Pergamon

Biochemical Pharmacology, Vol. 50, No. 6, pp. 879–883, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00194-8

Short Communication

THE LOW-AFFINITY DIHYDROPYRIDINE RECEPTOR AND Na^+/Ca^{2+} EXCHANGER ARE ASSOCIATED IN ADRENAL MEDULLARY MITOCHONDRIA

MERCEDES PALMERO,* LUIS M. GUTIERREZ,* MARIA J. HIDALGO,* JUAN A. REIG,* JUAN J. BALLESTA† and SALVADOR VINIEGRA*

*Departamento de Neuroquímica; and †Departamento de Farmacología, Instituto de Neurociencias, Universidad de Alicante, Ap. 374, 03080 Alicante, Spain

(*Received* 3 *November* 1994; *accepted* 25 *April* 1995)

Abstract—The effect of Ca^{2+} channel-acting drugs on bovine adrenal mitochondria Ca^{2+} movements was investigated. Mitochondrial Ca^{2+} uptake is performed by an energy-driven Ca^{2+} uniporter with a Km of 20.9 ± 3.2 μM and V_{max} of 148.1 ± 7.2 nmol $^{45}Ca^{2+}$ min^{-1} mg^{-1}. Ca^{2+} release is performed through an Na^+/Ca^{2+} antiporter with a Km for Na^+ of 4.2 ± 0.5 mM, a V_{max} of 7.5 ± 0.4 nmol $^{45}Ca^{2+}$ min^{-1} mg^{-1}, and a Hill coefficient of 1.4 ± 0.2. Ca^{2+} efflux through the mitochondrial Na^+/Ca^{2+} exchanger was inhibited by several dihydropyridines (nitrendipine, felodipine, nimodipine, (+)isradipine) and by the benzothiazepine diltiazem with similar potencies. In contrast, neither CGP 28392, Bay-K-8644, amlodipine, nor verapamil had any effect on Ca^{2+} efflux. Nitrendipine at 20 μM modified neither the Km nor the Hill coefficient for Na^+, whereas the V_{max} was reduced to 2.9 nmol $^{45}Ca^{2+}$ min^{-1} mg^{-1}, thus demonstrating noncompetitive modulation of the Na^+/Ca^{2+} exchanger. None of the Ca^{2+} channel-acting drugs assayed at 100 μM affected Ca^{2+} influx through the uniporter. Ca^{2+} channel blockers inhibited the Na^+/Ca^{2+} antiporter and displaced the specific binding of [^{3}H]nitrendipine to intact mitochondria with Ki values similar to the IC_{50}s obtained for the inhibition of the Ca^{2+} efflux. Ca^{2+} channel-acting drugs that did not inhibit the Na^+/Ca^{2+} exchanger (amlodipine, CGP 28392, Bay-K-9644, and verapamil, at concentrations of 100 μM or higher) had no effect on [^{3}H]nitrendipine binding. These results suggest that the adrenomedullary mitochondrial dihydropyridine receptor is associated with the Na^+/Ca^{2+} exchanger.

Key words: dihydropyridine; mitochondria; calcium; adrenal medulla; Na^+/Ca^{2+} antiporter

The movement of mitochondrial Ca^{2+} in excitable tissues is controlled by uptake through the Ca^{2+} uniporter [1] and release via the Na^+/Ca^{2+} exchanger [2, 3]. It is generally admitted that mitochondria do not behave as cytosolic calcium buffers under physiological conditions [4, 5]. Recent reports indicate that changes in intracellular Ca^{2+} levels in intact cells induce significant modifications in mitochondrial energy production [6, 7] due to the activation of intramitochondrial Ca^{2+}-dependent enzymes [8, 9].

