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Energy-Dependent Accumulation of Calcium Antagonists in Catecholamine Storage Vesicles

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ABSTRACT. The calcium antagonists verapamil, nitrendipine, mibefradil, and amlodipine accumulate in chromaffin granule ghosts with apparent equilibrium partition coefficients [(mol/mg membrane lipid)/(mol/mg solvent water)] of 246 ± 105 (N = 8), 2700 ± 600 (N = 4), 7400 ± 2200 (N = 4), and 8100 ± 1100 (N = 5), respectively. In the presence of 1.2 mM MgATP, the partition coefficients were 854 ± 206 (N = 10), 2300 ± 600 (N = 4), 32,700 ± 8,900 (N = 7), and 20,300 ± 5,000 (N = 11) for verapamil, nitrendipine, mibefradil, and amlodipine, respectively. Except for nitrendipine, the apparent partition coefficients in the presence of MgATP were significantly different from the control ($P < 0.001$). For amlodipine and verapamil, the vacuolar H^+-ATPase inhibitors bafilomycin A_1 (30 nM) and *N*-ethylmaleimide (2 mM) and the protonophore (uncoupler) carbonyl cyanide *m*-chlorophenylhydrazone (CCCP, 10 μM) completely blocked the increase in partition coefficients in response to MgATP. The extra amlodipine, mibefradil, and verapamil that accumulated in response to MgATP were released into the medium by CCCP (10 μM) by 18% (N = 5), 30% (N = 5), and 88% (N = 5) for amlodipine, mibefradil, and verapamil, respectively. Thus, amlodipine, mibefradil, and verapamil, but not nitrendipine, accumulate in catecholamine storage vesicles in response to $\Delta\mu_{H+}$ generated by the endogenous V-type H^+-ATPase, and are partially released by de-energetisation. Hence, these calcium antagonists can reach unexpectedly high concentrations in certain target cells, and give pharmacodynamic properties not shared by nitrendipine. BIOCHEM PHARMACOL **59**;2:123–129, 2000.

KEY WORDS. calcium antagonist; amlodipine; mibefradil; nitrendipine; verapamil; active uptake; vacuolar H^+-ATPase; chromaffin granules.

A number of commonly used calcium antagonists have been found to affect the bioenergetics (i.e. the transmembrane proton electrochemical gradient, $\Delta\mu_{H+}$) of catecholamine storage vesicles at low micromolar concentrations *in vitro* (see [1] for references). For some high-dose calcium antagonists, e.g. prenylamine and cinnarizine, the inhibitory effect on the bioenergetics fully accounts for their pronounced catecholamine-releasing effect [2, 3]. *In vitro* studies have also revealed that the more specific calcium antagonists of the dihydropyridine type, such as nifedipine, also affect the bioenergetics of catecholamine storage vesicles [1] at low micromolar concentrations. Even at such concentrations (10 μM), amlodipine (2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine), with a free amino group [4, 5], did not significantly inhibit the MgATP-dependent generation of a pH gradient across the chromaffin granule ghost vesicles [1]. However, when added to energised ghosts, amlodipine (10 μM), in contrast to felodipine, gave a transient increase in the intravesicular pH [1], which was interpreted to indicate that unprotonated amlodipine (pK_a = 8.6) entered the vesicles for subsequent protonation (internal pH ~5.5). On the basis of this finding, protonated amlodipine is expected to accumulate in energised chromaffin granule ghosts in a tyramine-like manner, i.e. non-carrier-mediated [6]. Furthermore, it has been shown that the phenylalkylamine L-calcium antagonist verapamil accumulates in gastric glands [7], most likely due to the tertiary nitrogen (pK_a = 8.8 [8]) and is expected to accumulate in catecholamine storage vesicles as well. Similarly, as the T-calcium channel blocker mibefradil has a pK_a value of 5.5 [9], this compound is also expected to accumulate by the same mechanism (i.e. "pH trapping").

The aim of the present work was to examine if the proton electrochemical gradient $\Delta\mu_{H+}$ (inside acidic and positive) generated by the V-type H^+-ATPase promotes the selected calcium antagonists to accumulate in chromaffin granule ghosts against a concentration gradient. The non-charged dihydropyridine nitrendipine (1,4-dihydro-2,6-dimethyl-4-(*m*-nitrophenyl)pyridine-3,5-dicarboxylic acid 3-ethyl, 5-methyl ester) was selected as a reference compound since it has no group that can be ionised in the relevant pH range and is thus not expected to distribute itself in response to $\Delta\mu_{H+}$.

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MATERIALS AND METHODS

Chromaffin Granule Ghosts

Chromaffin granule ghosts isolated from bovine adrenal medulla [10] were used as a model for synaptic vesicles [6]. The two types of vesicles have the same V-type H^+-ATPase (i.e. identical bioenergetics), although their amine carrier differs in specificity [11]. The energy-dependent generation of a pH gradient was assayed by dual-wavelength spectrophotometry as described [12].

