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Nitrendipine-Induced Stimulation of Renin Release by the Isolated Perfused Rat Kidney (42154)

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Abstract. The direct effects of the organic calcium antagonist nitrendipine upon renin release were assessed using the isolated rat kidney perfused at constant pressure. This model circumvents the indirect actions of vasodilating agents by artificially maintaining perfusion pressure constant, thereby avoiding the hypotensive effects associated with the systemic administration of such agents. Renin release as assessed by radioimmunoassay was stimulated 2.6-fold upon the administration of 10^{-6} *M* nitrendipine. Since this stimulation of renin release occurred in the absence of any alteration in perfusion pressure, we conclude that it represents a direct action of nitrendipine. This finding is in support of the current hypothesis concerning the inverse relationship between cytosolic Ca^{2+} and renin secretory rate, and suggests that Ca entry into the juxtaglomerular cells of the juxtaglomerular apparatus is sensitive to blockade by organic calcium antagonists such as nitrendipine. © 1985 Society for Experimental Biology and Medicine.

Theoretical considerations indicate that agents which lower cytosolic Ca^{2+} may exert a stimulatory effect upon renin secretion (1–3). Preliminary observations suggest that the administration of the dihydropyridine nitrendipine is associated with a stimulation of plasma renin activity in hypertensive patients (4, 5). Since nitrendipine prevents Ca^{2+} entry in vascular smooth muscle (6, 7), the observed increase in plasma renin activity may be a direct consequence of Ca^{2+} entry blockade at the level of the renin-secreting juxtaglomerular (JG) cells. Unfortunately, the interpretation of these findings is confounded by the extrarenal effects of nitrendipine. Since nitrendipine exerts a hypotensive effect upon systemic blood pressure (4, 5, 8), the stimulation of renin release under these conditions may simply reflect an indirect action which is secondary to stimulation of the renin–baroreceptor reflex (9).

To determine the ability of nitrendipine to directly alter renin secretion, we examined the effects of this agent upon renin release under conditions in which extrarenal effects were eliminated. The isolated perfused rat kidney represents a unique model for the study of renin secretion. This preparation retains the capacity to respond to direct-acting secretagogues and inhibitory agents (3, 10), while allowing renal perfusion pressure to be controlled independent of alterations in renal vascular resistance.

Methods. *Isolated perfused kidney.* Extracorporeal perfusion of the right kidney was performed in seven rats utilizing a modification of procedure of Nishiisutsuji-Uwo *et al.* (11), which has been previously described in detail (10). In summary, the right kidney was cannulated and initially perfused *in situ* by introducing the cannula through the superior mesenteric artery. The perfused kidney was excised and placed in a recirculating perfusion apparatus, designed to provide warmed (37°C), oxygenated media at a constant pressure. Care was taken to ensure that perfusion pressure, measured at the level of the renal artery, was maintained at 100 mm Hg throughout all experimental manipulations (12).

The perfusing media consisted of a Krebs–Ringers bicarbonate buffer, containing 5 m*M* D-glucose, 10 m*M* sodium acetate, 8 g/dl bovine serum albumin (fraction V, Reheiss Chemical Co.), and a complement of amino acids as previously described (10). The initial volume of media in the system was 150 ml. The media was equilibrated with 95% O_2/5% CO_2 and the pH was maintained at 7.4.

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Nitrendipine was added following 60 min of perfusion. All experiments were conducted in the presence of sodium illumination to avoid photo-induced degradation of the dihydropyridine.

Renin assay. Duplicate 1.5 ml media samples were collected in chilled syringes and placed immediately into chilled tubes containing 3 mg K_2 EDTA (Becton–Dickinson, Rutherford, N.J.) and kept frozen at −70°C until assayed. The renin activity of the media was analyzed by determining the amount of angiotensin I (AI) generated following the addition of 0.5 μg/ml of hog renin substrate, as described in a previous communication (10). The angiotensin I generated was determined by radioimmunoassay using antiangiotensin I-coated tubes (Gamma Coat PRA Determination Kit, Clinical Assays, Cambridge, Mass.).

