

INHIBITORY ACTION OF CALCIUM ANTAGONISTS ON ATP-DEPENDENT CALCIUM UPTAKE BY THE RENAL CORTICAL MICROSOMES

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Abstract—Effects of Ca^{2+} antagonists, verapamil and diltiazem, on uptake and release of Ca^{2+} by microsomes of the renal cortex were studied. Verapamil inhibited the ATP-dependent Ca^{2+} uptake by renal microsomes. Addition of 0.8 mM verapamil was required to produce 50% inhibition of the ATP-dependent Ca^{2+} uptake. Diltiazem also depressed the Ca^{2+} uptake of the microsomes. The inhibitory effect of both drugs on the Ca^{2+} uptake was not due to an increased permeability for Ca^{2+} , since release of Ca^{2+} from the microsomes was not significantly affected by either drug. It is proposed that verapamil and diltiazem inhibit Ca^{2+} transport by interfering with an active process of Ca^{2+} accumulation in microsomes of the renal cortex.

Verapamil is currently described as an inhibitor of calcium (Ca^{2+}) translocation across the cell membranes in different tissues (1-5). Abe et al. (6) reported the effect of verapamil on renal functions and suggested that the drug inhibited Ca^{2+} reabsorption in renal tubules. Several workers using isolated subcellular organelles have found that verapamil also inhibits the active Ca^{2+} transport by the mitochondria (7, 8) and sarcoplasmic reticulum (S.R.) (9, 10). Thus, the possibility arises that verapamil could act as a more general inhibitor of Ca^{2+} translocation across biological membranes. ATP-dependent Ca^{2+} uptake by microsomes in various non-muscular tissues has attracted considerable attention, for example in the kidney (11), but there is apparently no documentation concerning the effect of verapamil on Ca^{2+} transport by isolated microsomes from non-muscular tissues. In recent work, we found that ATP-dependent Ca^{2+} uptake of the kidney microsomes is affected by diuretics, thereby suggesting the Ca^{2+} uptake of the microsomes is related to Ca^{2+} reabsorption in the kidney (12). In the present work, an attempt was made to study the effect of verapamil on the Ca^{2+} uptake of renal microsomes.

MATERIALS AND METHODS

Preparation of microsomes: Male Wistar rats, weighing 180-250 g, were used in all experiments. A microsomal fraction was prepared from the renal cortex using the method described previously (12). The microsomes were used immediately after preparation for assay of Ca^{2+} uptake and release.

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Assay of Ca²⁺ uptake: Ca²⁺ uptake by microsomes was measured using the membrane filtration-technique (12). The standard medium used in our studies consisted of 100 mM KCl, 30 mM imidazole-HCl buffer (pH 6.6), 5 mM NaN₃, 20 mM ammonium oxalate, 5 mM MgCl₂, 5 mM ATP, 50 μ M CaCl₂, 0.1 μ Ci/ml ⁴⁵CaCl₂, 25 mM sucrose and 0.2–0.3 mg protein/ml of microsomes as the final concentration in a total volume of 1 ml. The incubations were held at 37°C for 20 min. Aliquots of the assay were passed through 0.3 μ m membrane filters and ⁴⁵Ca²⁺ in microsomes on the filters was determined by liquid scintillation spectrometry. ATP-dependent Ca²⁺ uptake was calculated by subtracting Ca²⁺ binding of microsomes in the absence of ATP in the medium from the total Ca²⁺ uptake in its presence.

Assay of Ca²⁺ release: The procedure employed in the Ca²⁺ release assay was similar to that previously reported (12). The assay of Ca²⁺ release was carried out after loading Ca²⁺ in microsomes for 20 min at 37°C in 1 ml of the standard medium described above. Ca²⁺ release was initiated by the addition of 0.2 ml of 100 mM KCl containing 12 mM glycoethyrdiamine tetraacetic acid (EGTA) and terminated by membrane-filtration 20 min later. The radioactive calcium in microsomes on the filters was determined by liquid scintillation spectrometry. The amount of Ca²⁺ release was calculated by subtracting Ca²⁺ content in the microsomes after incubation with EGTA from that before addition of EGTA.

Protein determination: Protein was determined by the method of Lowry et al. (13) using bovine serum albumin as a standard.

Drugs used: Verapamil was kindly provided by Eisai Co., Ltd. Calcium ionophore A23187 (Lilly Research Corporation, Indianapolis) was diluted in 50% ethylalcohol. The final concentration of ethylalcohol was 2.5% in the assay medium.

Statistical analysis: All data are expressed as a mean value \pm the standard error of the mean (S.E.M.) of four experiments. The differences between mean values were analysed by pair *t*-test and P values are given.

RESULTS

As shown in Fig. 1, verapamil inhibited the ATP-dependent Ca²⁺ uptake of microsomes of the renal cortex. Verapamil (2 mM) abolished most of the Ca²⁺ pump activity of the microsomes and an approximate 50% inhibition occurred with 0.8 mM. Diltiazem, another Ca²⁺ blocker with pharmacologically verapamil-like action, also reduced Ca²⁺ uptake of the microsomes (Table 1).

