0026-895X/91/050750-06$03.00/0

MOLECULAR PHARMACOLOGY, 40:750–755

Ethaverine, a Derivative of Papaverine, Inhibits Cardiac L-Type Calcium Channels

YU WANG and ROBERT L. ROSENBERG

Departments of Pharmacology (Y.W., R.L.R.) and Physiology (R.L.R.), University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599

Received May 22, 1991; Accepted August 1, 1991

SUMMARY

Ethaverine is a derivative of papaverine used in the treatment of peripheral vascular disease and is thought to cause vasodilation by reducing intracellular Ca^{2+} concentrations in vascular smooth muscle cells. We tested its effects on single, dihydropyridine-sensitive, L-type calcium channels from porcine cardiac muscle, incorporated into planar lipid bilayers. L-type calcium channels were activated by step depolarizations from a holding potential of −60 mV to a test potential of 0 mV, and unitary currents carried by 100 mM $BaCl_2$ were recorded. Channel activity was enhanced by the presence of the dihydropyridine agonist (+)-202-791 (0.5 μM) and the activated α subunit of the stimulatory GTP-binding protein, G_s. We found that 0.3–30 μM ethaverine on either side of the channel caused a reduction in the channel open probability (EC_{50} ~ 1 μM), with the higher concentrations inhibiting channel activity almost completely. In addition, the ethaverine caused a small reduction in the unitary current amplitude of single open channels (~20%). To test whether the effect of ethaverine on open probability was due to a displacement of the dihydropyridine agonist, we studied the effect of ethaverine on the binding of [^{3}H]nitrendipine to cardiac sarcolemma and found that ethaverine inhibited [^{3}H]nitrendipine binding with a K_i of ~8.5 μM. Ethaverine also inhibited the binding of [^{3}H]diltiazem and [^{3}H]verapamil, with K_i values of 1–2 μM. Because ethaverine is structurally related to verapamil, it is likely that ethaverine acts by binding to the verapamil binding sites on the L-type calcium channels to inhibit channel activation and dihydropyridine binding.

Ethaverine is the tetraethoxy derivative of papaverine and is used as a peripheral vasodilator and antispasmodic agent (1). This action appears to result from an inhibition of phosphodiesterase activity and a decrease in the intracellular Ca^{2+} concentration in smooth muscle cells (2). Our experiments were directed towards determining whether part of the action of therapeutic concentrations of ethaverine (~1 μM) (3) was due to its ability to inhibit the L-type calcium channels that are responsible for the influx of Ca^{2+} into smooth muscle that triggers the increase in tension (4).

We studied single L-type calcium channels from porcine cardiac sarcolemma in planar lipid bilayers (5, 6), in order to separate the effects of ethaverine on cAMP levels from its effects on calcium channels, to distinguish between effects on channel gating and ion permeation, and to determine whether there was any preferential sidedness of the action of ethaverine. In addition, we characterized the effects of ethaverine on the binding of the calcium channel blockers nitrendipine, diltiazem, and verapamil, in order to determine whether ethaverine interacted with known pharmacological sites on L-type calcium channels. Because the functional properties of cardiac and smooth muscle calcium channels are very similar (7), the effect of ethaverine on cardiac calcium channels is likely to be a good indicator of its effect on those channels in smooth muscle cells.

Materials and Methods

Chemicals. Synthetic 1-palmitoyl-2-oleoyl phosphatidylethanolamine and 1-palmitoyl-2-oleoyl phosphatidylserine were obtained from Avanti Polar Lipids (Pelham, AL). Decane was obtained from Aldrich. (+)-202-791 was a kind gift of Sandoz. Nitrendipine was a generous gift of Miles. [^{3}H]Nitrendipine, [^{3}H]diltiazem, and [^{3}H]verapamil were obtained from New England Nuclear. Ethaverine and cAMP-dependent protein kinase were obtained from Sigma Chemical Co. ATPγS and GTPγS were from Boehringer-Mannheim.

Sarcolemmal membranes. Surface membrane from porcine left ventricle were prepared as described (6, 8) and stored at −80° for up to 6 months.

G_s α subunit. Bacteria containing a plasmid with the gene for the α subunit of the stimulatory GTP-binding protein ($G_{s\alpha}$) were kindly provided by Drs. M. Linder and A. Gilman (Department of Pharmacology, The University of Texas Southwestern Medical Center, Dallas, TX). Expression and purification of the $G_{s\alpha}$ were as described by Graziano *et al.* (9), except that only two steps of purification were used, employing DEAE-Sephacel and hydroxyapatite chromatography. The resulting preparation was approximately 5–10% active $G_{s\alpha}$, as deter-

This work was supported by Grant NS-26660 from the National Institute of Neurological Diseases and Stroke and a Grant-in-Aid from the American Heart Association. This work was done during the tenure of an Established Investigatorship of the American Heart Association (R.L.R.).

