Effect of Adenosine$_1$-Receptor Blockade on Renin Release from Rabbit Isolated Perfused Juxtaglomerular Apparatus

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Abstract

Adenosine has been proposed to act within the juxtaglomerular apparatus (JGA) as a mediator of the inhibition of renin secretion produced by a high NaCl concentration at the macula densa. To test this hypothesis, we studied the effects of the adenosine$_1$ (A_1)-receptor blocker 8-cyclopentyl-1,3-dipropylxanthine (CPX) on renin release from single isolated rabbit JGAs with macula densa perfused. The A_1-receptor agonist, N^6-cyclohexyladenosine (CHA), applied in the bathing solution at 10^{-7} M, was found to inhibit renin secretion, an effect that was completely blocked by adding CPX (10^{-5} M) to the bath. Applied to the lumen, 10^{-5} M CPX produced a modest stimulation of renin secretion rates suppressed by a high NaCl concentration at the macula densa ($P < 0.05$). The effect of changing luminal NaCl concentration on renin secretion rate was examined in the presence of CPX (10^{-7} and 10^{-5} M) in the bathing solution and in vehicle control experiments. The control response to increasing luminal NaCl concentration was a marked suppression of renin secretion, that was maintained as long as luminal NaCl concentration was high and was promptly reversible when concentration was lowered. CPX did not alter renin release when luminal NaCl was low, but diminished the reduction caused by high NaCl ($P < 0.01$). It is concluded that A_1-receptors are located within the JGA, and that A_1-receptor activation inhibits renin release. A high NaCl concentration at the macula densa appears to influence A_1-receptor activation, but a low NaCl concentration does not. The findings support participation of adenosine in macula densa control of renin secretion. (*J. Clin. Invest.* 1990. 85:1622–1628.) macula densa • kidney • adenosine analogues

Introduction

In an in vitro preparation of the juxtaglomerular apparatus (JGA)[1] in which the tubular segment is perfused, we have recently established that a low NaCl concentration in the tubular perfusate stimulates renin secretion (1). It appears likely that the macula densa cells, which show a number of specialized features (2, 3), function as the luminal sensor for this response. They are in close contact with the extraglomerular mesangial cells, also called Goormaghtigh cells, which have been postulated to transmit the signal from the macula densa to the epithelioid granular cells where renin is generated, stored, and released. The cellular events by which changes in solute concentration in the tubular lumen lead to inverse changes in renin secretion are poorly understood. Stimulus-effector coupling may be achieved by the transport dependent generation of a humoral mediator.

Several observations suggest that adenosine is a possible candidate as an autacoid that may participate in the regulation of renin secretion. Exogenous adenosine has been shown to consistently inhibit renin release (4–6). There is some indirect evidence in the rat that generation of adenosine may depend on the rate of tubular NaCl transport (7). Since an increased NaCl concentration at the macula densa is probably associated with an increased local transport rate, increased ATP utilization could lead to increased release of adenosine into the juxtaglomerular interstitium (4, 7, 8). Adenosine, according to this proposal, would then serve as a local inhibitor of renin release. Inhibitory effects on renin release are produced via receptors of the adenosine$_1$ (A_1)-subtype. Activation of these receptors is typically achieved by low (nanomolar) adenosine concentrations and is often associated with inhibition of adenylate cyclase (9, 10). A_1-agonists have been found to inhibit renin release from isolated perfused kidneys (11), kidney slices (12), and isolated juxtaglomerular cells (13). Agonists of A_2-receptors, in contrast, stimulated renin secretion from these preparations (11–13); A_2-receptors are activated by high (micromolar) adenosine concentrations and often induce stimulation of adenylate cyclase (9, 10).

The present work was intended to examine the possibility that adenosine acts as a mediator of the inhibition of renin release caused by an elevation in macula densa NaCl concentration. Using two agents with a high degree of specificity for the adenosine$_1$ category of receptors experiments were performed to answer two specific questions: (*a*) Does the A_1-agonist N^6-cyclohexyladenosine (CHA) (14, 15) inhibit renin release in the presence of a low NaCl concentration at the macula densa, and (*b*) can the inhibition of renin secretion caused by a high NaCl be diminished or abolished by the A_1-antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX) (16, 17)?

Methods

Animals and technique

Details of the method for the measurement of renin secretion from isolated perfused and superfused JGA preparations have been de-

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1. *Abbreviations used in this paper:* CHA, N^6-cyclohexyladenosine; CPX, 8-cyclopentyl-1,3-dipropylxanthine; DCT, distal convoluted tubule; GU, Goldblatt Unit; JGA, juxtaglomerular apparatus; TAL, thick ascending limb of Henle.

