EFFECTS OF LABETALOL ON RENIN RELEASE FROM RAT KIDNEY CORTICAL SLICES

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Effects of labetalol, a combined α - and β -adrenoceptor blocking agent, on the changes in renin release in response to isoproterenol or norepinephrine were examined in comparison with those of propranolol and prazosin, using rat kidney cortical slices. Isoproterenol (10^{-9} to 10^{-5} M) produced a dose-related increasing effect on renin release from the slices, although the increased release was markedly attenuated by the higher dose (10^{-4} M). On the other hand, norepinephrine, at relatively higher concentrations (over 10^{-6} M), caused a significant decreasing action on the release. Labetalol alone (10^{-9} to 10^{-4} M) had no effect on basal renin release. However, the agent exerted a concentration-dependent blocking action on the renin response to 10^{-6} M isoproterenol. Similar results were observed with propranolol. The decreased response of renin release to 10^{-5} M norepinephrine was significantly inhibited by labetalol over 10^{-6} M. Labetalol, at a concentration of 10^{-5} M, abolished the decreased release by 10^{-5} M norepinephrine. On the other hand, the decreasing effect of 10^{-5} M norepinephrine was substantially reversed rather than abolished by prazosin over 10^{-7} M. These results indicate that labetalol inhibited the response of renin in rat kidney cortical slices, by β - and α_1 -adrenoceptor antagonistic action, respectively.

Keywords — rat kidney cortical slice; renin release; α -adrenoceptor; β -adrenoceptor; isoproterenol; norepinephrine; labetalol; prazosin; propranolol

INTRODUCTION

Labetalol, 5-[1-hydroxy-2-[(1-methyl-3-phenylpropyl)amino]-ethyl]salicylamide hydrochloride, is an antihypertensive agent with a unique mode of action involving both α - and β -adrenoceptor blockade. The agent lowers blood pressure partly by blocking the α -adrenoceptors in the peripheral arterioles and thereby decreasing the peripheral vascular resistance. The resulting reflex activation of sympathetic nerves to the heart is controlled by concurrent blockade of β -adrenoceptors in the heart.^{1,2)}

Renin release from the kidney is partly controlled by renal sympathetic nerves and circulating catecholamines. The activation of renal β -adrenoceptors stimulates the release from renin secretory cells.³⁾ Intrarenal administration of isoproterenol (IP) markedly elevates the renin secretory rates,⁴⁾ whereas treatment with β -adrenoceptor antagonists lowers plasma renin activity.⁵⁾ The latter effect is considered to be one of the mechanisms by which the agents exert an effective antihypertensive action.⁶⁾ On the other hand, a role of the renal α -adrenoceptors in control of renin release has not been estab-

lished. We recently demonstrated that α -adrenoceptor agonists such as norepinephrine (NE), methoxamine and phenylephrine inhibited renin release from rat kidney cortical slices *via* the stimulation of α_1 -adrenoceptors.⁷⁾

Although labetalol has been known to decrease plasma renin activity,⁸⁾ the direct action of the agent on renin release from the kidney has not been reported. The present study was carried out to investigate the effect of labetalol on the response of renin release to IP and NE in rat kidney cortical slices. Use of the *in vitro* kidney cortical slices enables examination of the direct action of agents on the release in the aspect of adrenergic mechanisms, since this preparation does not include other factors affecting renin release *in vivo*.

MATERIALS AND METHODS

Chemicals — Drugs used in this study were *l*-isoproterenol hydrochloride (Sigma), *l*-norepinephrine bitartrate (Sigma), *dl*-labetalol hydrochloride (Glaxo), *dl*-propranolol hydrochloride (Sigma) and prazosin hydrochloride (Taito-Pfizer). All drug solutions were freshly prepared before use. Isoproterenol hydrochloride and

norepinephrine bitartrate were dissolved in 0.9% NaCl. *dl*-Labetalol hydrochloride and *dl*-propranolol hydrochloride were dissolved in distilled water. Prazosin hydrochloride was dissolved in ethanol and diluted with distilled water; the final concentration of ethanol was lower than 0.1%. We verified that the final concentration of ethanol did not affect basal renin release from the slices.