ABBREVIATIONS: ATPγS, adenosine-5′-*O*-(3-thiotriphosphate); GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); HEDTA, *N*-hydroxyethylethylenediaminetriacetic acid; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid).

mined by [^{35}S]GTPγs binding assays. The $G_{s\alpha}$ was activated in the presence of 25 mM $MgCl_2$ and 10 μM GTPγS, at 30° for 60 min (10), before use.

Treatment of the sarcolemma with cAMP-dependent protein kinase. To maximize cAMP-dependent protein phosphorylation of the sarcolemmal proteins, membranes were incubated with 10 mM $MgCl_2$, 0.2 mM ATPγS, 250 units/ml catalytic subunit of cAMP-dependent protein kinase, and 1 mM cAMP, for 30 min at room temperature (22–24).

Planar lipid bilayers and calcium channel incorporation. Artificial membranes (100–150 μm) were formed from decane solutions of 15 mg/ml 1-palmitoyl-2-oleoyl-phosphatidylethanolamine and 5 mg/ml 1-palmitoyl-2-oleoyl-phosphatidylserine. Aqueous solutions contained 50 mM NaCl and 10 mM Na-HEPES, pH 7.0. After formation of the bilayer, 1 M $BaCl_2$ was added to the *cis* compartment to a final concentration of 100 mM. The dihydropyridine agonist (+)-202-791 (5 mM in ethanol) was added to both chambers to a final concentration of 0.5 μM, in order to maximally increase the open time and probability of the L-type calcium channels (11). (+)-202-791 is required in these experiments because the brief openings of unmodified channels would be unresolved from the intrinsic noise arising from the relatively large area of planar lipid bilayers. Activated $G_{s\alpha}$ was added to the *trans* chamber to a final concentration of ~6 nM, to slow L-type channel run-down (12, 13). Cardiac sarcolemma (~40 μg of protein/ml) was added to the *cis* chamber. Channel activity appeared spontaneously within 4 min (6).

Voltage-clamp of the bilayers and data acquisition were achieved as described (6), using a patch-clamp amplifier modified for greater capacity compensation (V. Pantani, Yale University Department of Cellular and Molecular Physiology) and a 80386-based computer running AxoBasic (Axon Instruments). Current records were filtered at 200 Hz and sampled at 1000 Hz. Voltages were defined as *trans* minus *cis*, so that the *trans* chamber represented the cellular interior (14) and currents from *cis* to *trans* are shown as downward transitions. All recordings shown were corrected for leak resistance and capacitative transients by digital subtraction of averaged records with no channel activity (5, 6).

Analysis of channel open probability was done by a computer program that calculated the total open time during each leak-subtracted depolarization from the (current·time) integral, normalized to that value expected for a single channel open for 100% of the depolarization. To correct for changes in unitary current amplitudes after drug addition, different values for the (current·time) normalization were used for each part of the experiment. Unitary current amplitudes were determined by eye, using computer-drawn lines to fit the closed and open channel levels. Fits of curves to theoretical equations were done using a quasi-Newtonian algorithm to minimize least squares (Systat).

[^{3}H]Nitrendipine binding assays. Cardiac sarcolemma were diluted, in an assay buffer containing 200 mM NaCl, 10 mM Na-HEPES (pH 7.4), 1 mM HEDTA, and 0.3 mM $CaCl_2$ (free Ca^{2+}, ~1 μM), to a final concentration of 0.09 mg of membrane protein/ml. Ethaverine was added to final concentrations of 0–100 μM, and [^{3}H]nitrendipine was added to a final concentration of 0.2 nM, unless otherwise indicated. Nonspecific [^{3}H]nitrendipine binding was determined in the presence of 500 nM unlabeled nitrendipine with the appropriate concentration of ethaverine. Samples (1 ml) were incubated in the dark for 90 min at room temperature (15). The membranes were collected on glass fiber filters (Whatman GF/B) and rinsed with four additions of 3 ml of ice-cold assay buffer. The amount of bound [^{3}H]nitrendipine was determined by liquid scintillation counting of the filters.

[^{3}H]Diltiazem and [^{3}H]verapamil binding assays. Cardiac sarcolemma were diluted to the same final concentration as for the [^{3}H]nitrendipine assay, in a buffer consisting of 50 mM Tris·HCl, 10 μM $CaCl_2$, and 10 μM $MgCl_2$, pH 7.4. To reduce nonspecific binding, 0.1 mg/ml bovine serum albumin was included in assay and rinse buffers, and filters were soaked in 0.3% polyethyleneimine (16, 17). The concentrations of [^{3}H]diltiazem and [^{3}H]verapamil were 50 and 25 nM, respectively, values selected to be ~60% of the K_d values for each ligand (16, 17). Nonspecific binding was determined in the presence of 10 μM unlabeled diltiazem or 25 μM unlabeled verapamil. Samples were incubated and filtered as described for the [^{3}H]nitrendipine binding assay, except that the filters were rinsed twice with 4 ml of the Tris·HCl buffer.

