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Phosphate, nitrendipine and valinomycin increase the Ca^{2+}/ATP coupling ratio of rat skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase

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Abstract

Nitrendipine and valinomycin act synergistically to stimulate ATP-dependent Ca^{2+} accumulation by rat skeletal muscle sarcoplasmic reticulum vesicles 3-fold. The stimulation is not caused by activation of the Ca^{2+}-ATPase or by inhibition of the sarcoplasmic reticulum Ca^{2+} channel, but is due to an increased efficiency of transport by Ca^{2+}-loaded vesicles. At low Ca^{2+} concentrations, nitrendipine + valinomycin inhibits Ca^{2+} uptake by increasing the Ca^{2+} K_M but does not effect equilibrium Ca^{2+} binding to the Ca^{2+}-ATPase ($K_d = 0.75$ μM). In the presence of 50 mM phosphate, nitrendipine + valinomycin increases the steady-state coupling ratio (Ca^{2+} accumulated per ATP hydrolyzed) from 0.6 to 1.9 by decreasing the rate of ATP hydrolysis by 72%, while reducing the Ca^{2+} accumulation rate by only 13%. The rates of both passive and Ca^{2+}-ATPase-mediated Ca^{2+} release are reduced by nitrendipine + valinomycin. The data indicate that nitrendipine and valinomycin act directly on the Ca^{2+}-ATPase to decrease the ATP hydrolysis rate, increase the Ca^{2+} K_M, decrease Ca^{2+} efflux, and increase the Ca^{2+}/ATP coupling ratio of Ca^{2+}-loaded vesicles.

Key words: Nitrendipine; Valinomycin; Sarcoplasmic reticulum; ATPase, Ca^{2+}-; Calcium ion transport; Ionophore; (Rat)

1. Introduction

In the relaxed state, the cytosolic Ca^{2+} concentration of skeletal muscle is maintained at submicromolar levels by Ca^{2+} transport into the sarcoplasmic reticulum. Ca^{2+} uptake is mediated by the Ca^{2+}-ATPase which couples the transport of two calcium ions to ATP hydrolysis.

There is a growing list of hydrophobic compounds that activate Ca^{2+} uptake by sarcoplasmic reticulum vesicles: valinomycin [1–3], nigericin [4], carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone [2], nitrendipine, BayK8644 [5], palmitate [6], gingerol [7], diethyl ether [8], nonylphenol [9], triphenylphosphine [10], trifluoperazine [10] and 3-nitrophenol [10]. The concentration at which these compounds activate Ca^{2+} accumulation is on the order of 0.02 to 1 mole per mole phospholipid.

The elucidation of the mechanism by which these hydrophobic compounds increase Ca^{2+} accumulation may provide insight into the mechanism by which the Ca^{2+}-ATPase mediates Ca^{2+} translocation across the membrane. In this study we investigate the mechanism by which valinomycin and nitrendipine stimulated Ca^{2+} uptake by sarcoplasmic reticulum.

2. Materials and methods

Arsenazo III was purchased from Aldrich (Milwaukee, WI). Adenosine 5′-triphosphate, acetyl phosphate, chlortetracycline, carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), valinomycin, gramacidin, lactate dehydrogenase, pyruvate kinase were obtained from Sigma (St. Louis, MO). Monensin, nigericin, and A23187 was supplied by Calbiochem-Behring (La Jolla, CA). Nitrendipine and BayK8644 was kindly supplied by Dr. Scriabine (Miles Laboratories, New Haven, CO).

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2.1. Preparation of sarcoplasmic reticulum vesicles

The back and hind leg skeletal muscles were removed from 300 g Sprague-Dawley rats and the microsomal fraction prepared as previously described [11]. The microsome fraction was placed on a 30–45% sucrose gradient and centrifuged 100 000 × *g* for 15 h at 4°C. The low-density sarcoplasmic reticulum fraction (30–34% sucrose) was removed from the gradient and diluted into 0.15 M KCl, 10 mM histidine (pH 6.8), and 2 mM $MgSO_4$ (KCl solution). The vesicles were concentrated by centrifugation (100 000 × *g* for 30 min) and then diluted with KCl solution to give a protein concentration of 15–25 mg/ml.