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scribed recently (18). In brief, the left kidney was removed from female white New Zealand rabbits with an average weight of ~ 1 kg. The kidney was decapsulated and sliced transversally through the papilla. Slices were microdissected under a stereo microscope in ice-cold dissection medium (described below). Specimens consisted of the late thick ascending limb of Henle (TAL), macula densa, early distal convoluted tubule (DCT), adherent glomerulus, and short arteriolar fragments (see Fig. 1 *a*). An experiment was only performed when the dissection was completed within 60 min.

The dissected specimen was transferred into a bathing chamber which contained freshly prepared bath solution. Under microscopic control either TAL or DCT were cannulated: the tubule was drawn into the holding-pipette, and the perfusion-pipette was advanced into the tubular lumen. When perfusion was established the bath temperature was raised to 38°C and kept constant between 37.5 and 38°C. During an initial equilibration time of 10 to 15 min the tubular walls were examined to ensure cellular integrity (Nomarski optics, magnification 400 to 1,000), and the presence of a macula densa was verified (see Fig. 1 *b*).

After the equilibration period, an outer concentric pipette, which served as a superfusion chamber, was advanced over the specimen and superfusion was established with prewarmed bath solution. A constant flow rate of 1 μl/min was maintained by a syringe pump. The pipettes were then immersed in a warmed mineral oil bath (37.5 to 38°C). Droplets, containing both luminal perfusate and superfusate, formed at the tip of the superfusion chamber and were collected at 8-min intervals (Fig. 1 *c*).

In experiments in which tubular perfusate was exchanged (series 2 and 3), [^{3}H]inulin was added to the perfusate in the middle experimental period to verify the completeness of perfusate exchange.

Protocols

Three experimental series were performed. In each experiment, collections were taken at 8-min intervals for measurement of single JGA renin secretion rate.

Series 1. A first series of six experiments studied the effects of the adenosine$_1$-receptor agonist CHA and the adenosine$_1$-receptor antagonist CPX on renin release. The tubular lumen was perfused with the low NaCl solution throughout the experiment. After a control period in which five timed 8-min collections were made, the superfusing bath was exchanged for a solution with identical composition containing 10^{-7} M CHA. In this experimental period, again five collections were taken. In the final experimental period, a second bath exchange was made, and again five collections were performed. In this period the CHA concentration was maintained at 10^{-7} M, and in addition 10^{-5} M CPX was applied. During all three periods adenosine deaminase (2 U/ml) was present in the bath.

In four control experiments only the vehicle for CHA (1% ethanol) was applied to the bath in the respective periods, but conditions were otherwise identical.

Series 2. A second series of experiments examined the effects of luminal CPX application on JGA renin release from five specimens. The luminal perfusate was the high NaCl solution during the first five collections. Then this solution was replaced with an identical solution with 10^{-5} M CPX added. After the tenth collection CPX was removed, and five more collections were taken. The bath was not exchanged in this series. In three control experiments only the vehicle was added to the high NaCl perfusate in the second period.

Series 3. A third series of experiments tested the effects of CPX on changes in renin release produced by altering the macula densa perfusate. The luminal perfusate was the low NaCl solution during the first five collections. It was exchanged to the high NaCl solution for the next five collections and then back to the low NaCl solution. The superfusing bath contained CPX and was not exchanged during the experiment. Two CPX concentrations were tested with this protocol: in five experiments CPX was applied in a concentration of 10^{-7} M, in eight experiments a CPX concentration of 10^{-5} M was used. 12 otherwise identical vehicle control experiments were also performed.