Accumulation of the Calcium Antagonists in Chromaffin Granule Ghosts

Chromaffin granule ghosts (~0.05 mg protein/mL in the experiments with amlodipine, nitrendipine, and mibefradil, and 0.15 mg protein/mL in the experiments with verapamil) were incubated (glass tubes were used to avoid binding of the drugs to plastic material) in 7 mM HEPES buffer, pH 7.0 supplied with 80 mM KCl at 25° with either [^{14}C]amlodipine (2–200 nM), [^{14}C]mibefradil (1–1000 nM), [^{3}H]nitrendipine (5–20 nM), or [^{14}C]verapamil (1 μM). In some experiments, the V-type H^+-ATPase inhibitors bafilomycin A_1 (30 nM, final concentration), *N*-ethylmaleimide (NEM,* 2 mM, final concentration), the protonophore (uncoupler) CCCP (10 μM, final concentration), or NH_4Cl (1 mM, final concentration) were present during incubation. Proton pumping was initiated by adding MgATP (1.2 mM, final concentration) and allowed to proceed for 10 min to reach a steady-state $\Delta\mu_{H+}$. The assay mixture (1–2 mL) was then centrifuged (Sorvall RC5C centrifuge, SS-34 rotor, 12,000 *g* for 40 min, 4°) to pellet the vesicles. The concentrations of radiolabelled compounds in the total assay mixtures and in the supernatants were determined by liquid scintillation counting (Beckman LS6000LL scintillation counter). Chromaffin granule ghosts incubated as above (10 mL total volume) with 1.2 mM MgATP and [^{14}C]amlodipine (28 nM), [^{14}C]mibefradil (7 nM), or [^{14}C]verapamil (1 μL) were collected by centrifugation (as above) and resuspended in the standard buffer (2 mL) containing MgATP (1.2 mM) by gentle homogenisation. The resuspended vesicles were termed amlodipine-, mibefradil- and verapamil-loaded ghosts, respectively. For each compound, the resuspended drug-loaded ghosts were divided into two aliquots. To one aliquot was added the uncoupler CCCP (10 μM, final concentration) while the other was untreated, and both were incubated at 25° for 10 min, and finally centrifuged as described above. The amounts of [^{14}C]amlodipine, [^{14}C]mibefradil, or [^{14}C]verapamil in the resuspended vesicles and the supernatants were measured as above.

Calculation of the Apparent Equilibrium Partition Coefficient

Lipophilic drugs bind to the phospholipids in biological membranes and can thus reach high concentrations expressed as mol/mg membrane lipid compared to the aqueous phase (mol/mg solvent water). Based on a phospholipid/protein ratio of 2.3 μmol phospholipid/mg protein for chromaffin granule ghosts, and a mean molar mass of the phospholipids of 750 [13], 1 mg of granule ghost protein corresponds to 1.7 mg phospholipids. The ratio of radiolabelled drug that accumulated in the ghosts (i.e. particle-bound or sedimentable drug) expressed as mol drug/mg membrane phospholipid to that in the water phase (mol drug/mg solvent water) corresponds to the definition of partition coefficient given by Mason *et al.* [14, 15]. It should be noted that the term "apparent partition coefficient" used in this work represents drug that is bound to the granule membranes per se as well as drug that is present in the internal free water space of the ghosts. A significant change in the apparent partition coefficient as a result of, for example, MgATP or CCCP (see Results section) indicates a significant change in the amount of particle-bound drug.

Other Analytical Methods

Protein was determined by the method of Bradford [16].

Chemicals

Racemic amlodipine and [^{14}C]amlodipine (specific activity 703 MBq/mmol, purity 86.4%) were gifts from Pfizer Central Research. The substances were supplied as dry material and dissolved in the assay medium. The concentration of [^{14}C]amlodipine was assayed spectrophotometrically (ϵ ($mM^{-1}cm^{-1}$) = 6.2 at 368 nm (in HEPES buffer, pH 7.5; determined by using non-radioactive amlodipine as a standard). Racemic [^{3}H]nitrendipine (specific activity 2904.5 GBq/mmol, dissolved in ethanol, 97% purity) was obtained from NEN Life Science Products. Nitrendipine was from ICN. Racemic nitrendipine or [^{3}H]nitrendipine was dissolved in ethanol/water (50/50, v/v). Mibefradil (dry material) and [^{14}C]mibefradil (dissolved in toluene/methanol (9:1), 1.86 GBq/mmol) were gifts from F. Hoffmann-La Roche Ltd. An appropriate amount of [^{14}C]mibefradil was transferred to a glass vial and the organic solvent allowed to evaporate at room temperature. The [^{14}C]mibefradil was then dissolved in 7.5 mM HEPES buffer, pH 7.0. Mibefradil was dissolved in the assay buffer. [^{14}C]Verapamil (supplied as dry material, and dissolved in the assay buffer) was a gift from Knoll. Bafilomycin A_1 and CCCP were from Sigma. Acridine orange was from Molecular Probes. All other chemicals were of reagent grade.