Results

L-Type calcium channels remain active in cell-free planar lipid bilayers for extended periods. L-Type calcium channels in excised membrane patches (e.g., Ref. 18) and in planar lipid bilayers (5, 6) are known to "run down," such that the channel activity diminishes dramatically over a period from seconds to minutes. This effect complicates any analysis of the effect of drugs that reduce channel open probability. The addition of activated $G_{s\alpha}$ has been shown to slow channel rundown (12, 13), so it was included in the *trans* (intracellular) compartment in all experiments, in order to prolong channel lifespan in the bilayers. In addition, in many experiments the

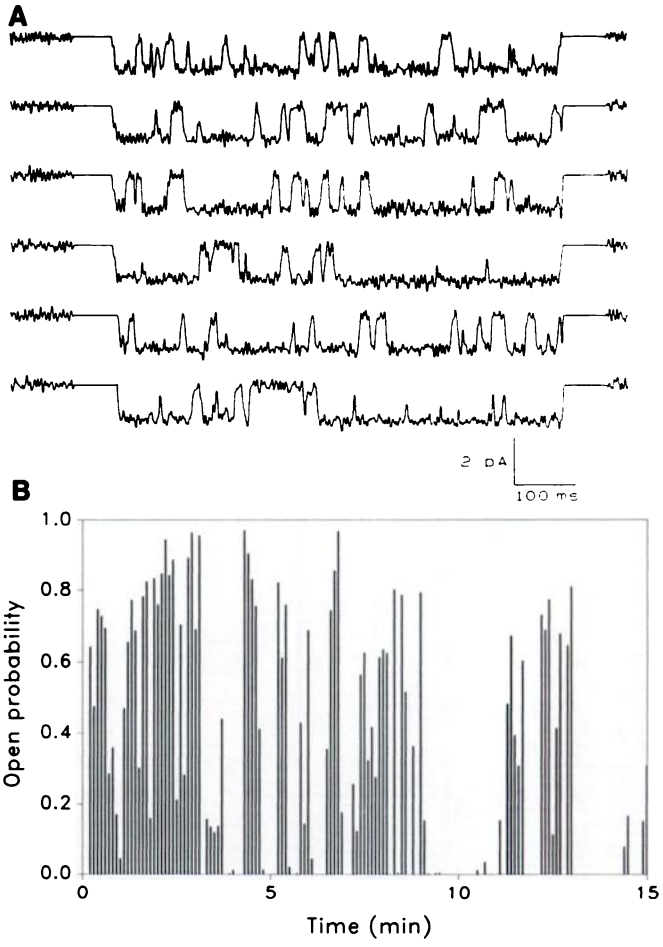

Fig. 1. An example of the activity of L-type calcium channels in planar lipid bilayers in the presence of internal $G_{s\alpha}$. Sarcolemma were treated with cAMP-dependent protein kinase and ATPγS, as described in Materials and Methods. A, Sequential recordings after leak and capacity compensation (5, 6). Channel openings are downward transitions. The electrically silent periods at the beginning and the end of each depolarization result from amplifier and A/D converter saturation following the voltage transitions. B, Plot of channel open probability (*vertical bars*) versus time, on a sweep-by-sweep basis, under control conditions. In this case, the activity of the channel was robust and sustained for ~15 min.