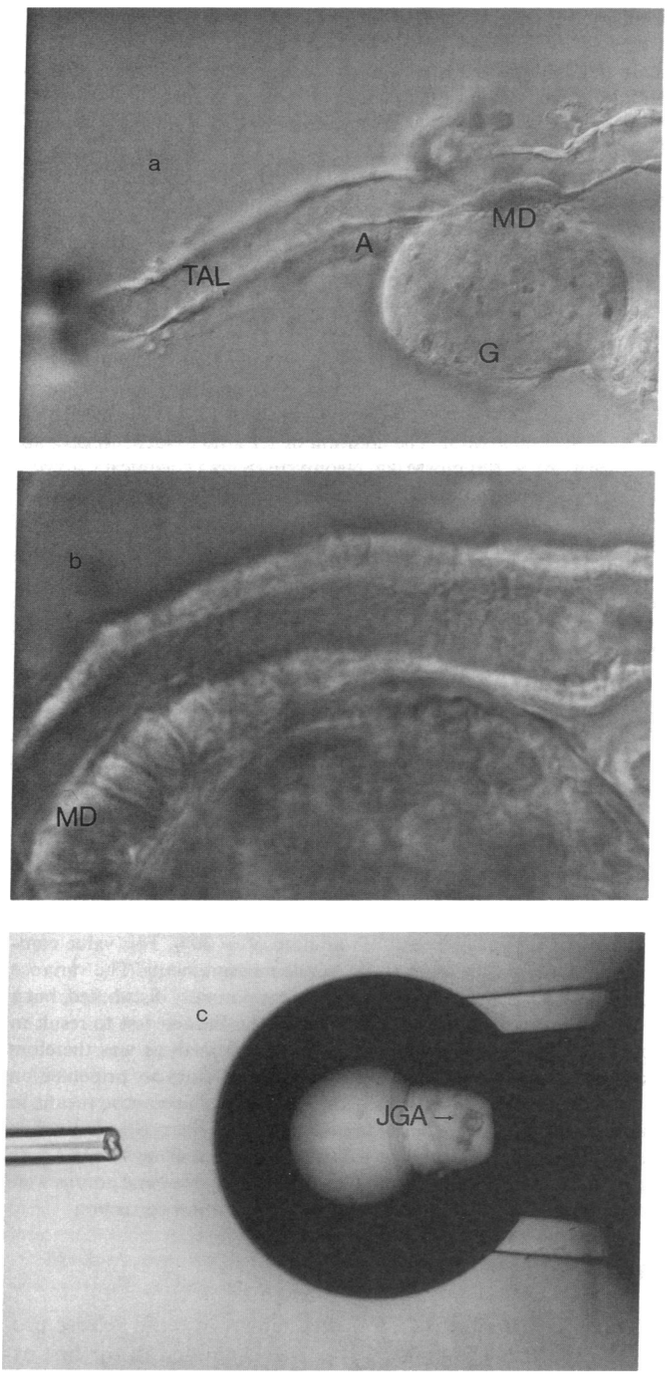

Figure 1. (*a*) Photomicrograph of an isolated perfused JGA (×400), microdissected from a New Zealand rabbit. Specimens consisted of a portion of the thick ascending limb (TAL), macula densa (MD), early distal convoluted tubule (not visible), adherent glomerulus (G) and arteriolar fragments (A). (*b*) Macula densa at higher magnification (×1,000). As a plaque of thick, cuboidal cells the macula densa (MD) can be distinguished from surrounding TAL-cells. (*c*) Droplet collection. Bath fluid containing the renin secreted by the cannulated JGA (JGA) formed droplets at the tip of the superfusing chamber. The droplets were collected at 8-min intervals.

Renin assay. Renin was measured with the antibody trapping technique developed by Lykkegård and Poulsen (19). Application of an antibody, binding to angiotensin I, reduced distinctly the cleavage of generated angiotensin I by angiotensinases. This permitted prolonged

incubation times, resulting in high assay sensitivity. Renin release is expressed in Goldblatt units (GU), determined by comparison with renin standards from the Institute of Medical Research (M.R.C., Holly Hill, London), which were included in each assay run. The detection limit of the assay was 1 nGU renin present in 5 μl fluid with an incubation time of 24 h at 37°C.

Solutions and reagents. The tubular perfusate was either the low NaCl or the high NaCl solution. Both solutions were bicarbonate-buffered Krebs solutions. The low NaCl solution consisted of 25 mM $NaHCO_3$, 0.96 mM NaH_2PO_4, 0.24 mM Na_2HPO_4, 5 mM KCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, and 5.5 mM glucose. The high NaCl solution was compiled identically except 115 mM NaCl were added. Both solutions were equilibrated with 5% CO_2 + 95% O_2 to a pH of 7.4 before use; their osmolarity was measured and adjusted to 300 mosM/kg with mannitol. Bath solution and dissection medium were prepared from Dulbecco's modified Eagle medium (DME/F-12; Sigma Chemical Co., St. Louis, MO). The addition of 1.2 g/liter $NaHCO_3$ gave an osmolarity of ~ 300 mosM/kg. Neomycin (Sigma Chemical Co.) was added in a concentration of 0.002 g/liter. The DME-solution was made fresh every fourth day; it was stored in sterilized containers at 4°C. Immediately before use the DME solution was bubbled with 5% CO_2 + 95% O_2 and its pH was adjusted to 7.4. Employed as dissection medium, 2.0 g/100 ml fetal calf serum (Gibco Laboratories, Grand Island, NY) was added; employed as superfusing bath, 0.25 g/100 ml human albumin (Sigma, St. Louis, MO) was added instead.