* *Abbreviations:* CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; and NEM, *N*-ethylmaleimide.

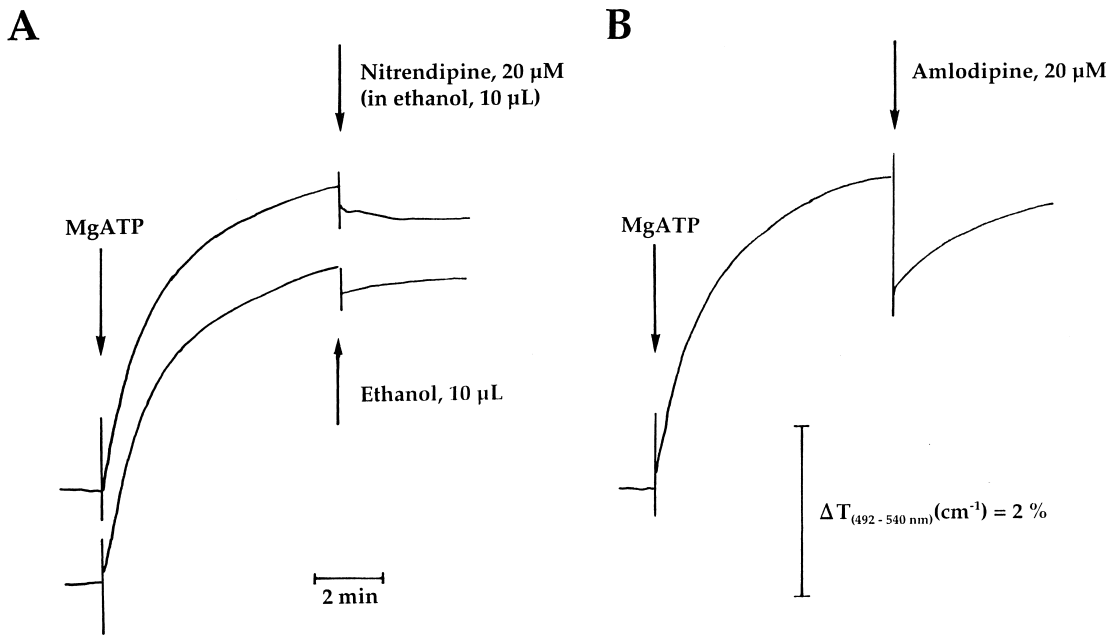

FIG. 1. The effect of the calcium antagonists nitrendipine (20 μM, final concentration) and amlodipine (20 μM, final concentration) on the time–course of the energy-dependent generation of a pH gradient in chromaffin granule ghosts as assayed by dual-wavelength spectrophotmety ($\Delta T_{495-540\ nm}$) using acridine orange (3 μM, final concentration) as a probe for the transmembrane pH difference (an upstroke indicates internal acidification). Chromaffin granule ghosts (0.05 mg protein/mL) were incubated in 7 mM HEPES buffer, pH 7.0, supplied with 80 mM KCl. Proton pumping was started by adding MgATP (1.2 mM, final concentration) as indicated by arrows. Panel A: Upper trace shows the effect of nitrendipine (dissolved in ethanol), and lower trace shows the effect of ethanol (i.e. the solvent effect). Panel B: The effect of amlodipine.

RESULTS

The Effect of Nitrendipine on the Bioenergetics of Chromaffin Granule Ghosts

The calcium antagonist nitrendipine (10 μM, final concentration) reduced the rate of MgATP-dependent (i.e. energy-dependent) intravesicular acidification by 23% (N = 5). However, nitrendipine did not transiently increase the intravesicular pH when added during proton pumping at a final concentration of 20 μM (Fig. 1A), in contrast to what we previously found for amlodipine (Fig. 1B). The non-charged dihydropyridine nitrendipine, therefore, represents an ideal control for the accumulation of ionisable (in the relevant pH range of 5.5–7.5) calcium antagonists in chromaffin granule ghosts.

The Effect of MgATP on the Apparent Equilibrium Partition of Selected Calcium Antagonists in Chromaffin Granule Ghosts

