Effects of W-7 and W-5 on Renin Release from Rat Kidney Cortical Slices

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Accepted July 25, 1984

It has been widely acknowledged that intracellular calcium (Ca) is essential for the secretory process of neurotransmitters and hormones (1). Recent studies have suggested that calmodulin acts as an intracellular Ca-receptor in mediating the role of Ca in the secretory process, since this process is inhibited by calmodulin-antagonists such as trifluoperazine (TFP) (2-4). On the other hand, it has been reported that renin release is suppressed by several substances which cause a rise in intracellular Ca level (5-8). In order to determine whether the Ca-calmodulin system is associated with the mechanisms of renin release, we investigated the effect of a calmodulin-antagonist on renin release from rat kidney cortical slices. In this study, N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was used as the calmodulin-antagonist. W-7 is thought to be more appropriate than other calmodulin-antagonists for experiments at a cellular level since this agent penetrates the cell membrane and interacts selectively with calmodulin (9). In addition, the effect of W-7 on renin release was compared with that of N-(6-aminohexyl)-1-naphthalenesulfonamide (W-5), which has virtually no calmodulin-antagonistic activity (10).

Male Wistar rats weighing 200-250 g were used. Under pentobarbital anesthesia (35 mg/kg, i.p.), bilateral nephrectomy was performed. The kidneys were decapsulated, immediately placed in ice-cold saline, and 4 thin cortical slices were cut from each kidney with a razor blade under low temperature. The slices were approximately 0.5 mm thick and weighed 40-50 mg. Krebs-Ringer bicarbonate solution (KRB, 118.5 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃, 10 mM glucose, pH 7.4) was used as an incubation medium. Two slices were placed in each flask which contained 3 ml of KRB and preincubated for 30 min at 37°C under an atmosphere of 95% O₂-5% CO₂ in a metabolic incubator. Subsequently, the slices were transferred in 3 ml of fresh KRB prewarmed at 37°C, and carefully washed. The wash solution was aspirated, and the slices were incubated for 3 consecutive 15 min periods in 3 ml of fresh KRB under the same conditions as the preincubation period. At the end of each period, the incubation medium was aspirated and centrifuged at 3000 rpm for 10 min at 4°C. The supernatant was stored at -20°C until the renin assay. Renin activity in the sample was measured by radioimmunoassay (11) of angiotensin I (AI) generated after incubation with partially purified rat renin substrate, which was prepared by our method, which was previously reported (12). All data were presented as the mean±S.E. of the values obtained in eight separate experiments. Statistical analysis was performed using one-way analysis of variance for repeated measures combined with Dunnett's multiple range tests (13).

Renin release from rat kidney cortical slices was stable throughout the 3 periods; the amounts of renin release in the first, the second and the third periods were 10.8±0.4, 10.3±0.3 and 10.2±0.4 ng AI/hr/mg wet tissue, respectively. First, the effects of W-7 and W-5 on renin release from the slices were examined by adding various concentrations of each agent to the incubation medium. The first period was taken to be the control period, and the slices were exposed to each agent in the second and the third periods. The amount of renin release in the
third period was expressed as a percentage of that in the control period. As shown in Fig. 1, W-7, at concentrations from 30 up to 100 \( \mu \text{M} \), produced a dose-related increase in renin release from the slices. At the highest concentration (100 \( \mu \text{M} \)), W-7 increased renin release by 44.1±7.2%. On the other hand, W-5 caused no significant change in renin release.

It has been reported that a high extracellular concentration of potassium (K) inhibits renin release from kidney cortical slices by increasing the intracellular Ca level (5, 7). So, we examined the combined effect of a high extracellular concentration of K and W-7 or W-5 on renin release from the slices. In these experiments, 60 mM K solution prepared by substituting KCl for NaCl in KRB was used as the high K medium, since this concentration of K was reported to inhibit renin release from kidney cortical slices independently of sodium concentration (14). The slices were exposed to W-7 or W-5 in all the incubation periods and to 60 mM K in the second and the third periods. The amount of renin release in the third period was expressed as a percentage of that in the first period. As shown in Fig. 2, renin release from the slices was decreased to 54.9±7.0% of the control value by 60 mM K. However, when the slices were incubated with 100 \( \mu \text{M} \) W-7, a suppressive effect of 60 mM K on renin release was virtually abolished. On the other hand, 100 \( \mu \text{M} \) W-5 did not influence the suppression of renin release by 60 mM K.

Recently, Churchill and Churchill (15) have indicated that TFP causes a concen-
tration-dependent increase in renin release from rat kidney cortical slices. In addition, TFP was reported to antagonize inhibition of renin release by angiotensin II (16), the effect of which is considered to be mediated by increased Ca influx (6). However, it remains unclarified whether the stimulatory effect of TFP on renin release is due to its calmodulin-antagonistic action, since this agent has a Ca-channel-blocking action (17, 18). According to the study of Kanamori et al. (19), W-7 interacts with intracellular calmodulin without affecting Ca influx. In the recent study of Kawamura and Inagami (20), W-7 was reported to cause a stimulation of renin release from isolated rat glomeruli, although the effect of W-5 on renin release was not examined. The present study demonstrated that W-5 did not produce a stimulatory effect on renin release from rat kidney cortical slices, in contrast with W-7. Since W-5 has virtually no calmodulin-antagonistic activity (10), it is likely that W-7 stimulates renin release from kidney cortical slices by inhibiting the Ca-calmodulin system. Furthermore, the results of the experiment using these agents and 60 mM K suggest that a suppression of renin release due to a high extracellular concentration of K is mediated by the activation of the Ca-calmodulin system. Thus, it seems that the Ca-calmodulin system plays an inhibitory role in renin release from the juxtaglomerular cells.

It is well known that an increase in cyclic AMP content in the juxtaglomerular cells leads to the stimulation of renin release (21). Recently, Hidaka et al. (10) have indicated that the inhibitory effect of W-7 on cyclic nucleotide phosphodiesterase is much more potent, compared with that of W-5. Accordingly, there is a possibility that W-7 produces the stimulation of renin release via the accumulation of cyclic AMP. For the clarification of this possibility, further experiments are under way.

Acknowledgement: We wish to thank Professor H. Hidaka, Department of Pharmacology, Mie University School of Medicine, Tsu, Japan, for the kind gift of W-7.

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