CHA and CPX were obtained from Research Biochemicals Inc. (Natick, MA). Both reagents were dissolved as stock solutions in ethanol. For experimental use the CHA-ethanol solution was applied in 100-fold dilution and the CPX-ethanol solution in 1,000-fold dilution. Adenosine deaminase was obtained from Sigma Chemical Co., [^{3}H]-inulin from ICN Radiochemicals (Irvine, CA).

Data analysis. Both single JGA renin content and the rate of renin secretion from a single JGA were observed to vary over an approximately three log range, consistent with previous observations (1, 20, 21). Within a single experiment, values have a much narrower range of variability, with a coefficient of variation of ~ 20%. This value compares favorably with other single tubule measurements. The variance of single JGA renin secretion rates was not normally distributed, but a log transformation was demonstrated by the Bartlett test to result in normalization of variance (1, 22). Statistical analysis was therefore performed only on log transformed data, and values are presented on log plots. In the text all values are presented as geometric means in nGU/min. The means of the log transformed values±SE are given in parentheses. Experimental periods were compared using ANOVA with a repeat measurements design. Control and experimental groups were compared using an unpaired *t* test with Bonferonni correction.

Results

Series 1. The effect of CPX on changes in renin release provoked by the A_1-agonist, CHA, was examined in the first experimental series. Results from four time control experiments and six experimental studies are shown in Fig. 2. Tubular perfusate was the low NaCl solution throughout these studies. No significant changes in renin secretion were observed in the time control vehicle studies, when bath exchanges were performed without adding the drugs. Constantly perfused with the low NaCl solution, renin release remained at a high level throughout the experiment, averaging at 148 nGU/min (2.17±0.32) in the first period, at 229 nGU/min (2.36±0.21) in the second period, and at 263 nGU/min (2.42±0.18) in the third period.

In the experimental series the values in the first period averaged at 158 nGU/min (2.20±0.16). In the second period, when the A_1-agonist CHA was added to the superfusing bath in a concentration of 10^{-7} M, renin secretion decreased signifi-