The two dihydropyridine L-type calcium antagonists [^{14}C]amlodipine (Fig. 2, Table 1) and [^{3}H]nitrendipine (Fig. 2) as well as the phenylalkylamine L-type calcium antagonist [^{14}C]verapamil (Table 1) were found to accumulate in chromaffin granule ghosts, with apparent equilibrium partition coefficients of 8100 ± 1100 (Table 1), 2700 ± 600 (Fig. 2), and 246 ± 105 (Table 1) for amlodipine, nitrendipine, and verapamil, respectively. Similarly, the tetralol derivative T-calcium channel blocker mibefradil also accumulates in chromaffin granule ghosts with an apparent partition coefficient of 7400 ± 2200 (Fig. 3). The partition coefficient for amlodipine increased significantly ($P < 0.001$) to 20,300 ± 5,000 (i.e. 2.5-fold increase) and for verapamil to 854 ± 206 (i.e. 3.5-fold increase) in response to 1.2 mM MgATP (Table 1). From Fig. 3, it may be seen that 1.2 mM MgATP increased the apparent partition coefficient of [^{14}C]mibefradil from 7400 ± 2200 (control, i.e. no MgATP) to 32,700 ± 8,900 ($P < 0.001$ vs control). These responses to MgATP were completely abolished by the proton pump inhibitors bafilomycin A_1 (30 nM, final concentration) and NEM (2 mM, final concentration) as well as by the protonophore (uncoupler) CCCP (10 μM, final concentration), which did not affect the apparent partition coefficients of amlodipine or verapamil in the absence of MgATP (Table 1). NH_4Cl (1 mM) also completely blocked the energy-dependent accumulation of verapamil into chromaffin granule ghosts (Table 1). On the other hand, 1.2 mM MgATP had no effect on the apparent [^{3}H]nitrendipine partition coefficient (Fig. 2).

Release of Accumulated Calcium Antagonists in Response to De-energetisation of Granule Ghosts

After loading chromaffin granule ghosts with [^{14}C]amlodipine in the presence of 1.2 mM MgATP, the ghosts were collected by centrifugation and resuspended in an amlodipine-free medium containing MgATP, giving a total amount of [^{14}C]amlodipine of 31.6 ± 6.7 nmol/L (N = 6). This

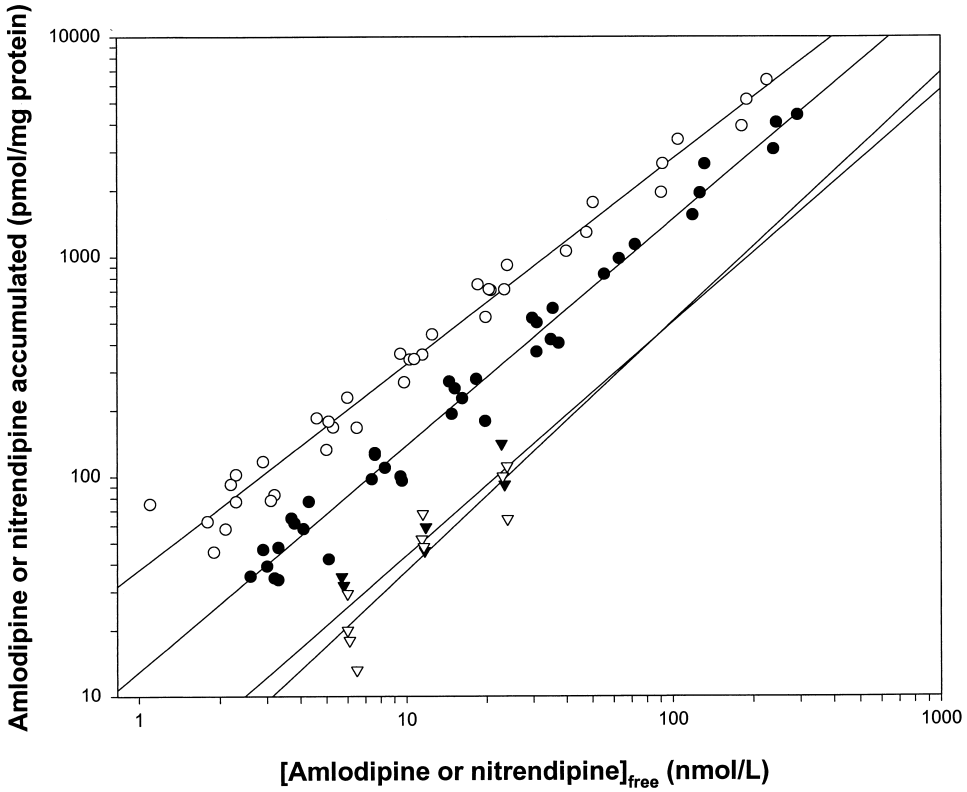

FIG. 2. Double logarithmic plot of the accumulation/binding of [^{14}C]amlodipine (circles) and [^{3}H]nitrendipine (triangles) in/to chromaffin granule ghosts in the absence (filled symbols) or presence (open symbols) of MgATP (1.2 mM, final concentration) in 7.0 mM HEPES buffer with 80 mM KCl for 10 min at 25°. For experimental details, see Methods section. The ghosts were then collected by centrifugation and the amount of dihydropyridine compound accumulated was determined on the basis of residual radioactivity in the supernatant. The experimental data with amlodipine represent the individual numbers obtained from six different preparations of granule ghosts, except at the three highest concentrations (only three preparations examined). The nitrendipine data represent values obtained from three or four different ghost preparations. None of the data in Fig. 2 are included in Table 1. Statistical analysis (by the SigmaPlot) revealed a significant difference between the two regression lines for amlodipine ($P < 0.0001$), but not between the two nitrendipine regression lines ($P = 0.23$).