DHPs‡ are often used pharmacologically and functionally to characterize the high-affinity DHP receptor associated with the L-type Ca^{2+} channel [10, 11]. In the mitochondria of several tissues, a low-affinity (Kd which is subμM to submM), high-capacity (B_{max} 1,000-fold higher than the plasma membrane receptor) binding site for [^{3}H]DHP has been identified [11, 12, 13]. This receptor displays properties that differentiate it from the high-affinity, low-capacity receptor associated with plasma membrane voltage-dependent calcium channels [11, 12, 13].

To date, there exist no conclusive results concerning the effect of Ca^{2+} antagonists upon mitochondrial Ca^{2+} movements. Buss et al. [14] reported that the DHP derivatives nitrendipine and nimodipine inhibited Ca^{2+} uptake at high concentrations (μM range), but other groups have found no effect of DHP on Ca^{2+} uptake [15]. However, various Ca^{2+} antagonists inhibit Na^+ stimulated Ca^{2+} efflux with IC_{50} values in the μM range [15]. Other authors [16] suggested that nitrendipine acts as an activator of the mitochondrial Na^+/Ca^{2+} exchange, and that the Ca^{2+} agonist Bay-K-8644 blocks the energy-driven Ca^{2+} uptake.

In this paper we characterize calcium uptake and release in adrenal medulla mitochondria and the modulation of Ca^{2+} movements by several calcium channel blockers and activators. This work indicates that mitochondrial DHP binding sites in the bovine adrenal medulla are associated with the Na^+/Ca^{2+} exchanger.

Materials and Methods

[^{3}H]Nitrendipine (70–87 Ci/mmol) was supplied by New England Nuclear and $^{45}Ca^{2+}$ (2mCi/ml) by Amersham. Nitrendipine, (+)isradipine, and nimodipine were kindly donated by Professor Hoffmeister of Bayer A.G. (Wupertal, Germany) and amlodipine by Pfizer Central Research (Sandwich, Kent, U.K.). Other materials were bought from Sigma.

Mitochondria isolation. Mitochondria were isolated essentially as described by Ballesta *et al.* [11]. The final pellet was resuspended in 1 ml of assay buffer containing: 220 mM mannitol, 70 mM sucrose, 30 mM KCl, 0.5 mg/ml BSA fatty acids free, 50 mM Tris/HCl pH 7.2 and kept at 4°C no longer than 10 h. Mitochondrial membrane and chromaffin granule isolation was performed as described in Ballesta *et al.* [11]. Protein concentration was estimated as described by Lowry *et al.* [18].

Mitochondrial calcium movements. All experiments were performed in an assay buffer consisting of the above buffer supplemented with: 5 mM succinate, 4 μM rotenone, 0.2 mM potassium dihydrogen phosphate, and 10 mM potassium acetate [2, 19, 20]. Mitochondria were incubated in assay buffer with 16 μM ADP for 5 min at 21°C. The uptake was initiated by

Corresponding author: Dr. S. Viniegra, Departamento de Neuroquímica, Ap. 374, 03080 Alicante, Spain. Tel. 34 (6) 565-9811; FAX 34 (6) 565-8557.

‡ Abbreviations: DHP, 1,4-dihydropyridine; DCCD, dicyclohexylcarbodiimide.