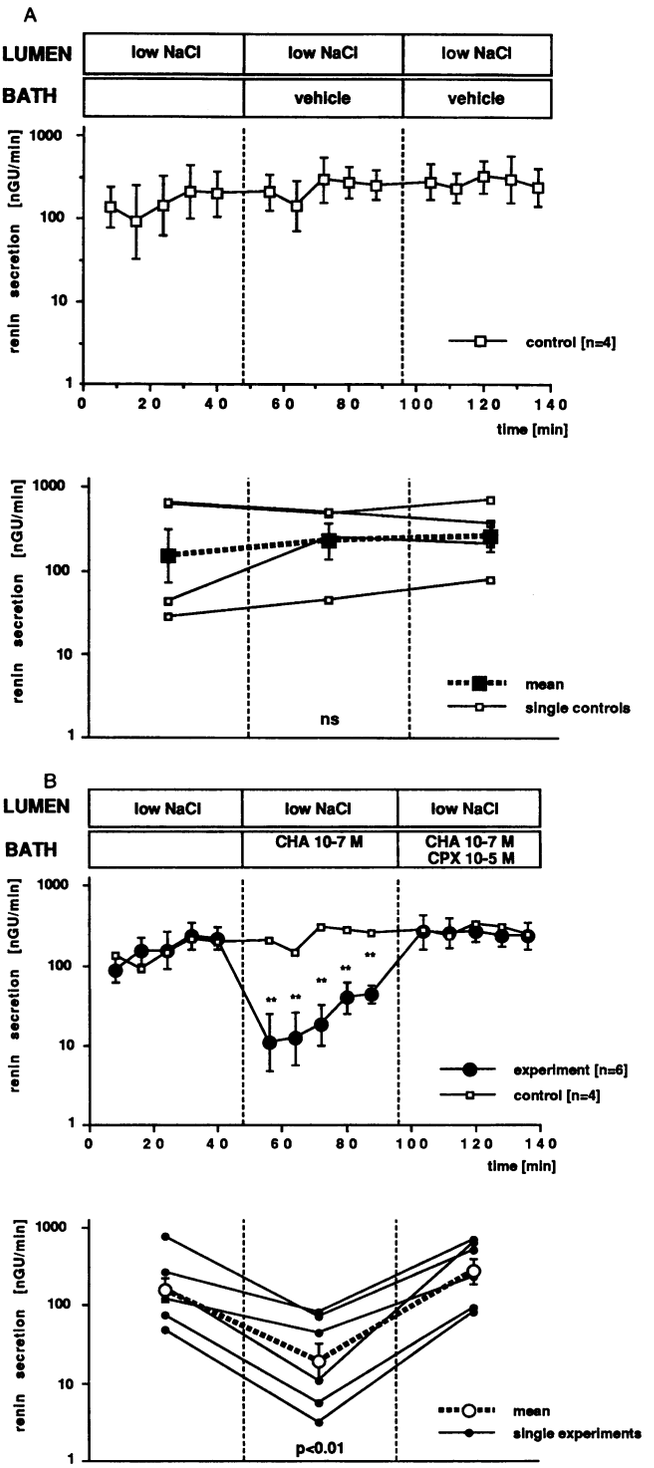

Figure 2. Series 1. Effects of CPX on changes in renin release provoked by the A_1-agonist, CHA. Renin secretion from single perfused JGAs is expressed as log nGU/min. (*A*) The control studies; the upper panel depicts the time course of renin secretion, the lower panel the mean of individual experiments. (*B*) The experimental studies as closed circles, with control results also included for comparison in the upper panel. No significant changes in renin release were noted in the control series (*A*). In the experimental series (*B*) addition of 10^{-7} M CHA into the bath decreased renin secretion rates significantly ($P < 0.01$). This inhibition was completely restored by additional application of 10^{-5} M CPX. Asterisks indicate significance levels. $^{**}P < 0.01$ compared to corresponding control value.

cantly ($P < 0.001$) to an average of 19 nGU/min (1.28±0.22), although the low NaCl perfusion was maintained. The inhibition of renin release was prompt and sustained. In the third period addition of 10^{-5} M CPX to the bath restored the renin secretion rates completely (average: 275 nGU/min (2.44±0.16)), despite the continued presence of CHA.

Series 2. The effect of luminal CPX application on renin release from five single JGAs and the measurements from three control studies are illustrated in Fig. 3. These studies were performed to test whether adenosine$_1$-receptor blockade reduces the inhibitory effect of a high macula densa NaCl concentration on renin release. In both the experimental and control series renin secretion rates in the first period were low as a result of suppression by the high NaCl perfusate; the means averaged 9 nGU/min (0.95±0.28) and 6 nGU/min (0.76±0.20), respectively. In the experimental group the renin secretion rates increased modestly, but significantly ($P < 0.05$), when CPX at 10^{-5} M was added to the tubular perfusate during the second period (average: 22 nGU/min [1.35±0.23]). When CPX was removed from the perfusate in the final recovery period renin secretion did not return to initial values; rather, a continued increase was noted (average: 60 nGU/min [1.78±0.17]). Renin secretion rate in the control series remained at very low values in the second period, but also showed a slight tendency to rise in the final period (averages: 5 nGU/min [0.76±0.20] and 16 nGU/min [1.20±0.16]).

Series 3. This series was performed to evaluate the effect of CPX on changes in renin release caused by altering the luminal NaCl concentration. Results are depicted in Fig. 4.

In control experiments changing the tubular perfusate from low NaCl in the first period to high NaCl in the second period reduced renin secretion significantly to 15% of the control value (from 54 nGU/min [1.73±0.22] to 8 nGU/min

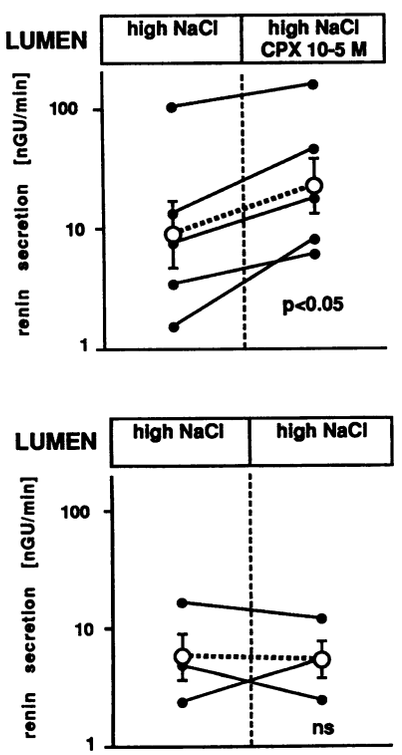

Figure 3. Series 2. Effect of luminal CPX application (10^{-5} M) on renin release. Both panels depict the average renin secretion per period for the single experiments, the upper panel (*A*) for the experimental series, the lower panel (*B*) for the control series. Renin release is expressed as log nGU/min.