represents the sum of amlodipine in the medium (3.8 ± 0.9 nM, Table 2) and particle-bound amlodipine (27.8 nmol/L), which can be divided into amlodipine that has accumulated passively (40% or 11 nmol/L) or in response to MgATP (60% or 16.4 nmol/L), according to the partition coefficients in the absence and presence of MgATP (see legend to Table 2). The addition of the protonophore (uncoupler) CCCP (10 μM, final concentration), which effectively dissipates the ΔpH in chromaffin granule ghosts in the presence of chloride [21], increased the concentra-

TABLE 1. The effect of bafilomycin A_1, CCCP, NEM, and NH_4Cl on apparent equilibrium partition coefficient

Incubation conditions	Apparent equilibrium partition coefficient	
	[^{14}C]amlodipine	[^{14}C]verapamil
CGG	8100 ± 1100 (N = 5)	246 ± 105 (N = 8)
CGG + bafilomycin A_1	8500 ± 1200 (N = 5)	325 ± 78 (N = 4)
CGG + CCCP	8000 ± 1500 (N = 10)	189 ± 20 (N = 4)
CGG + NEM	8100 ± 400 (N = 5)	285 ± 76 (N = 7)
CGG + NH_4Cl	not determined	232 ± 133 (N = 5)
CGG + MgATP	20300 ± 5000 (N = 11)*	854 ± 206 (N = 10)*
CGG + MgATP + bafilomycin A_1	7600 ± 900 (N = 5)	293 ± 124 (N = 4)
CGG + MgATP + NEM	7800 ± 1600 (N = 5)	268 ± 66 (N = 7)
CGG + MgATP + CCCP	8500 ± 2500 (N = 7)	295 ± 125 (N = 5)
CGG + MgATP + NH_4Cl	not determined	222 ± 72 (N = 5)

The effect of bafilomycin A_1 (30 nM), CCCP (10 μM), NEM (2 mM), and NH_4Cl (1 mM) on the apparent equilibrium partition coefficient of [^{14}C]amlodipine or [^{14}C]verapamil in the absence and/or presence of 1.2 mM MgATP in chromaffin granule ghosts (CGG). Data are given as means ± SD.

*Analysis of variance (two-way layout) of the means of all groups allowed the following conclusions at the 95% confidence level. The mean of the CGG + MgATP group is different from the means of all other groups ($P < 0.0001$), with no significant differences between any of the other groups.

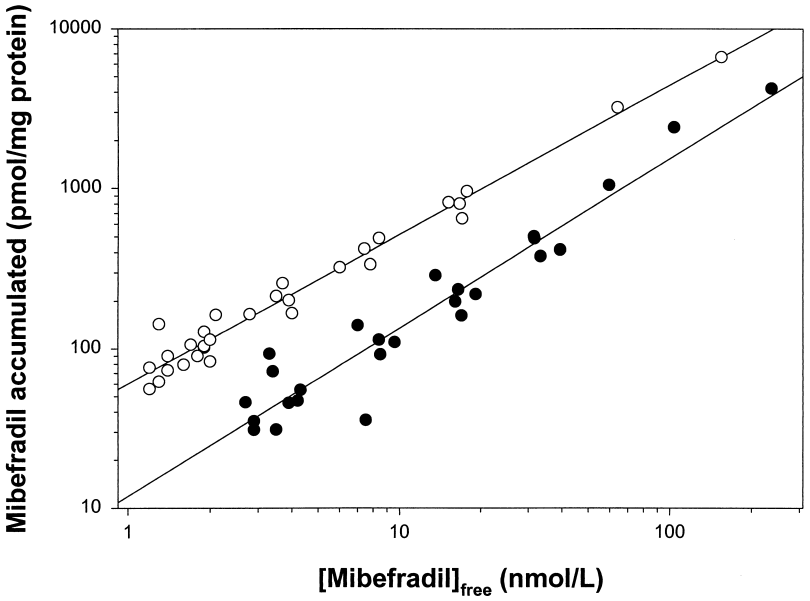

FIG. 3. The accumulation of mibefradil in the absence (filled symbols) and presence (open symbols) of 1.2 mM MgATP in chromaffin granule ghosts. Experimental conditions as in Fig. 1. The apparent partition coefficient for mibefradil is 7400 ± 2200 (control) and 32,700 ± 8,900 (in the presence of MgATP) ($P < 0.001$).

tion of free [^{14}C]amlodipine in the medium from 3.8 ± 0.9 to 6.9 ± 1.0 nM (Table 2, N = 5, $P < 0.001$). As CCCP does not affect the apparent amlodipine partition coefficients in the absence of MgATP (Table 1) and prevents the increase in its apparent amlodipine partition coefficient in response to MgATP (Table 1), it is concluded that CCCP induced a release of amlodipine from the amlodipine-loaded ghosts corresponding to 6.9 − 3.8 = 3.1 nM, i.e. ~19% (100 · 3.1/16.4) of the amlodipine pool that had accumulated in response to MgATP. In a similar way, when [^{14}C]verapamil- or [^{14}C]mibefradil-loaded ghosts were exposed to 10 μM CCCP, ~88% of the verapamil and ~30% of the mibefradil that had accumulated in response to MgATP were released (Table 2).