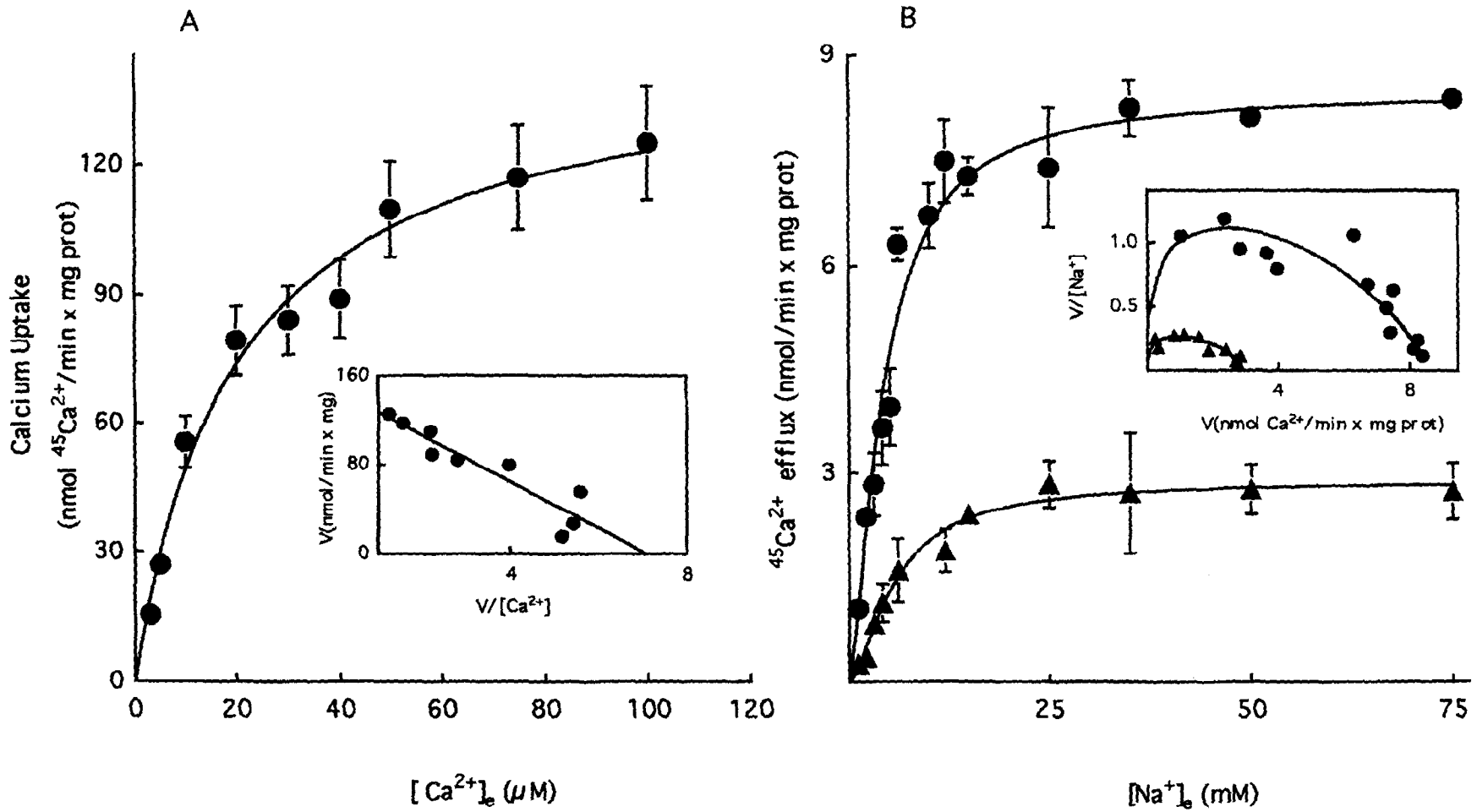

Fig. 1. (A) Dependence of Ca^{2+} uptake on external Ca^{2+} concentration. Mitochondria (1 mg) were incubated in 1 ml assay buffer. After 5 min incubation at 25°C, $^{45}Ca^{2+}$ uptake was initiated by adding a range of Ca^{2+} concentrations (3–100 μM, final concentration). Mitochondrial Ca^{2+} content at 5 s was measured as described in the Methods section. Experimental data were fitted to the Michaelis-Menten equation. Data are given as the means ± SEM of four experiments performed in duplicate with different batches of mitochondria. Inset: Linear transformation ($r^2 = 0.98$) of the same experimental data points using the Eadie-Hofstee equation. (B) Dependence of Ca^{2+} release on external Na^+ concentration: Effect of nitrendipine. Ca^{2+} efflux was stimulated on $^{45}Ca^{2+}$ preloaded mitochondria (1 mg/ml, final volume), for 1 min at 25°C with Na^+ (2–75 mM)/EDTA 0.1 mM in presence (▲) or absence (●) of 20 μM nitrendipine. Then, intramitocondrial $^{45}Ca^{2+}$ content was measured as described in Methods. Inset: Scatchard representation of the same experimental points. Data are given as the means ± SEM of four experiments performed in duplicate with different batches of mitochondria.