The generation of angiotensin I was conducted under the following conditions: 0.5 ml hog renin substrate (1 μg/ml) was added to 0.5 ml of media; 0.1 ml of phosphate buffer was added to adjust the pH to 6.0 and 0.01 ml of phenylmethylsufanylfluoride was added to inhibit the conversion of AI to AII; the samples were divided into pairs and one incubated for 5 hr at 37°C while the paired sample was maintained at 0–4°C over the same time period; subsequently, a routine radioimmunoassay for AI was performed. For the purposes of radioimmunoassay, a 0.025-ml aliquot of sample was mixed with a phosphate buffer and radioactive AI tracer mixture and incubated at room temperature for 3 hr in antibody-coated tubes. The renin activity obtained was expressed as nanogram AI generated/milliliter media/hour.

To circumvent errors related to alteration in the volume of the perfusate caused by sequential sampling, the renin data were converted to cumulative renin content of media as described in detail in a previous communication (10). In brief, the renin content refers to the product of the renin activity times the volume of the system at the time of sampling summated with the amount of renin removed by previous sampling. The data are expressed as cumulative renin content of the media (factored for kidney weight) and the renin release rate which is the change of renin content per unit time.

The data are presented as the mean followed by the standard error as an index of dispersion. The renin release data were analyzed utilizing a paired t test, the rate of renin release immediately prior to nitrendipine administration (i.e., 50–60 min) serving as the basal reference point. Probabilities less than 0.05 were considered to be statistically significant.

Results. The effects of nitrendipine on cumulative renin content of the media and the rate of renin release by the isolated rat kidneys are depicted in Fig. 1. During the initial 40 min of perfusion, renin content increased in a curvilinear fashion as previously documented (10). Renin release rate also increased during this initial, unstable period. After 40 min, however, the rate of renin release achieved a relatively constant value. Thus, at 40 min the rate (16 ± 6.3 ng AI/h/g) did not differ from that determined

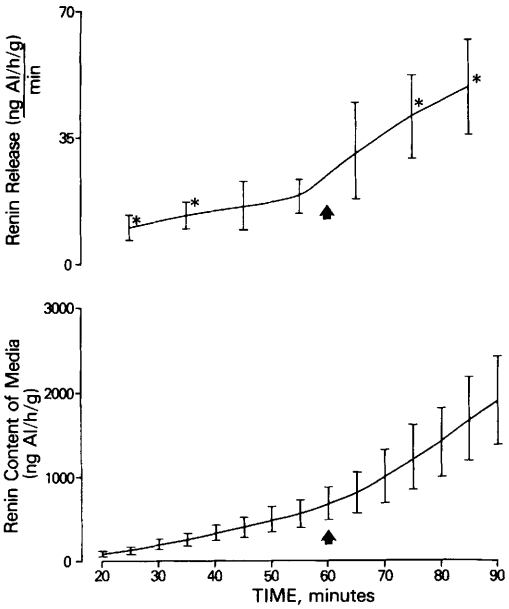

FIG. 1. Effects of administration of nitrendipine (10^{-6} M) upon cumulative renin content of media (lower panel) and renin release rate (upper panel) of seven isolated perfused rat kidneys. Renin release rate became constant after 40 min of perfusion. The addition of nitrendipine (at arrow, 60 min) resulted in a stimulation of renin release to a value 2.6 times the basal rate. Asterisks represent values which were significantly different from the renin release rate immediately prior to nitrendipine administration ($P < 0.05$).

at 60 min (18.5 ± 4.6 ng AI/h/g, $P > 0.5$). This observation is in accord with previous data from our laboratory in control (i.e., untreated) kidneys, demonstrating that the renin release rate attains a constant value after 40 to 50 min of extracorporeal perfusion (10).

