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EFFECTS OF DIHYDROPYRIDINE CALCIUM CHANNEL MODULATORS IN THE HEART: Pharmacological and Radioligand Binding Correlations

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Bay k 8644 produced a concentration-dependent positive inotropic effect followed by a negative inotropic effect in isolated and intact cardiac preparations. Nimodipine in low concentrations produced slight positive inotropy and in higher concentrations, the usual negative inotropic action. Radioligand binding experiments revealed equilibrium dissociation constants that, taken together with the pharmacological data, suggest that dihydropyridines bind to receptor subtypes and have varying intrinsic activities.

The calcium channel inhibitor drugs are a heterogeneous class of pharmacological agents that are being employed with increasing frequency in a wide spectrum of disease states, including angina pectoris, hypertension, migraine, certain arrhythmias, cardiomyopathies, peripheral vascular disorders and perhaps prevention of secondary myocardial infarction (1,2). While it is likely that multiple mechanisms are involved in these actions, it seems clear that at least one major site of action is the slow calcium channel in cell membranes of heart and vascular smooth muscle, inhibition of which results in relaxation (2). With the advent of high specific activity radioactive ligands of dihydropyridines, verapamil derivatives and diltiazem, it has become possible to relate specific binding to crude and partially purified membranes with effects on cardiac, skeletal and smooth muscle, brain and isolated intact cells (2,3). Recently, derivatives of dihydropyridines have become available that are of considerable interest because they produce effects opposite to those produced by the calcium antagonists. These drugs, including Bay k 8644 (4), YC-170 (5) and CGP 28392 (6), are termed calcium channel agonists. In

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terms of possible sites of action associated with the calcium channel, it appears that high (R_H) and low (R_L) affinity "receptor" binding sites exist and that the calcium channel agonists probably compete with the antagonists at R_H (7-10). In this paper, we provide evidence that Bay k 8644 produces concentration-related positive and negative effects in rat hearts, and dog Purkinje and ventricular trabecular tissues. Preliminary data were presented in abstract form (11).

EXPERIMENTAL PROCEDURES

Materials. Nitrendipine (NTD), nimodipine and Bay k 8644 were supplied by Miles Pharmaceuticals, courtesy of Dr. Alexander Scriabine. [^{3}H]NTD, specific activity 70-79.5 Ci/mmol, was purchased from New England Nuclear.

Methods.

Pharmacological Experiments. Myocardial contractile force in atrial and ventricular strips, trabeculae, and isolated hearts was measured with methods described elsewhere (12). Generally, the experiments were run at 37°C in Krebs-Henseleit solutions containing 1.3 to 2 mM Ca^{2+} and 5.9 mM K^+, pH 7.4. When required, the tissues were stimulated through needle electrodes with Grass S8 stimulators, voltage 20% above threshold. The dihydropyridine stock solutions (10^{-3} M) were prepared in ethyl alcohol, propylene glycol or DMSO. Control experiments were carried out with the appropriate solvent.

Radioligand Binding Experiments. Canine cardiac sarcolemma was isolated by the method of Van Alstyne et al. (13). Protein was determined by the Lowry procedure (14) using bovine serum albumin as standard.

Cardiac sarcolemma (25 µg protein) was incubated in a total volume of 1 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 150 mM NaCl and 1 mM $CaCl_2$ with varying concentrations of [^{3}H]NTD. The specific activity of [^{3}H]NTD used for studying high affinity binding sites ranged from 70-79.5 Ci/mmol. For measuring low affinity binding, the specific activity of [^{3}H]NTD was reduced to 8 Ci/mmol by diluting it with nonlabeled NTD. After 50 minutes of incubation, the bound [^{3}H]NTD was separated by a filtration method and the radioactivity was measured with a Beckman LS8100 liquid scintillation counter as described previously (9,10). The filter paper binding was measured by conducting experiments in the absence of sarcolemmal protein. All experiments were carried out in the presence of sodium lighting. The specific [^{3}H]NTD binding was linear with protein concentrations ranging from 5 to 100 µg/ml both at low and high [^{3}H]NTD concentrations.

Data Analysis. ED_{50s}, i.e., the drug concentration that produced a 50% increase in contractility, and I_{50s}, i.e., the drug concentration that produced a 50% decrease in contractility, were determined graphically in each experiment. Because of the varying negative inotropic effects of Bay k 8644, the I_{50} was determined from the contractile force decrease from maximal inotropy to control. Values are means ± SEM.