adding the indicated concentrations of Ca^{2+}, including $^{45}Ca^{2+}$ (1.5 μCi/ml) as a tracer. For calcium efflux, uptake was stopped after 2 min by the addition of 2 μM Ruthenium Red and 16 μM ADP. Ca^{2+} efflux was stimulated by adding 0.1 mM EDTA and different concentrations of NaCl. At increasing times, samples (0.1 ml) were taken up and filtered under vacuum through Whatman GF/C glass fiber filters, then washed twice with ice cold buffer. Filter-bound radioactivity was measured using a liquid scintillation counter. Analysis of kinetic data was performed using the Hill, Eadie-Hofstee, and Scatchard equations. When present, calcium channel blockers and activators were incubated at 25°C for 15 min prior to uptake. Analysis of dose-response curves was performed using the logistic equation.

[^{3}H]Nitrendipine binding. Binding was processed as described in Ballesta *et al.* [11] using intact mitochondria and the assay buffer described above. Nonspecific binding was determined in the presence of 20 μM unlabelled nitrendipine. Dose-response curves were fitted as described above. K_i and B_{max} values were calculated according to Cheng and Prusoff [21] and DeBlasi *et al.* [22], respectively.

Data analysis. Data are expressed as the mean ± SEM. Dose-response curves for mitochondrial Ca^{2+} efflux and inhibition of [^{3}H]Nitrendipine binding were analyzed using the ANOVA test. The concentration selected was the lowest that was significantly different from controls, according to the two-tailed unpaired Student's t test. IC_{50} and K_i values were compared using the one-way ANOVA test. The order of potencies was further assessed by the Tukey's DVS test. p values of <0.05 were considered significant.

Results and Discussion

Calcium uptake. The hyperbolic dependence of the uniporter on extramitochondrial Ca^{2+} concentration (Fig. 1A) is similar to that observed in other tissues [23, 24]. Ca^{2+} uptake was non-cooperative, with a $K_m = 20.9 \pm 3.2$ μM and $V_{max} = 148.1 \pm 7.2$ nmol Ca^{2+} min^{-1} mg^{-1} protein (Fig. 1A, inset). As in other tissues, Ca^{2+} uptake in adrenal medulla mitochondria is performed through an energy-driven uniporter [1] whose calculated K_m for external Ca^{2+} corresponds favorably with previously reported values for mitochondria from other tissues [4]. However, the V_{max} was slightly lower than that reported in most tissues. This reduction in the calculated V_{max} could be due to the presence of chromaffin granules in our preparation [11] causing an overestimation of mitochondrial protein; nevertheless, the contribution of the Ca^{2+} uptake uniporter that has been reported in the membrane of chromaffin granules [25] was less than 1% of total Ca^{2+} uptake (data not shown).

The Ca^{2+} channel activator (Bay K 8644 and CGP 28392) or blocker dihydropyridines (nitrendipine, nimodipine, nifedipine, felodipine, PN 200–110 and amlodipine), verapamil, and diltiazem at 100 μM were unable to modify mitochondrial Ca^{2+} uptake relative to the control. This absence of effect has been observed previously in cardiac mitochondria [15, 26], although other groups [14, 27] have reported an inhibitory effect on cardiac mitochondria with IC_{50} of 150 μM. This discrepancy could be explained by the very high dihydropyridine concentrations used and the possibility of a nonspecific effect.