2.2. Spectrophotometric measurement of Ca^{2+} accumulation by sarcoplasmic reticulum vesicles using arsenazo III [12]

The calcium uptake medium contained sarcoplasmic reticulum vesicles (76 μg/ml), 0.15 M KCl, 10 mM histidine (pH 6.8), 3 mM $MgSO_4$, 35 μM $CaCl_2$, 1.0 mM ATP and 100 μM arsenazo III at 20°C. The difference absorbance (660 nm – 685 nm) of the arsenazo III-Ca^{2+} complex was measured with an Aminco DW-2 dual beam spectrophotometer. The Ca^{2+} uptake values reported are the Ca^{2+} loading levels which are reached 200 s after initiation of Ca^{2+} uptake.

2.3. Spectrofluorometric measurement of Ca^{2+} uptake using chlortetracycline [13]

Chlortetracycline was used to monitor Ca^{2+} uptake when the extravesicular Ca^{2+} was buffered using EGTA since chlortetracycline monitors the intravesicular Ca^{2+} concentration. The Ca^{2+} uptake solution contained 0.15 M KCl, 10 mM histidine (pH 6.8), 2 mM $MgSO_4$, 82 μg protein/ml sarcoplasmic reticulum vesicles, 10 μM chlortetracycline, 5.0 mM acetyl phosphate and different concentrations of Ca^{2+} and EGTA. The fluorescence (ex. 390 nm, em. 530 nm) of the intravesicular chlortetracycline-Ca^{2+} complex was monitored.

2.4. Measurement of the Ca^{2+}-dependent ATPase activity of sarcoplasmic reticulum vesicles

ATPase activity was measured by a coupled enzyme assay [14] in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8), 5 mM $MgSO_4$, 1.0 mM EGTA, 1.0 mM ATP, 3 μg protein/ml sarcoplasmic reticulum vesicles, 21 U/ml pyruvate kinase, 6 U/ml lactate dehydrogenase, 0.75 mM phospho*enol*pyruvate, 0.15 mg/ml NADH, and various $CaCl_2$ concentrations. In some measurements, 5 μM A23187, a Ca^{2+} ionophore, was included to prevent accumulation of Ca^{2+} by the sarcoplasmic reticulum vesicles. ATP hydrolysis was monitored by measuring the decrease of the NADH absorbance at 340.

2.5. Measurement of passive Ca^{2+} binding to the Ca^{2+}-ATPase

Sarcoplasmic reticulum vesicles (2 mg/ml) were equilibrated in 3.5 ml of 0.15 M KCl, 10 mM histidine (pH 6.8), 2 mM $MgSO_4$, 0.20 mM $^{45}CaCl_2$ (1 μCi/ml) and various amounts of EGTA (0.3–2 mM) for 3 h. to vary the free Ca^{2+} concentration (0.1–2.0 μM). The vesicles were collected by centrifugation (100 000 × *g*, 30 min) and resuspended in 100 μl of 1% sodium dodecylsulfate. Aliquots were removed and the $^{45}Ca^{2+}$ determined by scintillation counting. Nonspecific Ca^{2+} sequestration was determined by measuring the amount of $^{45}Ca^{2+}$ in the pellet in the presence of an excess (2 mM) EGTA. Bound Ca^{2+} was calculated from the amount of counts associated with the vesicles minus the nonspecific counts.