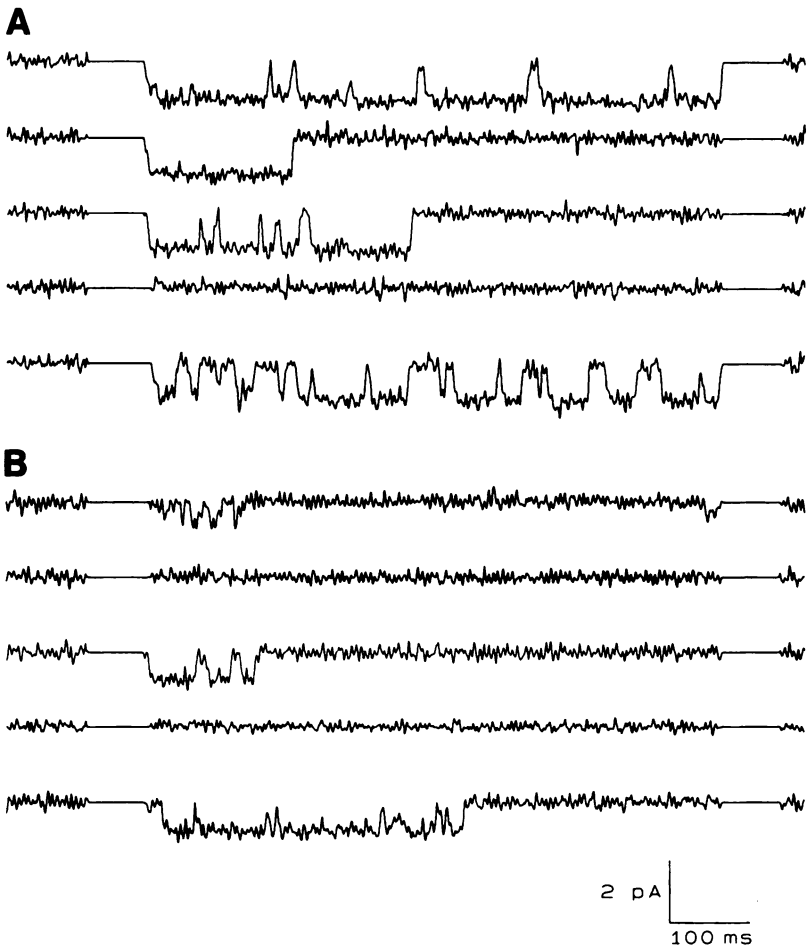

Fig. 2. Sequential single-channel recordings of an L-type calcium channel before (A) and after (B) exposure to 1 μM ethaverine on both sides of the bilayer. The effect was a decrease in open probability and a small decrease in the unitary current amplitude.

sarcolemma membranes were treated with the catalytic subunit of the cAMP-dependent protein kinase and ATPγS, in an attempt to bias the channel in favor of high initial activity. Initial open probability during the first 2 min of channel recording was 0.08 ± 0.07 (18 experiments, mean ± standard deviation) under control conditions and was 0.20 ± 0.14 (13 experiments) when sarcolemma were treated with the kinase and ATPγS.[1] In treated and untreated sarcolemma, there were large channel-to-channel variations in initial open probability, with some channels displaying robust activity (e.g., Fig. 1) and other channels displaying much lower, yet sustained, levels of activity.

An example of L-type channel activity recorded under our conditions is shown in Fig. 1A. Fig. 1B shows a plot of open probability during the length of the experiment, where each vertical bar represents the open probability for an individual depolarization. In the experiment shown, the channel survived for ~15 min before the activity diminished to a low level. In eight such experiments with $G_{s\alpha}$ present in the *trans* chamber, the mean lifespan of the channel activity after incorporation was 16.3 ± 3.7 min, compared with a mean lifespan of <1 min in the absence of $G_{s\alpha}$ (data not shown). Treatment with the cAMP-dependent protein kinase and ATPγS increased initial channel activity during the first 2 min and itself could increase the lifespan of the channels after incorporation.[2] These experiments indicated that the channel activity was sufficiently high and that the lifespan of the channel in the bilayer was sufficiently long for study of the effects of a channel blocker.

Ethaverine reduces channel open probability and unitary current amplitudes. Fig. 2 shows a selection of recordings under control conditions (Fig. 2A) and after the addition of 1 μM ethaverine to both sides of the bilayer (Fig. 2B). Two effects were seen, i.e., a reduction in open probability, manifested as a reduced number of channel openings during each depolarization and an increase in the number of depolarizations with no channel openings, and a small but consistent decrease in the unitary current amplitude. There was no significant change in channel open-time distributions, within our limited bandwidth resolution (200 Hz).

The effect on open probability is described in more detail in the plots of channel activity (Fig. 3). In Fig. 3A, ethaverine caused a partial reduction in open probability, from 0.22 in the control condition to 0.05 after the addition of 1 μM ethaverine to both sides of the channel. A subsequent addition of ethaverine to a final concentration of 3 μM caused a further reduction in open probability to 0.02. Fig. 3B shows an open probability plot from a different experiment, where 30 μM ethaverine was added after 3 min of robust channel activity. There was a clear immediate reduction in channel open probability from 0.45 to 0.02. Thus, although modest concentrations of ethaverine caused a partial inhibition of channel open probability that appeared similar to the spontaneous run-down of channel activity, the very large and immediate effects of 30 μM ethaverine

[1] Y. Wang and R. L. Rosenberg, manuscript in preparation.
[2] C. Townsend, Y. Wang, and R. L. Rosenberg, manuscript in preparation.