Calcium efflux. The Ca^{2+} efflux reached a V_{max} of 7.5 ± 0.4 nmol min^{-1} mg^{-1} protein, while the calculated K_m for Na^+ was 4.2 ± 0.5 mM (Fig. 1B). The Scatchard plot of the experimental data point showed an upward concavity (Fig. 1B, inset). The Hill coefficient was 1.4 ± 0.2. The contribution of Ca^{2+}/H^+ exchange at pH 7.2, in absence of Na^+, represented less than 15% of the total efflux in presence of 20 mM Na^+ (data not shown). In the mitochondria of excitable tissues, such as the heart and CNS, a Na^+/Ca^{2+} antiporter is the main release system, whereas in non-excitable tissues such as the liver and kidney, the Na^+/Ca^{2+} antiporter is only a minor component of Ca^{2+} release [19, 28], and the major Ca^{2+} efflux is via a Ca^{2+}/H^+ antiporter. Accordingly, our results demonstrate that in adrenal medullary mitochondria the Na^+/Ca^{2+} exchange is the main Ca^{2+} release system.

The calculated K_m for extramitochondrial Na^+ (4.2 ± 0.5 mM) was in good agreement with K_m of mitochondria from other tissues [29], whereas V_{max} (7.5 ± 0.4 nmol min^{-1} mg^{-1} protein) was slightly lower than that reported for most excitable tissues [29] although, as previously mentioned, the V_{max} value may have been underestimated because of the chromaffin granule protein in our preparation. The upward concavity of the Scatchard plot and the Hill coefficient (1.4) clearly indicates the likelihood that two Na^+ are exchanged for one Ca^{2+} by an electroneutral exchanger. Similar results have been reported in a number of tissues [19, 29, 30].

Table 1. Effect of different drugs on Na^+/Ca^{2+} exchange

Drug	IC_{50} (μM) (n)	% Maximal inhibition	K_i (μM) (n)
Nitrendipine	9.5 ± 1.1* (5)	44	2.9 ± 0.2*† (4)
(+)Isradipine	11.9 ± 2.8* (5)	37	14.0 ± 0.8* (4)
Felodipine	11.4 ± 2.5* (3)	63	18.5 ± 1.1* (3)
Nimodipine	34.9 ± 0.2* (3)	61	15.4 ± 2.8* (3)
CGP 28392	N.E. (75 μM)	–	N.E. (100 μM)
Bay-K-8644	N.E. (75 μM)	–	N.E. (100 μM)
Amlodipine	N.E. (200 μM)	–	N.E. (200 μM)
Diltiazem	10.9 ± 1.2* (4)	88	N.E. (100 μM)
Verapamil	N.E. (100 μM)	–	N.E. (1.5 mM)
DCCD	–	–	N.E. (100 μM)

Mitochondria (1 mg/ml) were incubated with different drugs for 15 min at 25°C. Intramitochondrial Ca^{2+} content was measured 10 min after adding 20 mM Na^+/0.1 mM EDTA (see Methods). For [^{3}H]nitrendipine binding, mitochondria (1 mg/ml) were incubated for 1 h at 25°C with 4 nM [^{3}H]nitrendipine in assay buffer containing a range of concentrations of different drugs (0.1–100 μM). Samples were processed and data fitted as described in Methods. Data were fitted to the logistic equation.

N.E. No effect at the highest drug concentration indicated in parentheses. Data correspond to means ± SEM of n experiments, where n represents the number of experiments performed in duplicate, from separate batches of mitochondria.

* $p < 0.01$ according to one-way ANOVA test (see Materials and Methods).

† Value for [^{3}H]nitrendipine represents Kd.

