experiments are needed to more clearly define the optimal structural requirements for both arterial myosin phosphorylation inhibition and myofibrillar ATPase stimulation, this common structural feature may serve as a template upon which to design and test future compounds which may modulate both contractile protein systems.

Development of Ca^{2+} binding protein modulators that function by inhibiting Ca^{2+}–calmodulin activation of arterial myosin light chain phosphorylation and also by stimulating Ca^{2+}–troponin C activation of cardiac myofilaments offers a novel cellular approach to a disease such as congestive heart failure, where a combination of vascular smooth muscle relaxation (vasodilation) and increased force development in cardiac muscle (positive inotropy) is often desired. Current therapeutic agents which are in various stages of development, such as milrinone, piroximone, and CI-914, provide this combination through purported selective inhibition of the high cAMP-affinity isozyme of phosphodiesterase, and subsequent increases in intracellular cAMP and modulation of Ca^{2+} flux and/or contractile protein mechanisms [see Ref. 34 for review]. Calcium binding protein modulators might offer a somewhat different therapeutic and side-effect profile, as the primary function should be alteration in the Ca^{2+} sensitivity (gain) of contractile protein interactions without changes in cyclic nucleotide formation or Ca^{2+} flux. In addition, depressed cardiac myofibrillar ATPase activity has been reported in some animal models of congestive heart failure [35, 36], suggesting that the contractile proteins are a locus which is affected by this disease. In this regard, we are currently assessing the relative effectiveness of Ca^{2+} binding protein modulators in directly improving myofibrillar function in myopathic animals.

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