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Stimulation of renal gluconeogenesis by verapamil and D-600*

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At least three "on-off" mechanisms are present in renal cortex that regulate the rate of glucose formation. Experiments *in vitro* with slices, tubules, and isolated perfused kidneys reveal that gluconeogenesis is stimulated by: (1) an increase in extracellular hydrogen ion concentration [1–6], (2) the presence of specific hormones, i.e. parathormone [7–10], catecholamines [11–13] and glucagon [11], and (3) calcium ion [1–3, 14, 15]. The mode of stimulation and the interaction of each of these mechanisms remain controversial [16], although there is substantial evidence to implicate cAMP in the endocrine and calcium-mediated effects. Beyond its role in renal cortex, Ca^{2+} has also been shown to mediate important metabolic and physiologic functions in heart [17], myometrium [18, 19], and in certain secretory tissues [20–23]. Ca^{2+}-associated activities in these tissues are inhibited by verapamil and D-600 [24–26]; presumably these agents block Ca^{2+} channels in the plasma membrane. In contrast to this seemingly generalized phenomenon, gluconeogenesis in renal tubules, a Ca^{2+}-stimulated function, is not blocked by verapamil and D-600. In fact, the gluconeogenic rate is accelerated in the presence of verapamil and D-600 and this response is independent of added Ca^{2+}.

Male, adult Sprague–Dawley rats (250–350 g) that had been fasted for 24 hr were used in all experiments. After the rats were stunned by cervical fracture, the kidneys were rapidly removed and placed in ice-cold saline or phosphate buffer. The detailed procedure for isolating tubules and the ultra-structural and biochemical characteristics of the preparation have been described [27]. Slices of cortex were made with the Stadie–Riggs microtome and then incubated in a buffered medium with collagenase, albumin and Ca^{2+}. After approximately 45 min, the suspension was filtered through three layers of gauze and the tubules were sedimented by centrifugation at 50 *g* for 2 min. The supernatant solution containing red blood cells and kidney cell debris was discarded and the tubules were resuspended in the Ca^{2+}-containing medium. After centrifugation and removal of the supernatant solution, the tubules were then suspended in a Ca^{2+}-free medium, spun down, washed once again in a Ca^{2+}-free medium, and then suspended in 2 vol. of Ca^{2+}-free medium.

All incubations were carried out in 25-ml Erlenmeyer flasks containing 2.5 ml of tubular suspension (approximately 12 mg protein) in a phosphate buffer (pH 7.4) at 37°. The incubation was started by addition of tubules to the medium which had been equilibrated at 37° under 100% O_2 for 5 min. Ethanolic solutions of verapamil and D-600 (kindly provided by Knoll Pharmaceutical Co.) were added in 10-μl volumes; 10 μl ethanol was added to control vessels. Substrates were added as neutral solutions so that the final concentration was 5×10^{-3} M. The incubation was terminated by immersion of the suspension in a boiling water bath for 30 sec. In those experiments where tubular calcium content was measured, the heating step was omitted and the tubules were rapidly separated from the incubation medium by centrifugation and then washed twice in Ca^{2+}-free medium before preparing a trichloroacetic acid filtrate of the tubules.

The glucose content of the supernatant solution prepared from the heat-treated tubular suspension was determined by the glucose oxidase method (Glucostat-Worthington Biochemical). Net glucose production by the tubules was taken as the difference in glucose content of the incubated suspensions in the presence of substrate and in the absence of added substrate. Oxygen consumption was measured at 37° in a closed system of 3 ml volume with the Clark electrode and continuously monitored on a Varian G-1000 recorder. A Techtron-AA5 atomic absorption spectrometer was used for determinations of the calcium content of the trichloroacetic acid extract of tubules.

In vitro, gluconeogenesis by rat renal cortex is limited by substrate availability [27–29]. In isolated tubules incubated in phosphate buffer, succinate and α-ketoglutarate appeared to be among the most effective precursors for new glucose formation [28, 29]. Exposure of tubules to 10^{-4} M verapamil or D-600 in short-term incubations (15 min) resulted in an increased rate of gluconeogenesis when either 5×10^{-3} M succinate or α-ketoglutarate was present as substrate (Table 1). Lowering the concentration of verapamil and D-600 to 10^{-5} M resulted in a lesser, but still significant stimulation of gluconeogenesis, while 10^{-6} M was without effect. Neither of these agents influenced the respiratory rate of the tubule preparation (control 30.9 nmoles/mg of protein/min, 10^{-4} M verapamil 30.7, 10^{-4} M D-600 31.0) in the presence or absence of added Ca^{2+} to the incubation.

Table 1. Effect of verapamil and D-600 on gluconeogenesis*

	Substrate	
	Succinate	α-Ketoglutarate
Control	24.2 ± 2.8	29.8 ± 5.6
Verapamil (10^{-4} M)	45.0 ± 8.3†	43.8 ± 14.5
D-600 (10^{-4} M)	32.6 ± 2.7†	44.6 ± 4.3†

Ca^{2+} (2.5 mM) was present. The values represent the mean ± S.E. for net glucose production (nmoles/mg of protein/15 min).

† These levels are statistically different ($P < 0.05$) from the corresponding controls.