DISCUSSION

The dihydropyridine calcium antagonists [^{14}C]amlodipine and [^{3}H]nitrendipine accumulate in chromaffin granule ghosts with apparent partition coefficients of about 8000 and 2700, respectively (Fig. 1, Table 1), whereas the phenylalkylamine verapamil has a much lower partition coefficient (250). Mibefradil also accumulates with a partition coefficient similar to that of amlodipine. More important, however, is our finding that energetisation of the ghosts by MgATP significantly increased the apparent partition coefficients of amlodipine (2.5-fold, Table 1), mibefradil (4.4-fold, Fig. 3), and verapamil (3.5-fold, Table 1), with no effect on that of nitrendipine (Fig. 2). The

TABLE 2. The release of [^{14}C]amlodipine, [^{14}C]mibefradil, or [^{14}C]verapamil from chromaffin granule ghosts, loaded with the calcium antagonists in the presence of MgATP in response to the uncoupler CCCP

	Control (+MgATP)			CCCP + MgATP	Net CCCP-induced release	
Drug	Total	Free	Estimated accumulation due to MgATP*	Free	Δ (CCCP-control)	Released (%)
Amlodipine (nmol/L)	31.6 ± 6.7	3.8 ± 0.9	17.6	6.9 ± 1.0†	3.1	3.1/17.6 ~ 18%
Mibefradil (nmol/L)	18.2 ± 1.3	1.5 ± 1.3	14.1	5.7 ± 1.7†	4.2	4.2/14.1 ~ 30%
Verapamil (μmol/L)	0.78 ± 0.05	0.42 ± 0.02	0.25	0.64 ± 0.02†	0.22	0.22/0.25 ~ 88%

Chromaffin granule ghosts were incubated in 7.0 mM HEPES buffer pH 7.0/80 mM KCl with either [^{14}C]amlodipine (28 nM), [^{14}C]mibefradil (7 nM), or [^{14}C]verapamil (1 μM) for 15 min at 25° in the presence of 1.2 mM MgATP (10 mL total volume). The ghosts were then collected by centrifugation (see Methods section), and the pellet resuspended by gentle manual homogenisation in the standard medium (2.0 mL) containing 1.2 mM MgATP. The resuspended vesicles, termed [^{14}C]amlodipine-, [^{14}C]mibefradil- or [^{14}C]verapamil-loaded ghosts, were then divided into two aliquots. To one aliquot was added CCCP (+CCCP + MgATP), the other was untreated (+MgATP), and both were allowed to incubate for 5 min at 25° before a second centrifugation. The [^{14}C]amlodipine, [^{14}C]mibefradil, or [^{14}C]verapamil was measured in the resuspended ghosts, and in the supernatants after the final centrifugation. For each drug, five different preparations of granule ghosts were examined. As the drugs are partly particle-bound and partly free in the medium, the terms nmol/L or μmol/L indicate amount of drug per L, irrespective of whether the drug is free or bound.

*The amount of drug accumulated in response to MgATP was calculated on the basis of the ratio between the partition coefficients in the presence and absence of MgATP, as found in Table 1 and Fig. 3.

†For each drug, the increase in the free medium drug concentration in response to CCCP was highly significant ($P < 0.001$, paired t-test).

increased accumulations of amlodipine and verapamil in response to MgATP was completely inhibited (Table 1) by the specific V-type H^+-ATPase inhibitors bafilomycin A_1 [17–19] and NEM [20] as well as by the protonophore (uncoupler) CCCP, known to dissipate transmembrane pH gradients in these vesicles [21]. The MgATP-dependent increase in verapamil partition was, as expected, also completely blocked by NH_4Cl (Table 1). In addition, CCCP induced a release of amlodipine (~20%), mibefradil (~30%), and verapamil (~88%) from the fraction that had accumulated in response to MgATP (Table 2). Since the partition coefficient of nitrendipine was not affected by MgATP, we conclude that amlodipine, mibefradil, and verapamil, but not nitrendipine, accumulate in chromaffin granule ghosts in response to ΔpH of the proton electrochemical gradient generated by the endogenous V-type H^+-ATPase, as expected for these charged amphiphilic molecules [6]. If the extra amlodipine taken up in response to MgATP accumulates in the intravesicular free water space of the granule ghosts (6 μL/mg protein [22]), the internal concentration of amlodipine is estimated to be approximately 3400 times higher than in the medium. Using the same method of calculation (data from Table 1), the concentration gradient for verapamil is only 144. By comparison, the concentration gradient reported for the energy-dependent uptake of dopamine varies from 650 [23] to >10,000 [24], depending on its free concentration in the medium. Thus, the concentration gradients obtained for amlodipine and verapamil are within the same range.