The radioligand binding data were analyzed using the nonlinear least squares Ligand program (15,16) converted to Applesoft by M.H. Teicher, MED-50 ligand program (Biomedical Computing Technology Information Center).

RESULTS AND DISCUSSION

Pharmacological Experiments.

Bay k 8644 produced striking concentration-dependent positive inotropy followed by negative inotropic effects in atrial and ventricular strips from rat, guinea pig, and dog, in isolated, perfused rat hearts, and in isolated dog Purkinje strands (Table I, Figure 1). It is of considerable interest that a "traditional" calcium antagonist, nimodipine, produced a consistent small but significant positive inotropy followed by the usual negative inotropic effect (Figure 1). Thus, it is probable that the calcium channel inhibitors are partial calcium antagonists, a phenomenon that was reported with respect to nifedipine, verapamil and diltiazem some years ago in isolated and intact cardiac preparations (17-19).

If the negative inotropic actions of Bay k 8644 were due simply to excessive accumulation of cellular calcium, i.e., "calcium overload," addition of calcium to tissues treated with high concentrations of the drug would produce further negative inotropic effects and irreversible damage. The opposite was observed, namely, calcium at least partially overcame the negative inotropy (Figure 2). An analysis of the effects of calcium in the presence and absence of Bay k 8644 revealed a shift of the calcium dose-response curve to

Table 1: Positive and Negative Inotropic Effects of Bay k 8644

	N	Positive Inotropic Effect Control to max ED_{50} (nM)	Negative Inotropic Effect Max to control I_{50} (μM)
Rat			
Langendorff	7	35 ± 15	6.8 ± 2.1
Atria	5	74 ± 45	12.4 ± 3.1
Ventricles	13	91.6 ± 21.7	9.9 ± 1.34
Guinea pig			
Atria	6	104 ± 28	4 ± 1.7
Ventricles	6	120 ± 34	6.6 ± 1.6
Dog			
Atria	8	30.5 ± 9.7	3.71 ± 1.48
Ventricles	8	29.5 ± 8.0	4.65 ± 2.31
Purkinje strands	2	67.5	7.5
Pig			
Coronary artery rings (11)	16	11.4 ± 1.0	5.7 ± 1.0

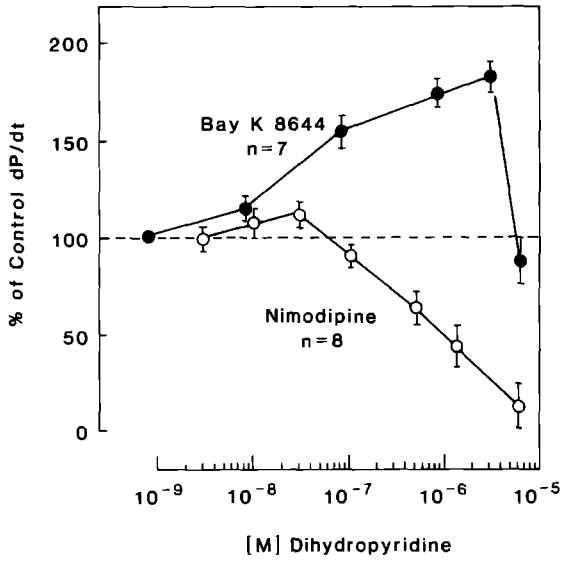

Figure 1. Concentration-dependent effects of Bay k 8644 (closed circles) and nimodipine (open circles) on contractility (dP/dt) of isolated, retrogradely perfused rat heart preparations. The drugs were added in a cumulative manner. Each point represents the mean response ± S.E.M. of 7 or 8 experiments.

the left (Figure 3, left panel). Calcium was also able to partially restore the contractility decreased by Bay k 8644 in a concentration-dependent manner (Figure 3, right panel). These data strongly suggest that Bay k 8644 acts as a calcium agonist at low concentrations and as a calcium antagonist at high concentrations. However, these pharmacological experiments offer only presumptive evidence regarding the receptors with which these drugs interact.