Following the addition of 10^{-6} *M* nitrendipine at 60 min, renin content increased markedly. The renin release rate increased to 30.8 ± 13.2, 40.4 ± 11.4, and 48.2 ± 13.0 ng AI/h/g/min during the three subsequent 10-min periods, respectively. The last value represents a 2.6-fold increase ($P < 0.025$). The stimulation of renin in response to the administration of nitrendipine contrasts with the constant renin release rate previously demonstrated in unmanipulated kidneys (10).

Discussion. Increasing experimental evidence suggests an inhibitory role of cytosolic Ca^{2+} in the control of renin secretion. Reducing extracellular Ca^{2+} stimulates renin secretion in both *in vitro* systems (3, 13, 14) and in intact preparations (15). On the other hand, increasing the transcellular Ca^{2+} gradient by elevating extracellular Ca^{2+} generally leads to an inhibition of the renin secretory process (13). Similarly, increasing cellular Ca^{2+} permeability either by the application of Ca^{2+} ionophores (3, 13), or by membrane depolarization (1, 16) is also associated with an inhibition of renin secretion. These observations suggest that, in contrast to many other secretory systems, renin release is stimulated by a reduction rather than increase in cytosolic Ca^{2+}.

Since the juxtaglomerular cells that secrete renin appear to be modified smooth muscle cells and since calcium antagonists lower cytosolic Ca^{2+} in smooth muscle, it might be anticipated that calcium antagonists stimulate renin release by such a mechanism. Churchill *et al.* have demonstrated that KCl-induced inhibition of renin release by renal cortical slices is prevented by D-600, a verapamil derivative (16), and diltiazem (17). In this preparation, however, these calcium antagonists do not alter basal renin release (16, 17). Verapamil (15, 18) and nifedipine (18) have also been reported to stimulate renin release when infused into the renal arteries of anesthetized dogs. Dietz *et al.* (18) reported, however, that with either of these agents renin release was stimulated only when systemic hypotension occurred. On the other hand, Roy *et al.* (19) reported that verapamil infusion reduced renin release by increasing GFR in the anesthetized dog, but caused a stimulation of renin secretion when administered to preparations with nonfiltering kidneys. These authors concluded that verapamil directly stimulates renin release in this model and that this action may be masked by other secondary effects.

An additional factor that confounds the interpretation of studies utilizing verapamil is that this agent not only acts as a Ca antagonist but also binds to adrenoceptors (20) and other types of receptors (21) when used in micromolar concentrations. Nitrendipine is a dihydropyridine which exhibits selective blockade of smooth muscle Ca^{2+} fluxes (6, 7). Unlike verapamil, nitrendipine does not appear to block the actions of agonists at the receptor level (20).

Although nitrendipine has previously been reported to increase renin activity in patients being treated for hypertension (4, 5), it is unclear if the increase in renin activity in these settings is attributable to a direct action of the drug or secondary to the lowering of blood pressure by this agent. In the present study, we observed that nitrendipine administration caused a stimulation of renin release by the isolated rat kidney. Since we utilized an experimental design that obviated alterations in perfusion pressure, the actions of nitrendipine in this setting reflect a direct effect and cannot be ascribed to a stimulation of renal baroreceptors. These findings are, thus, in accord with the formulation suggesting an inhibitory role of cytosolic Ca^{2+} in the control of renin secretion, and could indicate that the renin secreting JG cells are potential targets for Ca^{2+} channel modulators. Alternatively, this stimulation of renin secretion by nitrendipine may be mediated by other renal actions of this agent. For example, we have recently reported that nitrendipine is natriuretic in this model (22). It is therefore possible that nitrendipine may alter renin secretion through an effect upon sodium transport that is transmitted via the macula densa.

In conclusion, the present study demonstrates that nitrendipine is capable of stimu-

lating renin secretion by the isolated rat kidney independent of alterations in perfusion pressure, and without prior treatment with inhibitory agents. It remains to be determined if this action represents a direct effect upon renin-secreting juxtaglomerular cells or involves another intrarenal regulatory site.

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