2.6. Measurement of Ca^{2+} efflux from sarcoplasmic reticulum vesicles after active loading

Sarcoplasmic reticulum vesicles (0.16 mg protein/ml) were actively loaded with Ca^{2+} for 6 min at 20°C in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8) 1 mM $MgSO_4$, 10 μM $CaCl_2$, 10 μM chlortetracycline, 1 mM ATP, 0.75 mM phospho*enol*pyruvate, and 21 U/ml pyruvate kinase. The external Ca^{2+} concentration was then lowered to below 10 nM by the addition of 0.5 mM EGTA. The rate of Ca^{2+} efflux from the sarcoplasmic reticulum vesicles was monitored using chlortetracycline as a fluorescence indicator (ex. 390 nm, em. 530 nm) of the intravesicular Ca^{2+} concentration.

2.7. Measurement of Ca^{2+} efflux mediated by (Ca^{2+}-ATPase) pump reversal

Ca^{2+} release was measured by following the change in turbidity of vesicles loaded with Ca^{2+} phosphate precipitate [15]. Sarcoplasmic reticulum vesicles (0.10 mg protein/ml) were actively loaded up with Ca^{2+} in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8), 10 mM $MgSO_4$, 25 mM KH_2PO_4, 150 μM $CaCl_2$, 5.0 mM acetyl phosphate at 27°C for 1.5 h. During Ca^{2+} uptake, the turbidity of the solution increases due to the precipitation of calcium phosphate within the sarcoplasmic reticulum vesicle. The increase in the turbidity is directly proportional to the amount of Ca^{2+} accumulated. After the vesicles accumulated 1.5 μmol Ca^{2+}/mg protein, 5.0 mM EGTA was added and Ca^{2+} release was monitored by measuring the decrease in the turbidity at 600 nm. After the passive Ca^{2+} efflux rate was determined, 2.0 mM ADP was added to initiate pump reversal ($2Ca^{2+}_{in} + ADP + P_i \rightarrow 2Ca^{2+}_{out} + ATP$).

3. Results

3.1. Nitrendipine and valinomycin synergistically stimulate Ca^{2+} accumulation by sarcoplasmic reticulum vesicles

As previously reported, nitrendipine [5] and valinomycin [1,2,3] activate ATP-dependent Ca^{2+} accumulation by sarcoplasmic reticulum vesicles (Fig. 1). To determine if nitrendipine and valinomycin interact at a common site to stimulate Ca^{2+} accumulation, both were added together to see if their effect on Ca^{2+} uptake is additive. Valinomycin activation of Ca^{2+} accumulation is enhanced by 25 μM nitrendipine, and nitrendipine activation of Ca^{2+} accumulation is enhanced by 10 μM valinomycin demonstrating that nitrendipine and valinomycin act synergistically to stimulate Ca^{2+} uptake. The 3-fold increase in Ca^{2+} accumulation caused by nitrendipine + valinomycin (25 μM + 10 μM) is not due to the formation of intravesicular Ca^{2+} precipitate since the intravesicular Ca^{2+} concentration measured with chlortetracycline (a fluorescent indicator of intravesicular Ca^{2+} concentration) also increases about 3-fold (data not shown).