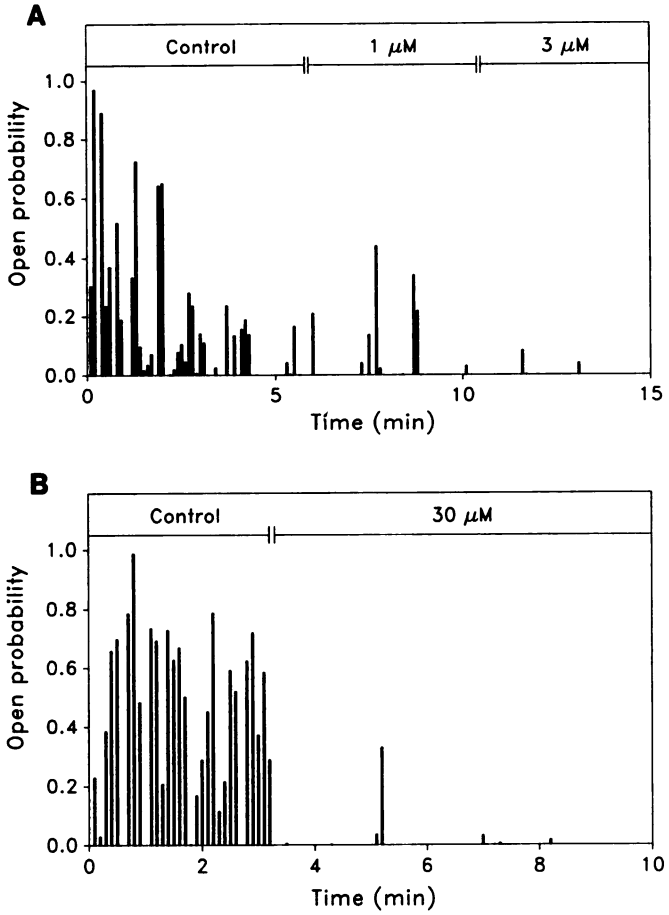

Fig. 3. Plots of channel open probability during exposure to the concentrations of ethaverine indicated at the *top* of each panel. A, Control open probability averaged 0.22. After addition of 1 μM ethaverine to both internal and external solutions, average open probability decreased to 0.05, with a large increase in the number of depolarizations that evoked no channel activity. Subsequent addition of ethaverine to a final concentration of 3 μM apparently caused a further decrease in open probability to 0.02. B, Effect of 30 μM ethaverine in a different experiment. Control open probability averaged 0.45 in this case, and the addition of 30 μM ethaverine to both solutions caused a sudden decrease in open probability to 0.02.

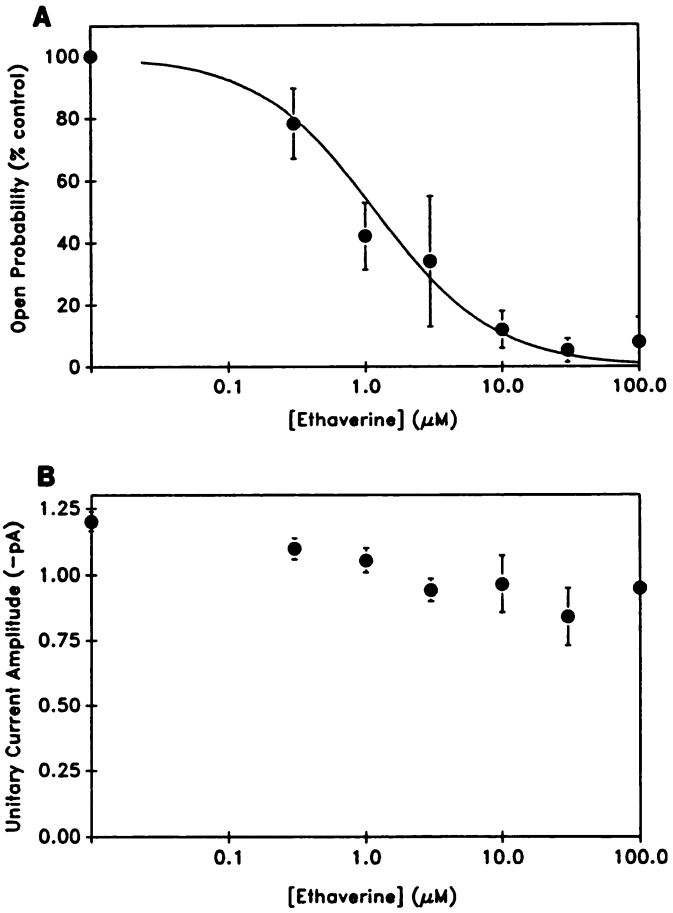

Fig. 4. Dose-response curves of the effect of ethaverine in both internal and external solutions. A, Effect on channel open probability. In each experiment, the average control open probability was determined from at least 25 depolarizations obtained before the addition of the drug, and the average open probability after drug addition was obtained from at least 30 depolarizations. Each data point represents the mean and standard error from three to five such experiments. Experiments with control open probabilities of <0.05 were not included in the analysis. *Smooth curve*, best fit to the equation $P_o = 100\%/(1 + [\text{ethaverine}]/EC_{50})$, which yielded an EC_{50} of 1.0 ± 0.18 μM. B, Effect on unitary current amplitude at the test potential of 0 mV. Except at 100 μM ethaverine, each data point represents the mean and standard error of the current amplitudes from at least two well resolved openings in three to five different experiments. The point at 100 μM ethaverine was from a single determination. The unitary current amplitudes measured in the presence of 3, 10, and 30 μM ethaverine were significantly different from control ($p < 0.02$, Student's *t* test). The limited data at 100 μM ethaverine made evaluation of the statistics there unreliable.

made it clear that ethaverine caused a decrease in channel activity independent of the run-down process. Repeated attempts to reverse the inhibition by ethaverine by perfusing the chambers with drug-free solutions were unsuccessful, because the bilayers became unstable and broke.

The effect of ethaverine in a large number of experiments is shown as dose-response curves (Fig. 4). In Fig. 4A, open probability, expressed as a percentage of the initial open probability obtained under control conditions in each experiment, is plotted versus the concentration of ethaverine. The data are described reasonably well by the curve assuming one-to-one binding of drug to channel, with an EC_{50} of 1 μM.