It has been demonstrated that Ca²⁺ ionophore A23187 makes biological membranes permeable to Ca²⁺ (14). The inhibitory effect of A23187 on the ATP-dependent Ca²⁺ uptake is, therefore, probably due to the functional integrity of A23187 which makes the membranes of kidney microsomal vesicles permeable to Ca²⁺ (12). The possibility that the Ca²⁺ blockers, verapamil and diltiazem, modify Ca²⁺ permeability of the microsomes was then examined (Table 2). After Ca²⁺ was loaded in the standard medium for 20 min at 37°C, Ca²⁺ release from the microsomes was initiated by the addition of EGTA to the medium. Verapamil and diltiazem did not significantly influence the Ca²⁺ release at concentrations which prominently inhibited ATP-dependent Ca²⁺ uptake of the microsomes.

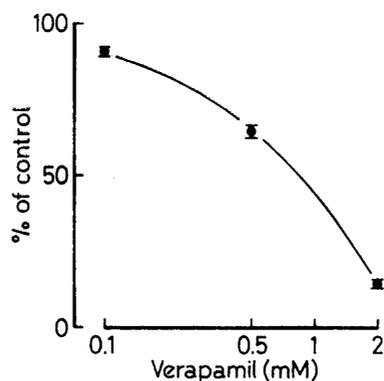


FIG. 1. Dose-response curve to verapamil in ATP-dependent Ca²⁺ uptake by microsomes of the renal cortex. Assay was carried out in the standard medium containing various concentrations of verapamil. Each point represents the mean value of four experiments. Vertical lines indicate the S.E.M.

TABLE 1. Effects of diltiazem on Ca²⁺ uptake by microsomes of the renal cortex

		ATP-dependent Ca ²⁺ uptake (nmoles Ca ²⁺ /mg protein/20 min)	% inhibition
Control		55.9 ± 9.8	
Diltiazem	0.5 mM	43.5 ± 8.0*	22.3

Each value is the mean ± S.E.M. of four experiments. Significantly different from control, *p < 0.01.

TABLE 2. Effects of verapamil and diltiazem on Ca²⁺ release from microsomes of renal cortex

	Ca ²⁺ contents in microsomes (nmoles Ca ²⁺ /mg protein)		Ca ²⁺ release from microsomes (A)-(B)
	Before incubation (A)	After incubation (B)	
Control	58.3 ± 9.8	48.6 ± 8.1	9.7 ± 2.1
Verapamil			
	0.5 mM	46.9 ± 8.7*	11.4 ± 2.2*
	2.0 mM	44.1 ± 7.3*	14.2 ± 3.7*
Diltiazem	0.5 mM	47.7 ± 7.8*	10.5 ± 3.3*

Calcium content in microsomes (A) was measured after loading Ca²⁺ for 20 min at 37°C in the standard medium for Ca²⁺ uptake assay. Calcium release was initiated by the addition of 2 mM EGTA (final concentration) with the substance indicated. The incubation was terminated 20 min later and the remaining Ca²⁺ content in microsomes (B) was determined. *Not significant from control.

DISCUSSION

In the present study, the possibility that ATP-dependent Ca^{2+} uptake of the microsomes is partly due to contamination of the mitochondria can be ruled out, since the experiments reported here were performed in the presence of sodium azide at a concentration which has been shown to produce complete inhibition of mitochondrial Ca^{2+} uptake (11). In addition, 2,4-DNP (15), an uncoupler of oxidative phosphorylation, had no significant effect on ATP-dependent Ca^{2+} uptake of the microsomes in the presence of sodium azide (data not shown).

The present results clearly indicate that verapamil and diltiazem produce a decrease in ATP-dependent Ca^{2+} uptake of the microsomes of the kidney cortex *in vitro*. This inhibitory effect is probably due to action on the active process of Ca^{2+} accumulation in the microsomes, since release of Ca^{2+} that had previously been taken up by the microsomes was not influenced by either drug. A concentration of 0.8 mM verapamil was sufficient to cause a 50% inhibition of Ca^{2+} uptake. This concentration of verapamil is approximately the same as the concentration reported to depress the active Ca^{2+} transport in rat everted intestinal sacs (0.5 mM) (4), mitochondria of rat cardiac muscle (1 mM) (7), mitochondria of rat renal cortex (0.2 mM) (8), S.R. of rabbit skeletal muscle (1 mM) (9) and S.R. of dog cardiac muscle (1 mM) (10). The effect of verapamil on Ca^{2+} uptake of the microsomes of the kidney may be analogous to that on the active Ca^{2+} transport in other tissues and subcellular organelles.

In our previous study (12), we found that marsalyl and high-ceiling diuretics, which increase urinary Ca^{2+} excretion, inhibit ATP-dependent Ca^{2+} uptake of the microsomes of the kidney cortex, but hydrochlorothiazide which decreases Ca^{2+} excretion, accelerates this uptake, suggesting a possible causal relationship between the effect of diuretics on Ca^{2+} reabsorption by the renal tubules and their influence on Ca^{2+} uptake of the microsomes. This relationship is further supported by the inhibitory effects of verapamil on Ca^{2+} reabsorption by the tubules and Ca^{2+} uptake of the microsomes.

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