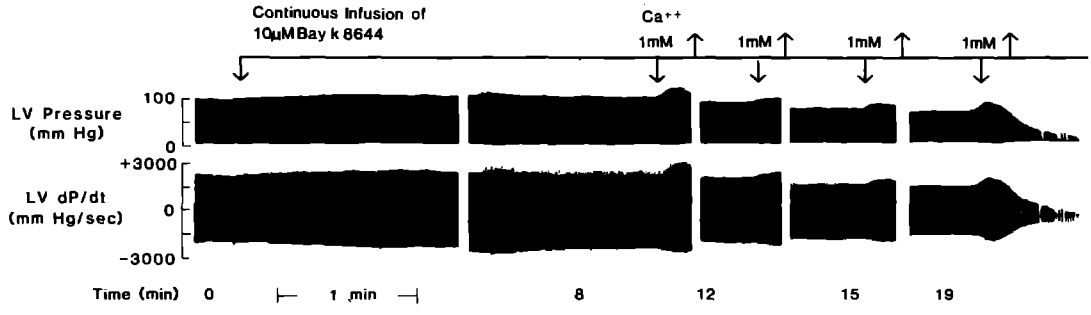

Figure 2. Reversal by Ca^{2+} of the Bay k 8644-induced negative inotropic effect in an isolated, retrogradely perfused rat heart preparation. A continuous infusion of 10 μM Bay k 8644 produced a transient increase in contractility followed by a time-dependent decrease in contractility. Short term (20 sec) infusions of 1 mM $CaCl_2$ (Ca^{2+}) were applied at the indicated times.

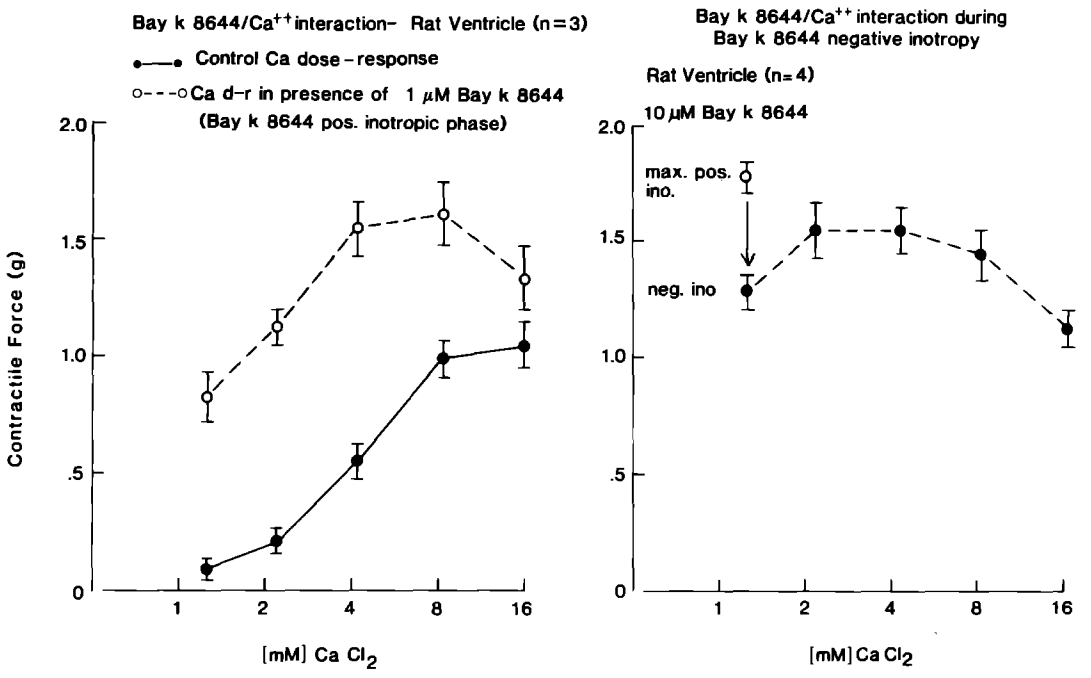

Figure 3. Effect of Ca^{2+} on the Bay k 8644-induced positive and negative inotropic effects in isolated rat ventricular strips. **Left panel:** Calcium dose-response curves in the absence (closed circles) and in the presence of 1 μM Bay k 8644 (open circles). **Right panel:** Rat ventricular strips were treated with 10 μM Bay k 8644 in the presence of 1.3 mM $CaCl_2$. Bay k 8644 induced first an increase in contractile force (open circle) followed by a decrease (arrow). During the negative inotropic phase, calcium dose-response curves were constructed (closed circles).

For this reason, we carried out detailed studies to analyze binding sites for [^{3}H]NTD in isolated, partially purified canine sarcolemmal vesicles.

Radioligand Binding Experiments.