The small decrease in unitary current amplitude in response to ethaverine is shown in Fig. 4B. The amplitudes at the doses of 3, 10, and 30 μM ethaverine were significantly different from control values. Although the effect was small, there was a general trend towards smaller current amplitudes at increasing concentrations of ethaverine, from 0.3 to 30 μM.

In the experiments shown, ethaverine was added to both sides of the channel. In order to test whether there was a sidedness of the effect of ethaverine, the drug was added to either the intracellular or extracellular side. In seven experiments with extracellular ethaverine and nine experiments with intracellular ethaverine (0.3, 10, and 30 μM), there were no significant differences in the effects, compared with the 13 experiments with these concentrations of ethaverine added to both sides. Thus, ethaverine appears to have sufficient lipid solubility to gain access to the site, regardless of its location.

Ethaverine displaces [^{3}H]nitrendipine from cardiac sarcolemma. Because the dihydropyridine agonist (+)-202-791 was used in all bilayer experiments to increase L-type channel open time and probability, we suspected that ethaverine was acting to reduce channel open probability by displacing the dihydropyridine agonist. Thus, we tested for the ability of

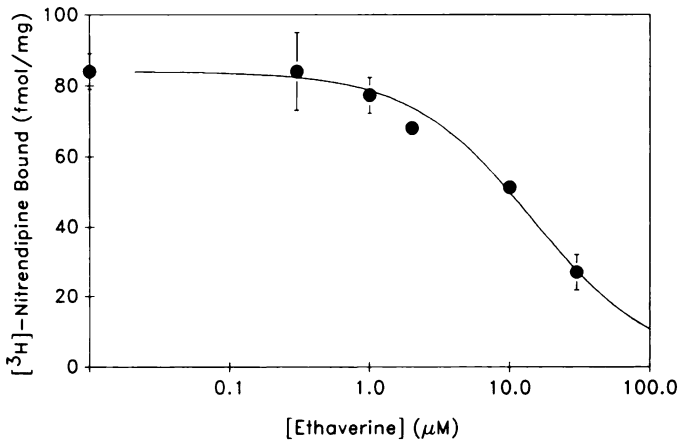

Fig. 5. Dose-response curve of ethaverine inhibition of specific [^{3}H] nitrendipine binding to cardiac sarcolemma. Each data point represents the mean and standard error from four individual determinations. The concentration of [^{3}H]nitrendipine was 0.2 nM. *Smooth curve,* best fit to the equation bound = $B_0/(1 + [\text{ethaverine}]/EC_{50})$, yielding a B_0 of 85 fmol/mg of membrane protein (the amount of the 0.2 nM [^{3}H]nitrendipine bound in the absence of ethaverine) and an EC_{50} of 14.6 μM.

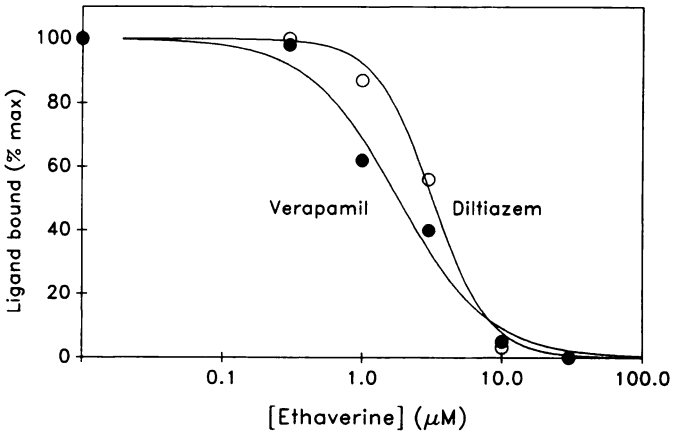

Fig. 6. Dose-response curve of ethaverine inhibition of the specific binding of 50 nM [^{3}H]diltiazem (○) and 25 nM [^{3}H]verapamil (●) to cardiac sarcolemma. Each data point is the average of two individual determinations of ligand binding. *Smooth curves,* best fits to the equation bound = $100\%/\{1 + ([\text{ethaverine}]/EC_{50})^n\}$. For [^{3}H]diltiazem, the EC_{50} was 3.2 mM and n was 2.1; for [^{3}H]verapamil, the EC_{50} was 1.8 mM and n was 1.3.

ethaverine to displace [^{3}H]nitrendipine from cardiac sarcolemma. Fig. 5 shows that ethaverine can compete with [^{3}H] nitrendipine for its binding site. The data were fit reasonably well with the curve that describes one-to-one displacement of the [^{3}H]nitrendipine, with an EC_{50} of 14 μM when 0.2 nM [^{3}H] nitrendipine was used. The EC_{50} was shifted to 45 μM when the sarcolemma were incubated with 2.0 nM [^{3}H]nitrendipine (data not shown). These values suggest that the K_i for ethaverine inhibition of [^{3}H]nitrendipine binding ($K_{i_{Eth}}$) is ~8.5 μM, using the Cheng-Prusoff equation ($K_{i_{Eth}} = EC_{50_{Eth}}/(1 + [\text{nitrendipine}]/K_{d_{Nit}})$; ref. 19) and a value of 0.3 nM for the K_d for [^{3}H] nitrendipine binding ($K_{d_{Nit}}$) (15, 17). The K_i of 8.5 μM is somewhat higher than the concentration of ethaverine required to cause a 50% reduction in open probability (1 μM) (see Discussion).