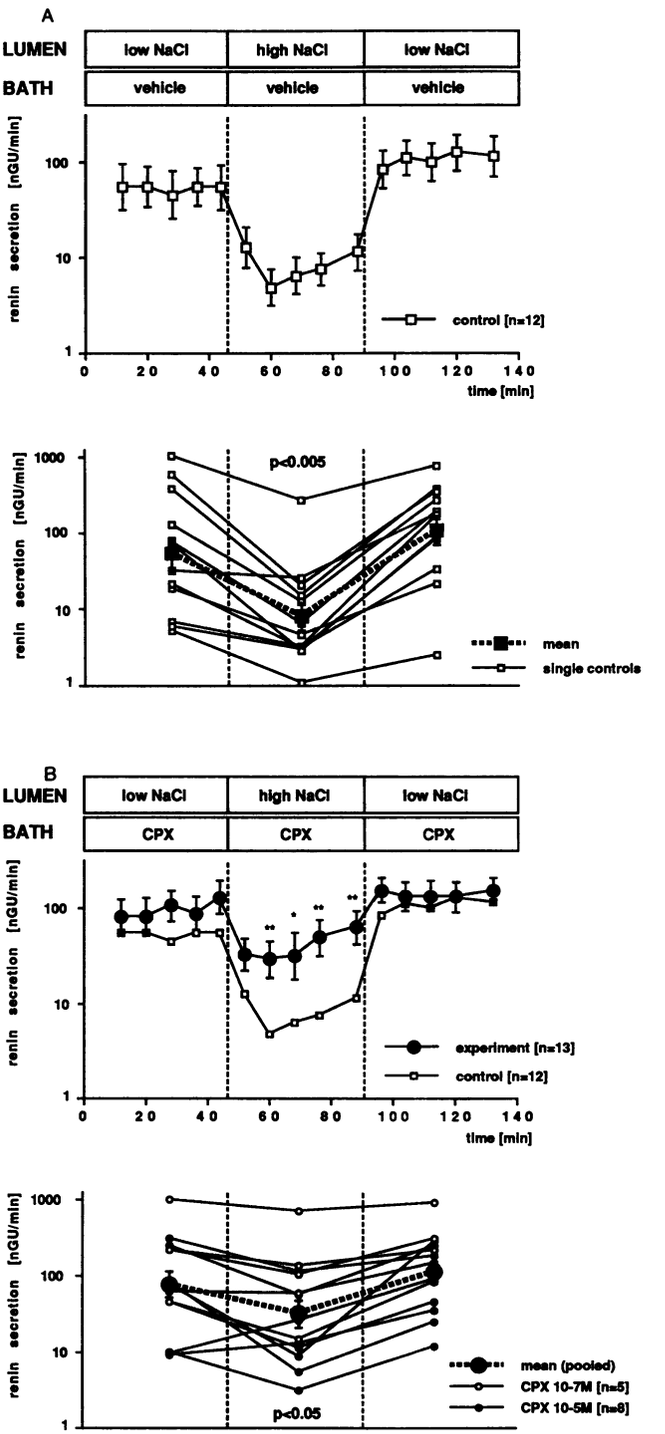

Figure 4. Series 3. Effect of CPX on changes in renin release provoked by altering the luminal NaCl concentration. (*A*) The control studies, (*B*) the experimental studies with closed symbols and the controls with open symbols. Renin secretion is expressed as log × nGU/min. The upper panels depict the time course of renin secretion; the lower panels show the average renin secretion per period for each single experiment. Consistent with previous observations a high NaCl perfusate strongly inhibited renin secretion in the control series. CPX, applied to the bath, significantly blunted this effect (*B*). The upper panel shows the pooled results for both CPX concentrations used. Effects of the different CPX concentrations are plotted in the lower panel. $*P < 0.05$, $**P < 0.01$ compared to corresponding control value.

[0.91±0.17]) ($P < 0.001$). This inhibitory effect of a high NaCl concentration at the macula densa was reversible: renin secretion increased to 107 nGU/min (2.03±0.19) after the perfusate was changed back to a low NaCl solution.

The addition of the A_1-antagonist CPX to the superfusing bath significantly blunted the inhibition of renin secretion produced by the high NaCl solution. This effect was seen with both CPX concentrations tested: 10^{-7} M ($n = 5$) and 10^{-5} M ($n = 8$). With 10^{-7} M CPX renin secretion fell only to 57% of the basal value (from 214 nGU/min [2.33±0.21] to 123 nGU/min [2.09±0.25]). In the accompanying controls ($n = 3$) renin secretion fell to 7% of the control value (from 363 nGU/min [2.56±0.29] to 25 nGU/min [1.40±0.47]). When 10^{-5} M CPX was present in the bath, renin secretion fell from 58 nGU/min (1.76±0.21) to 19 nGU/min (1.28±0.17). This change represents a decrease to 32% of the basal value. The values for the corresponding control studies ($n = 9$) were 28 nGU/min (1.45±0.20), falling to 5 nGU/min (0.74±0.13), 17% of the basal value. Thus, with both CPX concentrations the fall in renin secretion was significantly different from the corresponding control studies (for CPX 10^{-7} M: $P < 0.01$, for CPX 10^{-5} M: $P < 0.05$). There was no significant difference between the two CPX concentrations. Their pooled data are depicted in Fig. 4 *B*, with overall mean values being 95 nGU/min (1.98±0.17) falling to 39 nGU/min (1.59±0.18). CPX did not alter renin secretion rate significantly in the initial period, when luminal NaCl concentration was low. The average value was 95 nGU/min (1.98±0.17) compared to 54 nGU/min (1.73±0.22) in the control series. Both CPX concentrations reduced, but did not fully abolish the renin response to an increased NaCl concentration. In the presence of CPX the fall in renin secretion noted was significant ($P < 0.05$). In the first three collections after the perfusate had been exchanged, however, renin secretion returned to values not different from the control, indicating that in the presence of CPX high NaCl concentrations at the macula densa exert only a transient inhibitory effect on renin secretion.