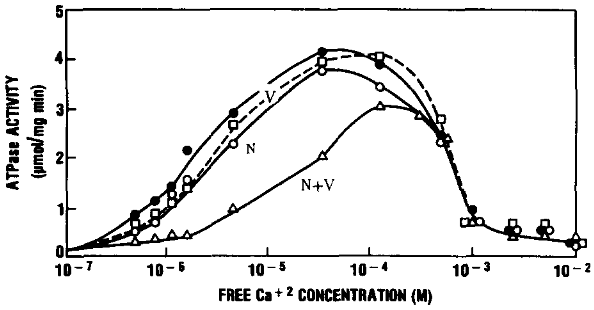

Fig. 2. Nitrendipine and valinomycin act synergistically to alter the relationship between the Ca^{2+} concentration and the rate of ATP hydrolysis by sarcoplasmic reticulum vesicles. ATPase activity was measured spectrophotometrically by a coupled enzyme assay in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8), 5 mM $MgSO_4$, 1.0 mM EGTA, 1.0 mM ATP, 3 μg protein/ml sarcoplasmic reticulum vesicles, 21 U/ml pyruvate kinase, 6 U/ml lactate dehydrogenase, 0.75 mM phospho*enol*pyruvate, 0.15 mg/ml NADH, 5 μM A23187, and the indicated $CaCl_2$ concentration. Nitrendipine (25 μM) (N, ○), valinomycin (10 μM) (V, □), or both nitrendipine (25 μM) and valinomycin (10 μM) (N + V, △) was included in the assay medium. Nitrendipine and valinomycin stock solutions were made in ethanol. In the control (●), 0.2% ethanol was included so that all the samples contained the equivalent amount of ethanol. ATPase activity was linear for at least 10 min.

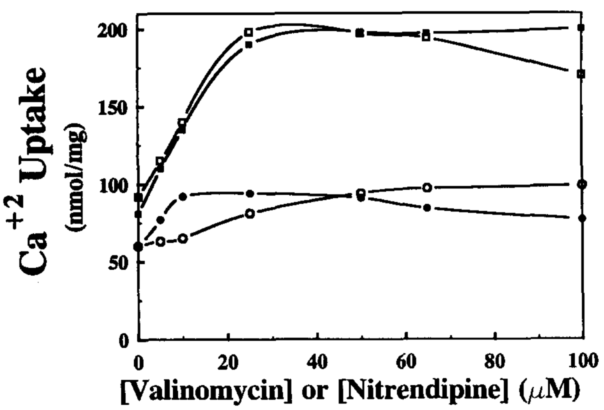

Fig. 1. Activation of Ca^{2+} accumulation by valinomycin and nitrendipine. Ca^{2+} uptake was measured spectrophotometrically using the Ca^{2+}-indicator, arsenazo III, as descibed in Materials and methods. The calcium uptake medium contained sarcoplasmic reticulum vesicles (76 μg/ml), 0.15 M KCl, 10 mM histidine (pH 6.8), 3 mM $MgSO_4$, 35 μM $CaCl_2$, 1.0 mM ATP and 100 μM arsenazo III at 20°C. Nitrendipine (○, □) or valinomycin (●, ■) were added to the Ca^{2+} uptake medium at the indicated concentrations. In one series, the valinomycin concentration was held constant (10 μM) while the nitrendipine concentration was varied (□); in another series the nitrendipine concentration was held constant (25 μM) while the valinomycin was varied (■). The stock solutions for valinomycin and nitrendipine were made in dimethylsulfoxide. The final dimethylsulfoxide concentration did not exceed 2% which does not effect Ca^{2+} accumulation. The Ca^{2+} uptake values reported are the Ca^{2+} loading levels which are reached 200 s after the Ca^{2+} transport was initiated.

3.2. Nitrendipine + valinomycin decreases the Ca^{2+}-ATPase activity

The effect of nitrendipine + valinomycin on the ATP hydrolysis rate was measured to determine if the Ca^{2+}-ATPase is activated by nitrendipine + valinomycin (Fig. 2). The Ca^{2+} ionophore, A23187, was added to the assay solution to prevent the accumulation of Ca^{2+} which would decrease the ATPase activity through product inhibition. When added separately, valinomycin and nitrendipine slightly decrease Ca^{2+}-dependent ATP hydrolysis without significantly changing the dependency of Ca^{2+}-ATPase on the Ca^{2+} concentration (half-maximal ATPase activity is observed at 1.8 μM Ca^{2+}). However, when added together, valinomycin + nitrendipine reduces the Ca^{2+}-dependent ATPase activity and increases the apparent Ca^{2+} K_M about 10-fold. The inhibition of Ca^{2+}-ATPase by nitrendipine + valinomycin indicates that the activation of Ca^{2+} uptake is not due to activation of the Ca^{2+}-ATPase.