Using an inhibition binding technique, we observed that the inhibition curves were dependent on the [^{3}H]NTD concentration (Figure 4). At a concentration of 0.3 nM, a monophasic inhibition curve was obtained. When we analyzed the data by a nonlinear least squares Ligand program, we found that a one site model fitted better than a two binding site model. Under these conditions, i.e., at low [^{3}H]NTD concentrations, only one set of high affinity (R_H) NTD binding sites was evident, with a K_D of 0.3 nM and a B_{max} of 1.8 pmol/mg protein. When the concentration of [^{3}H]NTD was increased to 7 nM or 25 nM, biphasic inhibition curves were obtained, and computer analysis

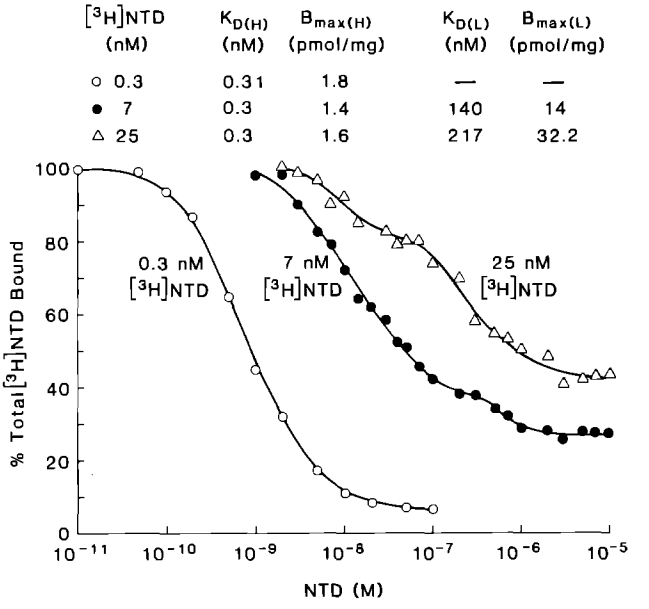

Figure 4. Inhibition binding studies for the identification of high and low affinity nitrendipine (NTD) binding sites. Canine cardiac sarcolemma (25 µg protein) was incubated with 0.3, 7 or 25 nM [^{3}H]NTD in the absence or presence of varying concentrations of nonlabeled NTD. The percentage inhibition of total [^{3}H]NTD binding by nonlabeled NTD is demonstrated.

revealed a good fit to a two site model. The low affinity site (R_L) had a K_D of 140-220 nM and B_{max} of 14-32 pmol/mg protein. The R_H is the same as that generally assumed to be the high affinity DHP binding site in membranes (2,3,22). The K_D for the low affinity NTD binding site was, however, somewhat higher than the values reported previously (22,23).

On the basis of the observation that different dihydropyridines label different numbers of high affinity binding sites in skeletal muscle membranes, Ferry et al. (20) suggested that this may be the reason for the different efficacies of dihydropyridine calcium antagonists. These authors also suggested that there might be a continuum of dihydropyridines from full agonists to full antagonists. However, they did not consider low affinity binding sites as pharmacologically relevant receptor binding sites. Very recent data suggest that high affinity dihydropyridine binding correlates with the positive inotropic effect of Bay k 8644 (7-10) but the high affinity binding does not correlate with the negative inotropic effects of dihydropyri-

dines (2,21,22). The low affinity dihydropyridine binding sites which were demonstrated both for NTD (9,22,23) and for Bay k 8644 (8,9) correlate with the negative inotropic effect.

Taken together, our binding and pharmacological data, i.e., K_D, ED_{50}, and I_{50} values, suggest a role for both the R_H and R_L sites with possible regulation by calcium agonists and antagonists. It is attractive to suggest that the R_H site may be part of, or closely related to, the calcium channel that controls calcium movement in such a way that a drug with high intrinsic activity, such as Bay k 8644, produces opening of calcium channels and significant positive inotropy, while NTD, nimodipine and other derivatives of nifedipine with low intrinsic activity at the R_H site, produce only a small positive inotropic effect. The converse would obtain at the R_L site. Using a pharmacological approach, i.e., isolated perfused guinea pig hearts and atria, treated with dihydropyridines, Thomas and co-workers have presented a two-site model very similar to that proposed here (24,25).

Certainly, we have little information at this time to be certain that these putative "sites" are really distinct entities. They could represent, for example, different conformations, subunits of a single protein, and/or different "states" or configurations of the calcium channel.

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