Discussion

The present studies provide further evidence that the fall in renin secretion produced by administration of adenosine is due to interaction of the nucleoside with A_1-receptors. Application of the A_1-receptor agonist, CHA, in a concentration of 10^{-7} M resulted in significant inhibition of renin release from single perfused JGAs. The selective A_1-receptor blocker, CPX, was found to reverse the inhibiting effect of CHA on renin release. These observations are in agreement with reported effects of 10^{-7} M CHA on renal cortical slices from rats (12), isolated perfused rat kidneys (11), and cultured JGA-cells (13). Higher concentrations of CHA (10^{-5} M) have been observed to either produce no significant effect (11) or to stimulate renin release (12), presumably due to interaction with A_2-receptors. Since the present data were obtained from isolated specimens lacking neuronal inputs and vascular connections, they provide evidence that renal A_1-receptor activation can inhibit renin release independent of changes in renal hemodynamics or sympathetic nerve activity, a conclusion that has also been inferred from previous findings (6, 12).

The cellular localization of the A_1-receptors within the JGA cannot be deduced with certainty, but our findings are consistent with their presence on granular cells or possibly on extraglomerular mesangial cells. A_1-agonist binding has been demonstrated in isolated rabbit glomeruli (23), and autoradiographic studies in human kidney suggest a predominant periglomerular localization (24). Studies of the vasoconstrictor properties of CHA also indicate the presence of A_1-receptors in arteriolar segments in the immediate vicinity of the glomerular vascular pole (25, 26). In a previous study adenosine was observed to inhibit renin secretion from glomeruli separated together with a long afferent arteriolar segment, but to have no effect on glomeruli with a short arteriole (27). This result suggests that A_1-responsive renin cells also occur at some distance from the glomerular tuft. The failure to observe inhibition of renin release from glomeruli with short arterioles is not necessarily in contradiction with the present findings, since in those studies adenosine was used at a concentration that probably activated both A_1- and A_2-receptors.

The conclusion from the CHA experiments that A_1-receptors are located within the juxtaglomerular apparatus is a necessary precondition for the hypothesis that adenosine might mediate the decrease in renin release produced by high NaCl concentration at the macula densa. In considering possible tests of this hypothesis it is instructive to compare this system with other responses in which adenosine has a putative autocrine or paracrine role. The proposal that adenosine may act as a local vasodilator mediating work-dependent adjustments in blood flow is supported by a rather sizable body of work on coronary artery flow regulation (28–30), but the idea has engendered substantial controversy, and such a role can by no means be considered established (31, 32). In isolated perfused heart preparations adenosine release from the epicardium has been shown to increase with manipulations which increase metabolic demand, such as administration of catecholamines (33). In the central nervous system, adenosine release into the interstitium increases with hypoxia (34). There is also some indirect evidence suggesting that adenosine release increases when transport work is increased in rectal salt glands (35). However, it has not proven possible to measure true interstitial concentrations of adenosine in any of these systems (32), and although much effort has been applied to developing estimates of this parameter (31, 32), there remain substantial uncertainties due to difficulties in estimating the true rate of adenosine generation and its rapid breakdown by adenosine deaminase and uptake by nucleoside transport systems. It appears unlikely that measurement of macula densa adenosine generation will be possible with available methods. In the system used in the present studies, the macula densa plaque is a small fraction of the perfused tubular segment, and even if adenosine were measurable in the effluent, the contribution of the macula densa would be uncertain. Critical tools in testing for a regulatory role of adenosine have been agents such as theophylline, which block adenosine receptors. Generally this has been considered the most useful pharmacological approach to study local effects of adenosine (36, 37). The development of potent antagonists specific for the A_1-receptor category, such as CPX, the agent used in the present studies, has further increased the utility of this approach.