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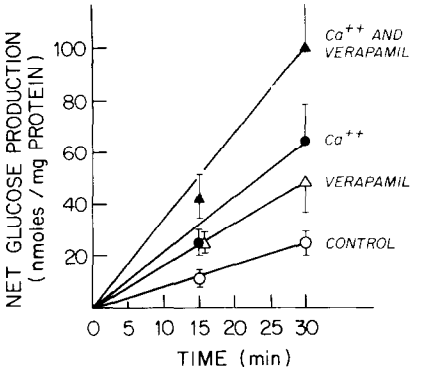

Fig. 1. Effect of verapamil (10^{-4} M) in the absence and presence of 2.5 mM Ca^{2+} on gluconeogenesis from succinate (5 mM). Each value represents the mean ± S.E. of at least four experiments; tubules were prepared from the kidney cortex of three rats for each experiment.

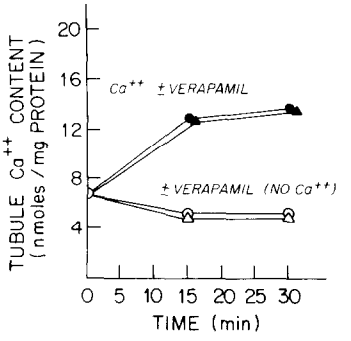

Fig. 2. Total calcium content after incubation of tubules in a Ca^{2+}-free and Ca^{2+}-containing medium in the presence and absence of 10^{-4} M verapamil. Each value represents the mean of at least four experiments and the S.E. does not exceed the limits of the mean symbol.

Addition of Ca^{2+} at a concentration of 2.5 mM increased the rate of gluconeogenesis (Fig. 1) and elevated the calcium content of the tubules (Fig. 2). After 30 min of incubation, there was a 3-fold increase in net glucose production due to added Ca^{2+}. Tubule calcium content increased from an initial value of 6.8 ± 1.2 to 12.8 ± 2.0 nmoles/mg of protein after 15 min and did not increase further after an additional 15 min of incubation. Tubules incubated in the absence of Ca^{2+} actually lost calcium to the medium. Verapamil, an agent that blocks Ca^{2+} channels in some tissues, exhibited no effect on net renal cortical calcium content (Fig. 2), but markedly stimulated the rate of new glucose formation regardless of the presence of Ca^{2+} in the external bathing medium.

Since Ca^{2+} is known to importantly regulate gluconeogenesis in renal cortex [1–3, 14, 15], it would be anticipated that agents which have been shown to block Ca^{2+} channels in the plasma membrane such as verapamil and D-600 would also block gluconeogenesis. Clearly the response of the renal cortex to these agents departs from the observations with all other tissues thus far studied. The present experiments suggest that the Ca^{2+} uptake mechanism in renal cortex did not recognize verapamil as a blocking agent. Verapamil did not affect the net uptake of calcium when tubules were incubated in the presence of Ca^{2+} and did not influence the net loss of calcium when tubules were incubated in a Ca^{2+}-free medium. These experiments do not, however, rule out the possibility of an intracellular redistribution of Ca^{2+} by verapamil.

In accord with the observed absence of an effect on net calcium transport in tubules, verapamil and D-600 did not inhibit gluconeogenesis. Rather, these agents effected a significant increase in the rate of gluconeogenesis. This stimulatory effect was observed when Ca^{2+} was deleted from the incubation medium, but was also apparent in the presence of Ca^{2+}. It appears that the verapamil-enhanced gluconeogenic rate was additive to the Ca^{2+}-stimulated rate, supporting the concept that the verapamil-stimulating mechanism could act independently of the Ca^{2+} mechanism.

An interesting relationship between H^+, HCO_3^-, pCO_2 and Ca^{2+} on renal cortical gluconeogenesis has been developing. H^+ and Ca^{2+} apparently stimulate gluconeogenesis by different mechanisms [2]. Alleyne *et al.* [1] have provided evidence to suggest that the concentration of HCO_3^- rather than the pH of the medium is responsible for the alteration of renal gluconeogenesis induced by acid-base changes in experiments *in vitro*. These authors further demonstrated that the bicarbonate effect is masked at an optimal Ca^{2+} concentration. In our experiments, a phosphate rather than a bicarbonate buffer was used. Since Ca^{2+} was present at an optimal concentration (2.5 mM), it is unlikely that the Ca^{2+}-stimulating effect on gluconeogenesis would have been different in a bicarbonate buffer. It is possible, however, that the verapamil and D-600 effect on gluconeogenesis might have differed in a bicarbonate buffer. Conceivably, these agents could stimulate gluconeogenesis by altering intracellular hydrogen ion or bicarbonate ion concentration. Alternatively, these agents might exert their effects by increasing the concentration of cyclic AMP, by influencing monovalent ion channels [30], or by some other independent mechanism.

These experiments with verapamil and D-600 are also revealing because they provide supporting data for the concept that the rate of gluconeogenesis is not an important determinant of the rate of mitochondrial energy generation. Guder *et al.* [29] initially demonstrated that cyclic AMP increased gluconeogenesis without affecting the respiration of renal cortical tubules. More recently, we have observed that gluconeogenesis may be blocked with quinolinic acid and hydrazine, without affecting respiration [27], and now we find that verapamil and D-600 stimulate gluconeogenesis, but do not affect respiration.

In summary, the rate of gluconeogenesis in renal cortex is stimulated by Ca^{2+}; this phenomenon is associated with Ca^{2+} uptake by the tissue. Verapamil and D-600, agents that block Ca^{2+} influx in myocardium, myometrium and pancreatic β-cells, are without effect on net Ca^{2+} content in isolated renal cortical tubules incubated in the presence or absence of calcium. However, both verapamil and D-600 stimulate gluconeogenesis by the tubules independent of the presence of Ca^{2+} in the incubation medium. The data suggest that the gluconeogenic sequence is activated by verapamil and D-600 despite the lack of recognition of these agents by cell membrane Ca^{2+} transport sites.

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