Modulation of calcium efflux by calcium channel activators and blockers. Mitochondrial Na^+/Ca^{2+} exchange from the adrenal medulla was inhibited by several dihydropyridines and the benzothiazepine diltiazem (Table 1), with the following order of potencies: nitrendipine = diltiazem = felodipine = (+)isradipine > nimodipine. In all cases Ca^{2+} efflux inhibition was incomplete. The benzothiazepine diltiazem was the most potent inhibitor of the Na^+/Ca^{2+} antiporter (approx. 90%); in contrast, neither activator dihydropyridine (Bay-K-8644, CGP 28392) nor amlodipine (an hydrosoluble dihydropyridine) modulated the Na^+/Ca^{2+} antiporter; verapamil was devoid of inhibitory effect up to 100 μM, although at higher concentrations (500 μM) it inhibited Ca^{2+} efflux slightly (approx. 25%). Similar results have previously been published for cardiac mitochondria [15, 26].

In the presence of 20 μM nitrendipine (Fig. 1B), the K_m for Na^+ (4.2 ± 0.5 mM) was virtually unaltered by nitrendipine (5.7 ± 0.6 mM). The Hill coefficient was also unchanged (1.5 ± 0.2 and 1.4 ± 0.2, in the presence and absence of the drug, respectively). In contrast, the V_{max} was dramatically reduced from 7.5 ± 0.4 nmol min^{-1} mg $protein^{-1}$ in the absence of nitrendipine to 2.9 ± 0.1 nmol min^{-1} mg $protein^{-1}$ in the presence of the drug. These results demonstrate that dihydropyridines modulate the mitochondrial Na^+/Ca^{2+} antiporter in a noncompetitive manner.

[^{3}H]Nitrendipine binding to intact mitochondria. The apparent Kd values for [^{3}H]nitrendipine binding were directly proportional to the protein concentration used in the assay (r^2 = 0.96) (Fig. 2), reaching a Kd value of 2.9 ± 0.2 μM at a protein concentration of 1 mg/ml, the concentration used in $^{45}Ca^{2+}$ efflux assays.

Nitrendipine was the most potent drug, inhibiting the binding of [^{3}H]nitrendipine to intact adrenal medulla mitochondria, followed by (+)isradipine, nimodipine, and felodipine with similar K_i values (Table 1). However, not all dihydropyridines used in this study were able to displace the specific binding of [^{3}H]nitrendipine to intact mitochondria. Interestingly, those DHP that had no effect on the Na^+/Ca^{2+} antiporter were unable to inhibit [^{3}H]nitrendipine binding to intact mitochondria, whereas DHP that inhibited mitochondrial Ca^{2+} efflux had IC_{50}s close to the determined K_i values (Table 1) for the inhibition of [^{3}H]nitrendipine binding. The fact that DHP drugs with no effect on Ca^{2+} efflux had no effect on [^{3}H]nitrendipine binding suggests that both effects run parallel. On the other hand, none of the benzothiazepines and phenylalkylamines assayed had any effect on [^{3}H]nitrendipine binding.

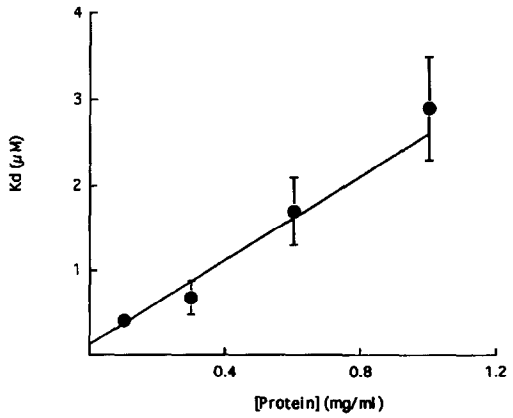

Fig. 2. Effect of protein concentration on the equilibrium dissociation constant (Kd). Mitochondria at different protein concentrations (0.05–0.5 mg/0.5 ml, final volume) were incubated in assay buffer with 4 nM [^{3}H]nitrendipine in the presence of unlabelled nitrendipine (25 nM–100 μM) for 1 h at 25°C and processed as described in Methods. Kd was calculated for each protein concentration as described in Methods. Data are given as the means ± SEM of four experiments performed in duplicate with different batches of mitochondria.