In the present studies two protocols were used to test whether the inhibition of renin secretion produced by a high NaCl concentration at the macula densa is blocked by CPX. In the first set of studies the tubular lumen of single JGAs was perfused with a high NaCl concentration, which suppressed renin secretion rates. Addition of CPX at a concentration of

10^{-5} M to the tubular perfusate resulted in significant stimulation of renin release. Although this finding was consistent with the hypothesis that the inhibition of renin secretion produced by high macula densa NaCl is, at least partially, an A_1-receptor mediated effect, the onset of the response was slow, did not appear to be reversible, and the stimulation achieved was substantially less than observed with a low NaCl solution. If adenosine effects are mediated by a direct effect on the granular cell, the time delay might reflect a lag in access of the luminally applied drug to the cell membrane. Delayed responses to methylxanthines have been reported in other systems (33). The fact that the response was not immediately reversible may reflect continued presence of CPX at the receptor site, since the drug is highly lipid soluble and the small tissue fragment may act as a reservoir, as well as delayed displacement from the receptor, which has a very high affinity for CPX (17).

A marked reduction of the inhibitory effect of high luminal NaCl concentrations on renin secretion was noted when CPX was applied in the bathing fluid (series 3). In control experiments an increase of NaCl concentration resulted in a substantial fall in renin secretion rate; this response was sustained for the 40-min experimental period and was promptly reversible when NaCl concentration was decreased. CPX did not affect the renin secretory rate when the luminal NaCl concentration was low. However, the inhibitory effect of a high NaCl perfusate was substantially blunted. Furthermore, the fall in renin secretion was transient: by thirty minutes, secretion rates did not differ from control values. These general findings were common to both CPX concentrations tested, 10^{-7} M and 10^{-5} M. The demonstration that CPX markedly blunts macula densa-mediated inhibition of renin secretion in this preparation suggests the involvement of endogenous adenosine in JGA-signal transmission. This conclusion supports recent work from Itoh, Carretero, and Murray (38). These authors observed that renin secretion from afferent arterioles with the macula densa attached was significantly lower than that from afferent arterioles without macula densa segments, and that theophylline increased renin secretion rates from afferent arterioles dissected together with the macula densa, but had no effect on arterioles dissected without macula densa. Adenosine$_1$-receptors may also participate in generating another effect of elevated macula densa NaCl concentration. Recent studies have shown that CPX significantly blunts the afferent arteriolar constriction elicited through the tubuloglomerular feedback mechanism (39).

Methylxanthines have the additional property of inhibiting the activity of cyclic nucleotide phosphodiesterases. This effect could potentially influence the interpretation of our results since an increase in the cellular level of cAMP is usually associated with an increase in renin release (40). The more selective antagonists such as CPX appear to have both a higher affinity for adenosine receptors and a lower phosphodiesterase inhibitory activity than less specific xanthines such as theophylline (9). For example, CPX was shown to inhibit adenosine receptors of rat fat cells with a K_i of 0.5 nM while 50% inhibition of phosphodiesterase activity required a concentration of 100 μM (17). Studies in the supernatant fraction of porcine coronary arteries also demonstrated only a minor inhibition of cAMP and cGMP hydrolysis by CPX (0.69×10^{-6} M) (41). On the other hand, it has been reported from studies in cultured opossum kidney cells that 5×10^{-5} M CPX produces a significant inhibition (74% ± 10%) of phosphodiesterase activity (42). Because of these discrepant results we cannot exclude the possibility that some inhibition of phosphodiesterase occurred in our experiments in which we used 10^{-5} M CPX. Nevertheless, this concentration of CPX did not elevate renin secretion rates at low NaCl concentration (series 3) compared to control as one would expect from increased cAMP levels. Furthermore, from published data it seems highly unlikely that CPX that 10^{-7} M detectably inhibits phosphodiesterases.

In summary, in the present studies in isolated perfused JGA CPX, an A_1-antagonist, blocked the inhibitory effect of an A_1-agonist on renin secretion. These results support the conclusions that A_1-receptor activation results in inhibition of renin release, that adenosine effects on renin release are independent of renal hemodynamics or renal nerve activity, and that adenosine$_1$-receptors are present on juxtaglomerular cells. Blockade of A_1-receptors by CPX was found to blunt the inhibitory effect of a high tubular NaCl concentration on single JGA renin release, but not to change renin release during perfusion of the macula densa segment with low NaCl concentrations. Thus, an elevation of NaCl concentration at the macula densa appears to be associated with A_1-receptor activation. These results suggest that adenosine may participate in macula densa-mediated renin release.

Acknowledgments

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