—SHORT COMMUNICATIONS

DIVALENT CATION TRANSPORT IN KIDNEY SLICES
III. INHIBITORY ACTION OF VERAPAMIL ON
MAGNESIUM GAIN

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It has been reported that verapamil, a substance which appears to block Ca^{2+} translocation across the plasma membrane (1–4), significantly increases urine flow and urinary excretion of sodium, potassium and calcium with no changes in renal blood flow and plasma electrolyte concentration (5). Our previous work indicated that verapamil inhibited not only Ca^{2+} accumulation but also Mg^{2+} accumulation by kidney cortical mitochondria (6). We suggested previously (7) that Mg^{2+} influx in kidney cortical slices is mediated by an energy-dependent process which is dissociated from Ca^{2+} flux. As little information is available concerning the effect of verapamil on Mg^{2+} transport through a plasma membrane, we studied the effect of verapamil on Mg^{2+} content of kidney cortical slices *in vitro*.

Male Wistar rats, weighing 180–250 g, were used in all experiments. Tissue slices were prepared from the kidney cortex as described previously (8). The slices were leached at 0°C for 120 min in 150 mM NaCl containing 0.1 mM EDTA (disodium ethylene-diamine-tetraacetate). EDTA (0.1 mM) neutralized with NaOH (0.067 mM). After leaching, about 200 mg of slices were incubated for 30 min at 37°C in 20 ml of Krebs-Ringer bicarbonate buffer containing 118 mM NaCl, 24.9 mM NaHCO_3 , 1.18 mM KH_2PO_4 , 4.74 mM KCl, 1.18 mM MgSO_4 , 2.54 mM CaCl_2 and 5.56 mM glucose. The incubation medium was aerated with 95% O_2 + 5% CO_2 . Mg^{2+} and Ca^{2+} content of slices were estimated using an atomic absorption spectrophotometer (Hitachi 170–10) as described in previous papers (7, 8). Verapamil and diltiazem were kindly provided by Eisai Co., Ltd. and Dr. T. Morita of Kanebo Co., Ltd., respectively.

Fresh kidney cortex used for experiments contained 7.22 ± 0.11 $\mu\text{moles Mg/g}$ wet wt. and 2.11 ± 0.06 $\mu\text{moles Ca/g}$ wet wt. When the slices were kept for 120 min at 0°C in a solution containing 150 mM NaCl and 0.1 mM EDTA, Mg^{2+} and Ca^{2+} losses from the slices were observed, as indicated in Table 1: these being values before incubation. Aerobic incubation in Krebs-Ringer bicarbonate buffer induced an increase of Mg^{2+} and Ca^{2+} in the slices. Verapamil inhibited not only increase in the contents of Ca^{2+} but also that of Mg^{2+} . Incubation with 1 mM verapamil completely inhibited the increase of Mg^{2+} content during

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TABLE 1. Effect of verapamil and diltiazem on Mg^{2+} and Ca^{2+} content of kidney cortical slices

Conditions	Tissue divalent cations (μ moles/g wet wt.)	
	Mg^{2+}	Ca^{2+}
Before incubation	3.93 ± 0.11 (24)	1.11 ± 0.04 (23)
After incubation		
Control	5.53 ± 0.13 (26)	3.53 ± 0.07 (23)
Verapamil 1×10^{-4} M	4.60 ± 0.06^c (6)	3.49 ± 0.09 (4)
5×10^{-4} M	4.06 ± 0.03^c (6)	2.99 ± 0.04^c (4)
1×10^{-3} M	3.82 ± 0.09^c (6)	3.07 ± 0.04^a (6)
Diltiazem 1×10^{-4} M	5.05 ± 0.11^b (12)	3.36 ± 0.10 (9)
1×10^{-3} M	5.06 ± 0.14^b (16)	3.14 ± 0.07^c (13)

The slices were leached for 120 min at 0°C in a solution containing 150 mM NaCl and 0.1 mM EDTA before incubation. Leached slices were incubated aerobically for 30 min at 37°C in a Krebs-Ringer bicarbonate buffer. Indicated concentrations of verapamil and diltiazem were added to the incubation medium. Mean values $\pm$ S.E. are given for the numbers of experiments shown in parentheses. a; $p < 0.05$ vs. control, b; $p < 0.025$, c; $p < 0.005$.

the aerobic incubation. Inhibitory effects of verapamil were more potent in the case of Mg^{2+} . While, diltiazem, another Ca^{2+} -blocker, also inhibited the increases in levels of Mg^{2+} , the ability to block increases in Mg^{2+} was considerably less than that of verapamil.

The present findings showed that Mg^{2+} gain was significantly inhibited by 0.1 mM verapamil, which had no effect on Ca^{2+} increase in kidney cortical slices. It is noteworthy that verapamil, which has been used extensively as a Ca^{2+} antagonist in studies of the role of Ca^{2+} in biological events, exerted a significant inhibitory effect on the maintenance of the Mg^{2+} content in rat kidney cortical slices. Our results suggest that verapamil has a direct effect on the flux of Mg^{2+} in the cortical slices. The magnitude of this effect in other tissues remains to be investigated.

We found that increases in Mg^{2+} in kidney cortical slices are mediated by an energy-dependent process (7) and that ATP-dependent accumulation of Mg^{2+} by mitochondria isolated from kidney cortex is suppressed by verapamil (6). From the present results it is difficult to compare the effect of verapamil on Mg^{2+} accumulation by mitochondria and that of this drug on Mg^{2+} increase in the slices. However, the finding that verapamil inhibited both energy-dependent processes of Mg^{2+} transports in slices and mitochondria should be given consideration. The metabolism of cells is affected by Mg^{2+} (9–11). Therefore, the effect of verapamil on biological phenomena may be due to an inhibition of Mg^{2+} translocation, in addition to the inhibition of Ca^{2+} entry into the cells.

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A RECEPTOR RESERVE OF THE ALPHA-INHIBITORY RECEPTOR FOR ALPHA-ADRENOCEPTOR STIMULANTS IN THE TAENIA CAECUM OF GUINEA PIG

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Adrenoceptors were classified as α - and β -receptors by Ahlquist (1). The α -receptor is generally associated with the excitatory functions and the β -receptor with the inhibitory functions in various smooth muscles. Stimulation of the α - and β -receptors in intestinal smooth muscles produces a relaxation (2). Our knowledge of the receptor reserve of α -receptor for the α -adrenoceptor stimulants has been, however, obtained from data in the excitatory responses (3, 4). Little is known of the receptor reserve for the α -agonists in inhibitory mechanisms.

Male guinea pigs, weighing 300 to 400 g, were sacrificed by a blow on the neck and a piece (3 to 4 cm) of taenia was removed from the caecum and suspended in a 20 ml organ bath filled with Locke Ringer's solution (NaCl 154, KCl 5.6, CaCl₂ 2.2, MgCl₂ 2.1, NaHCO₃ 5.9 and glucose 2.8 mM) kept at 37°C and bubbled with air. The physiological solution also contained propranolol hydrochloride (3×10^{-6} M) which blocks the β -adrenoceptor action of α -adrenergic stimulants. Norepinephrine hydrochloride, epinephrine bitartrate and phenylephrine hydrochloride were used as α -adrenoceptor stimulants and dibenamine