The lack of effect of verapamil on [^{3}H]nitrendipine binding was unexpected, as it is assumed that verapamil modulates the binding of [^{3}H]nitrendipine to mitochondrial membranes in an allosteric manner [11]. A possible explanation is that the allosteric effect of verapamil is not apparent in these experiments because in the present study the ionic strength and pH of the assay medium (20 mM NaCl and pH 7.2) differed from the previous report (500 mM NaCl and pH 6).

It has been suggested that in guinea pig liver mitochondria Ca^{2+} antagonist binding sites are associated with the inner mitochondrial membrane anion channel (IMAC) [17]. Intramitocondrial Ca^{2+} inhibits IMAC activity [31], and thus, the experimental conditions used exclude the possibility that the inhibition of the Na^+/Ca^{2+} antiporter we observed was a consequence of IMAC inhibition. Furthermore, DCCD was not able to inhibit [^{3}H]nitrendipine binding (Table 1). Thus, it seems logical to hypothesize that adrenomedullary mitochondrial dihydropyridine binding sites are associated with the Na^+/Ca^{2+} exchanger. Further experiments are required, either to reconstitute or to sequence and express the mitochondrial DHP binding site(s) in order to resolve this dilemma.

Acknowledgements—We would like to thank Dr. Frazer I. Smillie for his critical reading of the manuscript. The technical assistance of Miguel A. Company is gratefully acknowledged. This work was supported by Grants PB87-0093-C03-02 and PM91-0022-C02-02 from the Dirección General de Investigación Científica y Técnica (DGICYT).

REFERENCES

1. Lehninger AL, Carafoli E and Rossi CS, Energy linked ion movements in mitochondrial systems. *Adv Enzymol* **29:** 259–320, 1967.
2. Carafoli E, Tiozzo R, Lugli G, Crovetti F and Kratzing C, The release of calcium from heart mitochondria by sodium. *J Mol Cell Cardiol* **6:** 361–371, 1974.
3. Crompton M, Capano M and Carafoli E, The sodium-induced efflux of calcium from heart mitochondria. *Eur J Biochem* **69:** 453–462, 1976.
4. Richter C and Kass GEN, Oxidative stress in mitochondria: Its relationship to cellular Ca^{2+} homeostasis, cell death, proliferation, and differentiation. *Chem Biol Interactions* **77:** 1–23, 1991.
5. McCormack JG and Denton RM, Ca^{2+} as a second messenger within mitochondria. *Trends Biochem Sci* **11:** 258–262, 1986.
6. Duchen MR, Ca^{2+}-dependent changes in the mitochondrial energetics in single dissociated mouse sensory neurons. *Biochem J* **283:** 41–50, 1992.
7. Rizzuto R, Simpson AWM, Brini M and Pozzan T, Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. *Nature* **358:** 325–327, 1992.
8. McCormack JG, Characterization of the effects of Ca^{2+} on the intramitochondrial Ca^{2+} sensitive enzymes from rat liver and within rat liver mitochondria. *Biochem J* **231:** 581–595, 1985.
9. Hansford RG, Dehydrogenase activation by Ca^{2+} in cells and tissues. *J Bioenerg Biomembr* **23:** 823–854, 1991.
10. Ferry DR and Glossman H, Identification of putative calcium channels in skeletal muscle microsomes. *FEBS Lett* **148:** 331–337, 1982.
11. Ballesta JJ, Hidalgo MJ, Palmero M, Gutiérrez LM, Reig JA, Garcia AG and Viniegra S, Separate [^{3}H]nitrendipine binding sites in mitochondria and plasma membranes of bovine adrenal medulla. *Br J Pharmacol* **101:** 21–26, 1990.
12. Brush KL, Perez M, Hawkes MJ, Pratt DR and Hamilton SL, Low affinity binding sites for 1,4-dihydropyrididnes in mitochondria and in guinea pig ventricular membranes. *Biochem Pharmacol* **23:** 4153–4161, 1987.
13. Zernig G and Glossman H, A novel 1,4-dihydropyridine-binding site on mitochondrial membranes from guinea-pig heart, liver and kidney. *Biochem J* **253:** 49–58, 1988.