Because nitrendipine + valinomycin cause an apparent increase in the Ca^{2+} K_M, ATP-dependent Ca^{2+} accumulation at different Ca^{2+} concentration was measured to determine if nitrendipine + valinomycin affect Ca^{2+} accumulation in a similar manner. In the absence of nitrendipine + valinomycin, the steady-state Ca^{2+} loading level decreases only 32% when the free Ca^{2+} concentration is lowered from 25 μM to 0.3 μM

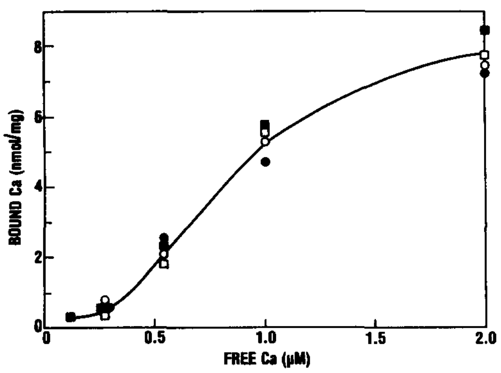

Fig. 3. Equilibrium binding of Ca^{2+} to Ca^{2+},Mg^{2+}-ATPase is not influenced by nitrendipine or valinomycin. Sarcoplasmic reticulum vesicles (2 mg/ml) were equilibrated in 3.5 ml of 0.15 M KCl, 10 mM histidine (pH 6.8), 2 mM $MgSO_4$, 0.20 mM $^{45}CaCl_2$ (1 μCi/ml) and various amounts of EGTA (0.3–2 mM) for 3 h. to vary the free Ca^{2+} concentation (0.1–2.0 μM) (○). In some solutions 10 μM valinomycin (□), 25 μM nitrendipine (●) or both valinomycin and nitrendipine (■) was included. The vesicles were collected by centrifugation (100000 × g, 30 min) and resuspended in 100 μl of 1% sodium dodecylsulfate. Aliquots were removed and the $^{45}Ca^{2+}$ determined by scintillation counting. Nonspecific counts were determined by measuring the amount of $^{45}Ca^{2+}$ in the pellet in the presence of an excess (2 mM) EGTA. Bound Ca^{2+} was calculated from the amount of counts associated with the vesicles minus the nonspecific counts.

(60 nmol Ca^{2+}/mg protein → 42 nmol Ca^{2+}/mg protein), but in the presence of nitrendipine + valinomycin, there is an 87% decrease (228 nmol Ca^{2+}/mg protein → 40 nmol Ca^{2+}/mg protein). Thus, the apparent Ca^{2+} K_M for transport, like the Ca^{2+} K_M for activation of ATP hydrolysis, is increased by nitrendipine + valinomycin.

The apparent decrease in the Ca^{2+}-binding affinity caused by nitrendipine + valinomycin (Fig. 2) is not observed for equilibrium Ca^{2+} binding (Fig. 3). There are 7.8 nmol high-affinity Ca^{2+} binding sites per mg protein; half-maximal binding is reached at 0.74 μM Ca^{2+}, and the Hill coefficient for Ca^{2+} binding is 1.75. Nitrendipine and valinomycin have no effect on equilibrium Ca^{2+} binding. These data suggest that there is no direct effect of nitrendipine + valinomycin on Ca^{2+} binding to the active site of the Ca^{2+}-ATPase.

3.3. Effect of nitrendipine + valinomycin on the coupling ratio of the Ca^{2+}-ATPase

Although the experiment in Fig. 1 demonstrates that nitrendipine + valinomycin stimulate Ca^{2+} accumulation, Fig. 2 shows that the rate of ATP hydrolysis decreases in the presence of nitrendipine + valinomycin. If the rate of Ca^{2+} transport isn't increased by nitrendipine and valinomycin, perhaps the coupling ratio (efficiency) is altered. Following initiation of Ca^{2+} transport by ATP, the coupling ratio is 2 Ca^{2+} per ATP hydrolyzed [18]. But as the vesicles fill up with Ca^{2+}, the ATPase activity decreases due to product inhibition (back-inhibition), and the apparent Ca^{2+}/ATP coupling ratio decrease as the rate of Ca^{2+} efflux increases.