The effects of calcium antagonists on, for example, the plasma noradrenaline level are often explained on the basis of a variable degree of reflex sympathetic stimulation [25]. It is interesting to note that the non-ionic dihydropyridines nifedipine and felodipine activate the adrenergic system more than what is observed for verapamil [26–28]. Neither nifedipine nor felodipine will be able to accumulate in catecholamine storage vesicles, as shown for nitrendipine in the present work. The other two compounds shown to accumulate in catecholamine storage vesicles (present study), amlodipine and mibefradil, also do not increase or only slightly increase adrenergic activity [29, 30]. To partially explain such differences in biological systems, we propose that calcium antagonists that accumulate in catecholamine storage vesicles *in vitro* also do so *in vivo*. These calcium antagonists may reach unexpectedly high concentrations, e.g. in sympathetic fibers. It has been shown that amlodipine at higher concentrations blocks the calcium flux through N-type calcium channels expressed in *Xenopus* oocytes [31]. As noradrenaline release is an exocytotic process requiring calcium influx through N-calcium channels, calcium antagonists which accumulate in catecholamine storage vesicles may thus have unique pharmacodynamic properties counteracting adrenergic activity.

[^{14}C]Amlodipine and amlodipine were gifts from Pfizer Norway AS and Pfizer Central Research, Sandwich, U. K. Mibefradil and [^{14}C]mibefradil were gifts from F. Hoffmann-La Roche Ltd., Basle, Switzerland and Roche Norway AS. [^{14}C]Verapamil was a gift from Knoll AG, Ludwigshafen, Germany and MEDA AS (Norway). We thank Dr. Roger Strand, Department of Biochemistry and Molecular Biology/Center of the Study of Science and Humanities, University of Bergen and Pfizer Norway AS for help with the statistical analysis of the data in Table 1. The expert technical assistance of Sissel Vik Berge is greatly appreciated. This work was partly presented as posters (nrs 764 and 765) at the XVI World Congress of the International Society for Heart Research, May 27–31 1998, Rhodes, Greece.

References

1. Terland O, Grønberg M and Flatmark T, The effect of calcium channel blockers on the H^+-ATPase and bioenergetics of catecholamine storage vesicles. *Eur J Pharmacol* **207:** 37–41, 1991.
2. Grønberg M, Terland O, Husebye ES and Flatmark T, The effect of prenylamine and organic nitrates on the bioenergetics of bovine catecholamine storage vesicles. *Biochem Pharmacol* **40:** 351–355, 1990.
3. Terland O and Flatmark T, Drug-induced parkinsonism: Cinnarizine and flunarizine are potent uncouplers of the vacuolar H^+-ATPase in catecholamine storage vesicles. *Neuropharmacology* **38:** 879–882, 1999.
4. Arrowsmith JE, Campbell SF, Cross PE, Stubbs JK, Burges RA, Gardiner DG and Blackburn KJ, Long-acting dihydropyridine calcium antagonists. 1.2-Alkoxymethyl derivatives incorporating basic substituents. *J Med Chem* **29:** 1696–1702, 1986.
5. Burges RA, Gardiner DG, Gwilt M, Higgins AJ, Blackburn KJ, Campbell SF, Cross PE and Stubbs JK, Calcium channel blocking properties of amlodipine in vascular smooth muscle and cardiac muscle *in vitro:* Evidence for voltage modulation of vascular dihydropyridine receptors. *J Cardiovasc Pharmacol* **9:** 110–119, 1987.
6. Johnson RG, Accumulation of biological amines into chromaffin granules: A model for hormone and neurotransmitter transport. *Physiol Rev* **68:** 232–307, 1988.
7. Herling AW and Ljungström M, Effects of verapamil on gastric acid secretion *in vitro* and *in vivo*. *Eur J Pharmacol* **156:** 341–350, 1988.
8. Hasegawa J, Fujita T, Hayashi Y, Iwamoto K and Watanabe J, pKa determination of verapamil by liquid–liquid partition. *J Pharm Sci* **73:** 442–445, 1984.
9. Ertel SI and Clozel JP, Mibefradil (Ro 40-5967): The first selective T-type Ca^{2+} channel blocker. *Exp Opin Invest Drugs* **6:** 569–582, 1997.
10. Terland O and Flatmark T, Oxidoreductase activities of chromaffin granule ghosts isolated from the bovine adrenal medulla. *Biochim Biophys Acta* **597:** 318–330, 1980.
11. Peter D, Jimenez J, Liu Y, Kim J and Edwards RH, The chromaffin granule and synaptic vesicle amine transporters differ in substrate recognition and sensitivity to inhibitors. *J Biol Chem* **269:** 7231–7237, 1994.
12. Grønberg M and Flatmark T, Studies on Mg^{2+}-dependent ATPase in bovine adrenal chromaffin granules. With special reference to the effect of inhibitors and energy coupling. *Eur J Biochem* **164:** 1–8, 1987.
13. Husebye ES and Flatmark T, Characterization of phospholipase activities in chromaffin granule ghosts isolated from the bovine adrenal medulla. *Biochim Biophys Acta* **920:** 120–130, 1987.
14. Mason RP, Moisey DM and Shajenko L, Cholesterol alters the binding of Ca^{2+} channel blockers to the membrane lipid bilayer. *Mol Pharmacol* **41:** 315–321, 1992.
15. Herbette LG, Mason PE, Gaviraghi G, Tulenko TN and Mason RP, The molecular basis for lacidipine's unique phar-