Preparation and Incubation of Kidney Cortical Slices — Male Wistar rats weighing 200–250 g were used. Bilateral nephrectomy was performed under pentobarbital anesthesia (35 mg/kg, i.p.). The kidneys were decapsulated, immediately placed in ice-cold physiological saline, and 4 thin cortical slices were cut from each kidney with a razor blade. The slices were approximately 0.5 mm thick and the wet weight was 40–50 mg. Krebs-Ringer bicarbonate solution (KRBS, 118.5 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 , 25 mM NaHCO_3 , 10 mM glucose, pH 7.4) was used as the incubation medium. Two slices were placed in each flask which contained 3 ml of KRBS and the preparations were preincubated at 37 °C in an atmosphere of 95% O_2 –5% CO_2 in a metabolic incubator. After preincubation for 30 min, the medium was discarded. The slices were carefully washed with 3 ml of KRBS and incubated for 3 consecutive 15 min periods in 3 ml of fresh KRBS. At the end of each period, the medium was removed by aspiration and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant fluid was stored at –20 °C until assayed for renin concentration.

Renin Assay — Renin concentration in the sample was measured by radioimmunoassay of angiotensin I (AI)⁹⁾ generated after incubation with partially purified rat renin substrate, which was prepared according to the procedure described previously.¹⁰⁾ The incubation medium consisted of: 1) samples containing renin, 50 μl ; 2) renin substrate (200 ng as AI equivalent) dissolved in 0.25 M phosphate buffer (pH 7.0) containing 20 mM disodium ethylenediaminetetraacetic acid, 0.5 ml; 3) 5% diisopropylphosphorofluoridate, 20 μl ; 4) 6.6% 8-hydroxyquinoline sulfate, 10 μl ; and 5) 10% dimercaprol, 3 μl . After incubation at 37 °C for 15 min, the reaction was terminated by heating for 5 min in a boiling water bath. Renin concentration was expressed as nanograms of AI generated per hour of the incubation per milliliter of the sample. The amount of renin release during each incubation period of kidney cortical slices was calculated as the renin concentration in the medium, multiplied by the volume of the medium and divided by the tissue wet weight (nanograms of AI per hour per milligram).

Statistics — The results are presented as the mean $\pm$ S.E. Dunnett's multiple comparison test¹¹⁾ was used to estimate significant changes between the control values and those obtained after drug treatment. In all comparisons, differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Renin release from rat kidney cortical slices was stable throughout the 3 periods. The amounts of renin released in the first, the second

FIG. 1. Effects of Labetalol, Propranolol and Prazosin on Basal Renin Release from Rat Kidney Cortical Slices

Columns and bars represent means $\pm$ S.E.M. ($n = 6$). a) $p < 0.05$; b) $p < 0.01$, compared with the values in the absence of each antagonist. $\square$ labetalol; ▨ propranolol; $\blacksquare$ prazosin.

and the third periods were 8.9 ± 0.4 , 8.8 ± 0.4 and 8.9 ± 0.4 ng AI/h per mg wet tissue, respectively.

In the experiments performed to examine the effects of each agonist or antagonist alone on the release, the slices were exposed to each drug in the second and the third periods. For experiments in which the blocking actions of each antagonist on agonist-induced renin secretory effects were examined, the slices were exposed to each antagonist in all the incubation periods and to each agonist in the second and the third periods. In the following results, the amount of renin released in the third period was expressed as a percent change from that in the first period which served as the control.

Figure 1 illustrates the effects of each antagonist alone on renin release from rat kidney cortical slices. At concentrations of 10^{-9} to 10^{-4} M, labetalol had no effect on basal renin release. Propranolol and prazosin, at concentrations of 10^{-9} to 10^{-5} M, were also without effect. However, at the highest concentration (10^{-4} M), propranolol and prazosin increased renin release by 36.5% and 32.9%, respectively. Capponi and Vallotton¹²⁾ also observed the increasing effect of propranolol on renin release from kidney cortical slices. This action is not due to the acti-

vation of β -adrenoceptors, since the agent apparently does not possess an intrinsic sympathomimetic activity.¹³⁾ Rather, the membrane-stabilizing properties of propranolol¹⁴⁾ may be involved in the above increasing action, since a membrane-stabilizing action is manifested with a decrease in the calcium permeability of the cell membrane,¹⁴⁾ a stimulatory signal for renin release.¹⁵⁾ The mechanisms by which prazosin increases basal renin release is unknown.