Nitrendipine + valinomycin increases the steady-state Ca^{2+} loading level reached within 200 seconds after inititation of Ca^{2+} transport (Fig. 1) with little effect of the ATPase activity (Fig. 4 and Table 1, look at the values for solutions with no phosphate added). (ATPase inhibition by nitrendipine + valinomycin shown in Fig. 2 was observed under conditions where Ca^{2+} accumulation was prevented by the Ca^{2+} ionophore A23187.) After reaching maximum Ca^{2+} loading, the coupling ratio is 0 since there is no net Ca^{2+} influx; the Ca^{2+} pump only replaces Ca^{2+} as it leaks out. The coupling ratio is increased by adding phosphate which precipitates Ca^{2+} inside the vesicle (Fig. 4 and Table 1) [19,20]. In the presence of 50 mM phosphate, the coupling ratios are 0.6 and 1.9 in the absence and presence of nitrendipine + valinomycin, respectively. Since a coupling ratio of 2 is expected for a perfectly coupled system, this leads to the conclusion

Table 1
The steady-state rate of Ca^{2+}-dependent ATP hydrolysis and Ca^{2+} uptake at various phosphate concentrations

Phosphate (mM)	A23187 (1 μM)	Control		Plus nitrendipine and valinomycin	
		Ca^{2+}-ATPase (μmol/mg per min)	steady-state Ca^{2+} uptake rate (μmol/mg per min)	Ca^{2+}-ATPase (μmol/mg per min)	steady-state Ca^{2+} uptake rate (μmol/mg per min)
0	–	0.65	0	0.77	0
0	+	3.37	0	2.47	0
10	–	0.69	0.02	0.46	0.37
10	+	3.22	0	1.95	0
20	–	0.81	0.19	0.56	0.45
20	+	3.32	0	1.42	0
30	–	0.81	0.22	0.39	0.49
30	+	2.98	0	1.40	0
50	–	0.97	0.6	0.27	0.52
50	+	2.81	0	0.88	0

The experimental conditions are described in Fig. 4.

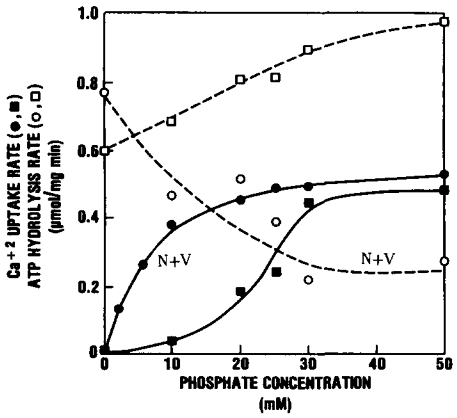

Fig. 4. Nitrendipine + valinomycin increases the coupling ratio between Ca^{2+} uptake and ATP hydrolysis. The Ca^{2+} uptake medium contained 0.15 M KCl, 10 mM histidine, 5 mM $MgSO_4$, 2.6 μg protein/ml sarcoplasmic reticulum vesicles, 0.75 mM phospho-*enol*pyruvate, 21 U/ml pyruvate kinase, 6 U/ml lactate dehydrogenase, 0.15 mg/ml NADH, 100 μM $CaCl_2$, and 1.0 mM ATP at 20°C. The rate of ATP hydrolysis (○, □) was monitored by measuring the decrease in the NADH absorbance at 340 nm. The rate of Ca^{2+} uptake (●, ■) was monitored spectroscopically by including 100 μM arsenazo III in the Ca^{2+} uptake medium. The decrease in the absorbance of the arsenazo III-Ca^{2+} complex at 660 nm was measured in a Aminco DW-2 dual beam spectrophotometer using 685 nm as a reference wavelength. The ATPase activity and Ca^{2+} accumulation rates were measured 200 s after initiation of Ca^{2+} transport at which time both were linear. Nitrendipine (25 μM) and valinomycin (10 μM) was included in some of the samples ((N + V, ●, ○).

that nitrendipine + valinomycin almost completely blocks uncoupled Ca^{2+} efflux from the sarcoplasmic reticulum.