macokinetics: Optimal hydrophobicity results in membrane interactions that may facilitate the treatment of atherosclerosis. *J Cardiovasc Pharmacol* **23**(Suppl 5): S16–S25, 1994.
16. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* **72:** 248–254, 1976.
17. Bowman EJ, Siebers A and Altendorf K, Bafilomycins: A class of inhibitors of membrane ATPases from microorganisms, animal cells, and plant cells. *Proc Natl Acad Sci USA* **85:** 7972–7976, 1988.
18. Hanada H, Moriyama Y, Maeda M and Futai M, Kinetic studies of chromaffin granule H^+-ATPase and effects of bafilomycin A_1. *Biochem Biophys Res Commun* **170:** 873–878, 1990.
19. Crider BP, Xie XS and Stone DK, Bafilomycin inhibits proton flow through the H^+ channel of vacuolar proton pumps. *J Biol Chem* **269:** 17379–17381, 1994.
20. Flatmark T, Grønberg M, Husebye E and Berge SV, Inhibition by *N*-ethylmaleimide of the MgATP-driven proton pump of the chromaffin granules. *FEBS Lett* **149:** 71–74, 1982.
21. Husebye ES and Flatmark T, The content of long-chain free fatty acids and their effect on energy transduction in chromaffin granule ghosts. *J Biol Chem* **259:** 15272–15276, 1984.
22. Kobold G, Langer R and Burger A, Does the carrier of chromaffin granules transport the protonated or the uncharged species of catecholamines? *Naunyn Schmiedebergs Arch Pharmacol* **331:** 209–219, 1985.
23. Huyghe BG and Klinman JP, Activity of membranous dopamine β-monooxygenase within chromaffin granule ghosts. Interaction with ascorbate. *J Biol Chem* **266:** 11544–11550, 1991.
24. Phillips JH and Apps DK, Stoichiometry of catecholamine/proton exchange across the chromaffin-granule membrane. *Biochem J* **192:** 273–278, 1980.
25. Grossman E and Messerli FH, Effect of calcium antagonists on plasma norepinephrine levels, heart rate, and blood pressure. *Am J Cardiol* **80:** 1453–1458, 1997.
26. Kailasam MT, Parmer RJ, Cervenka JH, Wu RA, Ziegler MG, Kennedy BP, Adegbile IA and O'Connor DT, Divergent effects of dihydropyridine and phenylalkylamine calcium channel antagonist classes on autonomic function in human hypertension. *Hypertension* **26:** 143–149, 1995.
27. Muiesan G, Agabiti-Rosei E, Castellano M, Alicandri CL, Corea L, Fariello R, Beschi M and Romanelli G, Antihypertensive and humoral effects of verapamil and nifedipine in essential hypertension. *J Cardiovasc Pharmacol* **4**(Suppl 3: S325–S329, 1982.
28. Halperin AK, Icenogle MV, Kapsner CO, Chick TW, Roehnert J and Murata GH, A comparison of the effects of nifedipine and verapamil on exercise performance in patients with mild to moderate hypertension. *Am J Hypertens* **6:** 1025–1032, 1993.
29. Leenen FHH and Fourney A, Comparison of the effects of amlodipine and diltiazem on 24-hour blood pressure, plasma catecholamines, and left ventricular mass. *Am J Cardiol* **78:** 203–207, 1996.
30. Schmitt R, Kleinbloesem CH, Belz GG, Shroeter V, Feifel U, Pozenel H, Kirch W, Halabi A, Woittiez AJJ, Welker HA and Brummelen P, Hemodynamic and humoral effects of the novel calcium antagonist Ro 40-5967 in patients with hypertension. *Clin Pharmacol Ther* **52:** 314–323, 1992.
31. Furukawa T, Nukada T, Suzuki K, Fujita Y, Mori Y, Nishimura M and Yamanaka M, Voltage and pH dependent block of cloned N-type Ca^{2+} channels by amlodipine. *Br J Pharmacol* **121:** 1136–1140, 1997.