Ethaverine displaces [^{3}H]diltiazem and [^{3}H]verapamil from cardiac sarcolemma. Because ethaverine, like verapamil, is derived from papaverine and because of the known interactions between the dihydropyridine and arylalkylamine calcium channel antagonists (20), we attempted to determine whether the action of ethaverine was due to an interaction at the verapamil binding site. We reasoned that ethaverine binding to the verapamil binding site could inhibit dihydropyridine binding by the allosteric mechanism described (20).

Fig. 6 shows that ethaverine inhibited the binding of both [^{3}H]diltiazem and [^{3}H]verapamil, with EC_{50} values of 3.2 and 1.8 μM, respectively. This translates roughly into K_i values of 2 μM for [^{3}H]diltiazem and 1 μM for [^{3}H]verapamil, assuming that the K_d values were 81 nM and 42 nM for [^{3}H]diltiazem and [^{3}H]verapamil, respectively (16, 17). The pseudo-Hill coefficient for the displacement of [^{3}H]diltiazem was close to 2, indicating the possibility for multiple or cooperative interactions between ethaverine and diltiazem binding sites.

Discussion

These results indicate that ethaverine at low concentrations (~1 μM) inhibits L-type calcium channel activity. The effective concentration range is very close to the mean plasma concentration of 1.2 μM (0.5 μg/ml) reached during a typical dosage regimen (3). The inhibition probably results from ethaverine binding to the arylalkylamine (e.g., verapamil) site (16), because of the structural similarities between the compounds (2), but binding of ethaverine to the dihydropyridine binding site(s) (15) and to the diltiazem binding site (17) has not been excluded. Ethaverine causes a decrease in channel open probability in the presence of dihydropyridine agonist by reducing the occupancy of the agonist drug and is likely also to inhibit channel activity in the absence of dihydropyridines by acting as an arylalkylamine inhibitor (20). In keeping with this, a single experiment (not shown) showed that micromolar concentrations of ethaverine caused a marked reduction in whole-cell cardiac calcium channel currents in the absence of dihydropyridines.

The effects of ethaverine in our experimental system are unlikely to result from an inhibition of phosphodiesterase activity (2), because, even if a membrane-bound form of the enzyme were present in the bilayers, there was no cAMP in the intracellular compartment. However, in therapeutic circumstances, the effect of ethaverine on phosphodiesterases may contribute significantly to the mechanism for vasodilation, especially when one considers that arylalkylamines are less potent in blocking calcium channels in smooth muscle, compared with those in cardiac cells (21).

The difference in the dose responsible for an inhibition of the open probability in the presence of (+)-202-791 (1 μM), compared with that responsible for the effect on [^{3}H]nitrendipine binding (8.5 μM), is probably due to the higher affinity of [^{3}H]nitrendipine than dihydropyridine agonists (15). In addition, a direct inhibitory effect of ethaverine interacting with the arylalkylamine binding site would be reflected in an increased extent of channel block at lower doses.

The small, dose-dependent effect of ethaverine on the unitary current amplitudes was probably independent of its effect on channel gating. The rare channel openings in the presence of ethaverine at concentrations as high as 30 μM (Fig. 3) were of approximately normal duration but had a smaller amplitude. We saw no evidence of the increased noise of the open-channel current that would be expected if ethaverine was undergoing rapid binding-unbinding transitions. Instead, it is likely that

the binding of ethaverine causes a reduction in the current amplitude by alteration of the permeation or selectivity mechanisms of the channel or by induction of very rapid opening-closing transitions that are unresolved at 200-Hz filtering. This additional action is possible for the "classical" arylalkylamines also, because their effects have not been characterized on single-channel currents.

Ethaverine has received less experimental attention than its parent compound, papaverine. For comparison, papaverine is a well known inhibitor of phosphodiesterases (22) that can cause positive inotropy and chronotropy at low concentrations (23, 24), probably due to the increase in intracellular cAMP. However, high concentrations (>50–100 μM) can significantly inhibit the pacemaker rate in heart or the duration of cardiac action potentials, perhaps due to an inhibition of calcium channel activity (23, 24). In comparison, ethaverine acts as an inhibitor of cardiac calcium channels at concentrations similar to those that inhibit phosphodiesterase activity (2).

Acknowledgments

The authors thank Dr. Mark Sirgo and Ms. Patricia Koplas for many helpful discussions and Ms. Joan East for skilled assistance with the sarcolemmal membrane and G_{as} preparations.