When kidney cortical slices were incubated with IP at concentrations of 10^{-9} to 10^{-5} M, the agent produced a concentration-dependent increase in renin release from the slices. Maximum increase was observed at a dose of 10^{-6} M IP; the amount of renin release was increased by approximately 60%, compared with the control value. The extent of the enhancement was similar to that obtained in our previous study¹⁶⁾ and in that of other workers^{17,18)} in which the same preparation was used. However, the increasing action of IP was markedly attenuated by the higher dose (10^{-4} M) of the agent (Fig. 2). The decreased response appears to be attributable to α -adrenoceptor agonistic action of IP, since the above attenuation of renin response by 10^{-4} M IP was not observed in the presence of phenoxybenzamine (unpublished data).

FIG. 2. Effects of IP and NE on Basal Renin Release from Rat Kidney Cortical Slices
Columns and bars represent means $\pm$ S.E.M. ($n = 8$). a) $p < 0.05$; b) $p < 0.01$, compared with the values in the absence of each agonist. $\square$ IP; hatched NE.

FIG. 3. *Inhibitory Effects of Labetalol and Propranolol on the IP-Induced Increase in Renin Release from Rat Kidney Cortical Slices*

Slices were incubated in the presence of 10^{-6} M IP. Columns and bars represent means $\pm$ S.E.M. ($n = 8$). a) $p < 0.05$; b) $p < 0.01$, compared with the values in the absence of each antagonist.

□ labetalol; ▨ propranolol.

On the other hand, NE exerted a dose-related inhibitory action on renin release. As shown in Fig. 2, the release was significantly decreased by the agent over 10^{-6} M. Maximum effect was observed at a concentration of 10^{-4} M NE; the amount of renin release was decreased by about 45%, compared with the control value.

Figure 3 shows the effects of labetalol and propranolol on renin release from the slices in response to 10^{-6} M IP. Labetalol exerted a dose-

related blocking action on the increased release induced by IP. Significant blocking actions of labetalol were observed at doses over 10^{-6} M. The increased release due to IP was virtually abolished (95% inhibition) by 10^{-5} M labetalol. Similar results were observed with propranolol, although the blocking action on the IP-induced renin release was slightly more potent than that of labetalol. Labetalol and propranolol possess blocking actions on both subtypes of β_1 - and β_2 -

FIG. 4. *Inhibitory Effects of Labetalol and Prazosin on the NE-Induced Decrease in Renin Release from Rat Kidney Cortical Slices*

Slices were incubated in the presence of 10^{-5} M NE. Columns and bars represent means $\pm$ S.E.M. ($n = 6$). a) $p < 0.05$; b) $p < 0.01$, compared with the values in the absence of each antagonist.

□ labetalol; ■ prazosin.

adrenoceptors.^{2,5)} Our study did not elucidate whether the IP-induced renin release is mediated by activation of β_1 - and/or β_2 -adrenoceptors. However, based on the finding that atenolol causes competitive antagonism against the IP-induced renin release from rat kidney cortical slices,¹⁹⁾ the adrenoceptor involved in the release in this preparation may be the β_1 -type.

The decreasing effect of 10^{-5} M NE on renin release was blocked by labetalol, in a dose-dependent manner. Approximately 85% inhibition of the decreased release was observed with 10^{-6} M labetalol. At a concentration of 10^{-5} M, the antagonist abolished the renin response to 10^{-5} M NE. Blakeley and Summers²⁰⁾ have reported that labetalol acts as a postsynaptic α -adrenoceptor antagonist in the isolated cat spleen with little effect on presynaptic α -adrenoceptors. We demonstrated previously that NE decreased renin release from the slices *via* α_1 -adrenoceptors.⁷⁾ In addition, prazosin, an α_1 -adrenoceptor antagonist, potently antagonized the decreased release in response to 10^{-5} M NE. Taken together, the antagonistic effect of labetalol on NE-induced decrease in renin release appears to be due to α_1 -adrenoceptor blocking action of the agent.

In the presence of prazosin over 10^{-7} M, the response to 10^{-5} M NE was reversed rather than blocked; about 50% increase in renin release was observed at a dose of 10^{-6} M prazosin (Fig. 4). This increase in the release was suppressed by propranolol (data not shown). Accordingly, it is conceivable that the NE-induced decrease in renin release in rat kidney cortical slices *via* α_1 -adrenoceptors is predominant, compared with the β -adrenergic effect of this catecholamine. On the other hand, labetalol did not reverse the renin response to NE, thereby suggesting that the enhancing effect of NE on renin release is masked by the β -adrenoceptor antagonistic action of labetalol.

Our data clearly indicate that labetalol directly antagonizes the response of renin release in the kidney, by β - and α_1 -adrenoceptor antagonistic action, respectively. Such actions may be involved in the antihypertensive effect of this agent.

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