14. Buss WC, Savage DD, Stepanek J, Little SA and Mcguffee LJ, Effect of calcium channel antagonists on calcium uptake and release by isolated rat cardiac mitochondria. *Eur J Pharmacol* **152:** 247–253, 1988.
15. Vaghy PL, Johnson JD, Matlib MA, Wang T and Schwartz A, Selective inhibition of Na^+-induced Ca^{2+} release from heart mitochondria by diltiazem and Ca^{2+} antagonist drugs. *J Biol Chem* **257:** 6000–6002, 1982.
16. Baydoun AR, Markham RM, Morgan RM and Sweetman AJ, Bay K 8644, modifier of calcium transport and energy metabolism in rat heart mitochondria: A new intracellular site of action. *British J Pharmacol* **101:** 15–20, 1990.
17. Zernig G, Graziadei I, Moshammer T, Zech C, Reider N and Glossman H, Mitochondrial Ca^{2+} antagonist binding sites are associated with an inner mitochondrial membrane anion channel. *Mol Pharmacol* **38:** 362–369, 1990.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RL, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
19. Crompton M, Moser R, Lüdi H and Carafoli E, The interrelations between the transport of sodium and calcium in mitochondria of various mammalian tissues. *Eur J Biochem* **82:** 25–31, 1978.
20. Hefron JJA and Harris EJ, Stimulatium of calcium ion efflux from liver mitocondria by sodium ions and its response to ADP and energynstate. *Biochem J* **194:** 925–929, 1981.
21. Cheng YC and Prusoff WH, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* **22:** 3099–3108, 1973.
22. DeBlasi A, O'Reilly K and Motulsky HJ, Calculating receptor number from binding experiments using same compound as radioligand and competitor. *Tips* **10:** 227–229, 1989.
23. Crompton M, Sigel E, Salzmann M and Carafoli E, A kinetic study of the energy-linked influx of Ca^{2+} into heart mitochondria. *Eur J Biochem* **69:** 429–436, 1976.
24. Affolter H and Carafoli E, Hyperbolic kinetics of the electrophoretic carrier of Ca^{2+} uptake in liver mitochondria. *Eur J Biochem* **119:** 199–205, 1981.
25. Phillips JH, Transport of Ca^{2+} and Na^+ across the chromaffin granule membrane. *Biochem J* **200:** 99–107, 1981.
26. Matlib MA, Lee SW, Depover A and Schwartz A, A specific inhibitory action of certain benzothiazepines and benzodiazepines on the sodium-calcium exchange process of heart and brain mitochondria. *Eur J Pharmacol* **89:** 327–328, 1983.
27. Baydoun AR, Markham RM, Morgan RM and Sweetman AJ, Nitrendipine inhibits the uptake of calcium into rat heart mitochondria. *British J Pharmacol* **88:** 387P, 1986.
28. Crompton M, Künzi M and Carafoli E, The calcium induced and sodium induced effluxes from heart mitochondria. Evidence for a sodium-calcium carrier. *Eur J Biochem* **79:** 549–558, 1977.
29. Carafoli E, Intracellular calcium homeostasis. *Ann Rev Biochem* **56:** 395–433, 1987.
30. Haworth RA, Hunter DR and Berkoff HA, Na^+ releases Ca^{2+} from liver, kidney and lung mitochondria. *FEBS Lett* **110:** 216–218, 1980.
31. Beavis AD, Properties of the inner membrane anion channel in intact mitochondria. *J Bioenerg Biomem* **24:** 77–90, 1992.