References

1. Trainor, F. S., R. E. Phillips, D. D. Michie, S. R. Zellner, L. Hogan, and J. M. Chubb. Effects of ethaverine hydrochloride on the walking tolerance of patients with intermittent claudication. *Angiology* **37**:343–351 (1986).
2. Greenslade, F. C., C. K. Scott, K. L. Newquist, K. M. Krider, and M. Chasin. Heterogeneity of biochemical actions among vasodilators. *J. Pharm. Sci.* **71**:94–100 (1982).
3. Meyer, M. C., G. Raghow, and A. B. Straughn. Plasma levels of ethaverine after oral administration to humans. *Biopharm. Drug Dispos.* **4**:401–404 (1983).
4. Nelson, M. T., J. B. Patlak, J. F. Worley, and N. B. Standen. Calcium channels, potassium channels and voltage dependence of arterial smooth muscle tone. *Am. J. Physiol.* **259**: C3–C18 (1990).
5. Rosenberg, R. L., P. Hess, J. P. Reeves, H. Smilowitz, and R. W. Tsien. Calcium channels in planar lipid bilayers: insights into mechanisms of ion permeation and gating. *Science* (*Washington D. C.*) **231**:1564–1566 (1986).
6. Rosenberg, R. L., P. Hess, and R. W. Tsien. Cardiac calcium channel in planar lipid bilayers. *J. Gen. Physiol.* **92**:27–54 (1988).
7. Bean, B. P. Classes of calcium channels in vertebrate cells. *Annu. Rev. Physiol.* **51**:367–384 (1989).
8. Kuwayama, H., and T. Kanazawa. Purification of cardiac sarcolemmal vesicles. *J. Biochem.* (*Tokyo*) **91**:1419–1426 (1982).
9. Graziano, M. P., M. Freissmuth, and A. G. Gilman. Expression of G_{as} in *Escherechia coli*. *J. Biol. Chem.* **264**:409–418 (1989).
10. Northup, J. K., M. D. Smigel, and A. G. Gilman. The guanine nucleotide activating site of the regulatory component of adenylate cyclase. *J. Biol. Chem.* **257**:11416–11423 (1982).
11. Hess, P., J. B. Lansman, and R. W. Tsien. Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature* (*Lond.*) **311**:538–544 (1984).
12. Yatani, A., J. Codina, Y. Imoto, J. P. Reeves, L. Birnbaumer, and A. M. Brown. A G protein directly regulates mammalian cardiac calcium channels. *Science* (*Washington D. C.*) **238**:1288–1292 (1987).
13. Imoto, Y., A. Yatani, J. P. Reeves, J. Codina, L. Birnbaumer, and A. M. Brown. α-Subunit of G_s directly activates cardiac calcium channels in lipid bilayers. *Am. J. Physiol.* **255**:H722–H728 (1988).
14. Rosenberg, R. L., and X.-h. Chen. Characterization and localization of two ion-binding sites within the pore of L-type calcium channels. *J. Gen. Physiol.* **97**:1207–1225 (1991).
15. Janis, R. A., P. J. Silver, and D. J. Triggle. Drug action and cellular calcium regulation. *Adv. Drug Res.* **16**:309–591 (1987).
16. Garcia, M. L., M. J. Trumble, J. P. Reuben, and G. J. Kaczorowski. Characterization of verapamil binding sites in cardiac membrane vesicles. *J. Biol. Chem.* **259**:15013–15016 (1984).
17. Garcia, M. L., V. F. King, P. K. S. Siegl, J. P. Reuben, and G. J. Kaczorowski. Binding of Ca^{2+} entry blockers to cardiac sarcolemmal membrane vesicles. *J. Biol. Chem.* **261**:8146–8157 (1986).
18. Nilius, B., P. Hess, J. B. Lansman, and R. W. Tsien. A novel type of cardiac calcium channel in ventricular cells. *Nature* (*Lond.*) **316**:443–446 (1985).
19. Cheng, Y., and W. H. Prusoff. Relationship between the inhibition constant (K_I) and the concentration of an inhibitor that causes a 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).
20. Murphy, K. M. M., R. J. Gould, B. L. Largent, and S. H. Snyder. A unitary mechanism of calcium antagonist drug action. *Proc. Natl. Acad. Sci. USA* **80**:860–864 (1982).
21. Yu, J., and R. Bose. Calcium channels in smooth muscle. *Gastroenterology* **100**:1448–1460 (1991).
22. Carpenedo, F., R. M. Gaion, and G. Fassina. Calcium and papaverine interaction with soluble cardiac phosphodiesterase. *Biochem. Pharmacol.* **24**:2069–2073 (1975).
23. Sanguinetti, M. C., and T. C. West. Effect of papaverine on Ca^{++}-dependent action potentials in guinea-pig myocardium depolarized by potassium. *J. Pharmacol. Exp. Ther.* **219**:715–722 (1981).
24. Sanguinetti, M. C., and T. C. West. Influence of papaverine on spontaneous activity of isolated right atria from small mammals. *J. Pharmacol. Exp. Ther.* **228**:500–509 (1984).

Send reprint requests to: Robert L. Rosenberg, Department of Pharmacology, CB 7365, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.