3.4. Nitrendipine + valinomycin decreases the rate of passive Ca^{2+} efflux from sarcoplasmic reticulum vesicles

Nitrendipine + valinomycin decreases the rate of passive Ca^{2+} efflux measured following inhibition of Ca^{2+} transport by EGTA addition (Fig. 5). Valinomycin, nitrendipine and valinomycin + nitrendipine inhibit the initial rate of Ca^{2+} efflux by 74%, 65%, and 88%, respectively. Similar results were obtained with vesicles passively loaded with 50 mM $CaCl_2$. However, the initial rate of Ca^{2+} efflux after EGTA addition is about 0.06 μmol Ca^{2+}/mg per min while the steady-state rate of Ca^{2+}-dependent ATP hydrolysis by vesicles loaded to the same initial Ca^{2+} level is about 23 times greater indicating that Ca^{2+} efflux rate during ATP-dependent Ca^{2+} transport is 46 times the passive Ca^{2+} efflux rate. The difference between the passive Ca^{2+} efflux rate and the rate of Ca^{2+} efflux observed during ATP-dependent Ca^{2+} accumulation could be caused by Ca^{2+}-ATPase-mediated Ca^{2+} efflux which occurs only in the presence of ATP and extravesicular Ca^{2+} as described by Gerdes and Moller [21,22].

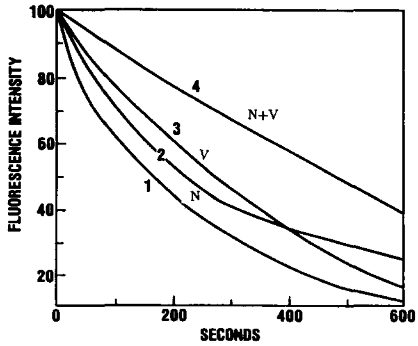

Fig. 5. Nitrendipine and valinomycin decrease the rate of Ca^{2+} efflux from sarcoplasmic reticulum vesicles. Sarcoplasmic reticulum vesicles (0.16 mg protein/ml) were actively loaded with Ca^{2+} for 6 min at 20°C in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8) 1 mM $MgSO_4$, 10 μM $CaCl_2$, 10 μM chlortetracycline, 1 mM ATP, 0.75 mM phophoenolpyruvate, and 21 U/ml pyruvate kinase. The external Ca^{2+} concentration was then lowered to below 10 nM by the addition of 0.5 mM EGTA. The rate of Ca^{2+} efflux from the sarcoplasmic reticulum vesicules was monitored using chlortetracycline as a fluorescence indicator of the intravesicular Ca^{2+} concentration. The excitation and emission wavelengths were set at 390 nm and 530 nm, respectively. Trace 1 is the control. For trace 2, 25 μM nitrendipine (N) was added at the time that EGTA was added. Valinomycin (V) (10 μM) was added with the EGTA for trace 3. Both valinomycin and nitrendipine (N + V) was added in trace 4.

Nitrendipine + valinomycin also inhibits Ca^{2+} efflux mediated by reversal of the Ca^{2+} pump (Fig. 6). Under these conditions nitrendipine, valinomycin and ni-

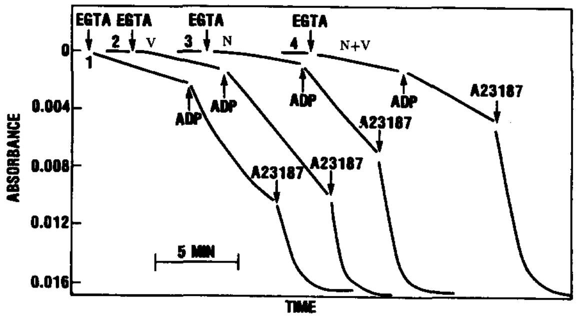

Fig. 6. Nitrendipine and valinomycin decrease the rate of Ca^{2+} efflux through the reverse reactions of the Ca^{2+}-ATPase. Sarcoplasmic reticulum vesicles (0.10 mg protein/ml) were actively loaded with Ca^{2+} in a solution containing 0.15 M KCl, 10 mM histidine (pH 6.8), 10 mM $MgSO_4$, 25 mM KH_2PO_4, 150 μM $CaCl_2$, 5.0 mM acetyl phosphate at 27°C for 1.5 h. During Ca^{2+}, uptake the turbidity of the solution increases due to the precipitation of calcium phosphate within the sarcoplasmic reticulum vesicle. The increase in the tubidity is directly proportional to the amount of Ca^{2+} accumulated. After the vesicles accumulated 1.5 μmol Ca^{2+}/mg protein, 5.0 mM EGTA was added and the rate of passive Ca^{2+} efflux was monitored (by measuring the turbidity change) for about 5 min. Then 2.0 mM ADP was added to initiate pump reversal. At the end of each measurement 1 μM A23187 was added to release all the Ca^{2+}. Trace 1 is the control in which 0.2% ethanol was added. In the other traces, valinomycin (V) (10 μM) (trace 2), nitrendipine (N) (25 μM) (trace 3) or both (N + V) (trace 4) were added to the solution along with the EGTA.

trendipine + valinomycin decrease the rate of pump reversal by 20%, 27% and 72%, respectively.

4. Discussion

These experiments indicate that the steady-state Ca^{2+} loading level of sarcoplasmic reticulum vesicles in the presence of ATP and relatively high Ca^{2+} concentrations is increased by the interaction of nitrendipine + valinomycin with the Ca^{2+}-ATPase. Nitrendipine + valinomycin interacts with the Ca^{2+}-ATPase to alter the Ca^{2+} K_m (Fig. 2), ATP hydrolysis rate (Figs. 2 and 4), the inhibition of the Ca^{2+} ATPase by phosphate (Fig. 4 and Table 1), and the rate of pump reversal (Fig. 6). The most dramatic effect of nitrendipine + valinomycin is the establishment a tight coupling between Ca^{2+} accumulation and ATP hydrolysis during Ca^{2+} accumulation in the presence of high phosphate. One explanation for this is that nitrendipine + valinomycin prevents slippage of the Ca^{2+} pump by preventing uncoupled, Ca^{2+}-ATPase-mediated Ca^{2+} efflux. A coupling ratio of 2 Ca^{2+}: ATP hydrolyzed is observed for the initial turnover of the Ca^{2+}-ATPase and for Ca^{2+} accumulation in the presence of oxalate which precipitates Ca^{2+} at relatively low Ca^{2+} concentration [24,25]. But in the presence of phosphate, the coupling ratio is not 2, probably because the intravesicular Ca^{2+} is not lowered as much as it is in the presence of oxalate. Indeed, the intravesicular Ca^{2+} concentration (measured with chlortetracycline) is decreased only 26% by 50 mM phosphate, probably because the Ca^{2+} transport rate is higher than the phosphate influx rate. A role for the Ca^{2+}-ATPase in mediating uncoupled Ca^{2+} fluxes across the sarcoplasmic reticulum membrane has previously been proposed [3,21–23,26–30], but this is the first demonstration that pump slippage can be blocked pharmacologically. The inhibition of slippage by nitrendipine + valinomycin comes with a price; at low Ca^{2+} concentrations, nitrendipine + valinomycin inhibits the Ca^{2+}-ATPase. It is possible that pump slippage is necessary to obtain highly-cooperative Ca^{2+} binding in the presence of ATP.

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