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FORSKOLIN AND CALCIUM: INTERACTIONS IN THE CONTROL OF RENIN SECRETION AND PERFUSATE FLOW IN THE ISOLATED RAT KIDNEY

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SUMMARY

1. Forskolin (activator of adenyl cyclase), high concentrations of K^+ and high renal perfusion pressure (manoeuvres known to increase Ca^{2+} permeability), and calmidazolium (the specific blocker of calmodulin) were used to investigate the mechanisms whereby adenosine 3′,5′-phosphate (cyclic AMP) and Ca^{2+} interact to control renin secretion and perfusate flow in the isolated perfused rat kidney.

2. Forskolin stimulated renin secretion and caused vasodilation in a dose-dependent manner in medium containing 5 mM-Ca^{2+}. Reducing the Ca^{2+} concentration to 1·25 mM did not affect the renin stimulatory response but blunted the vasodilation.

3. High K^+ concentration reversed the forskolin-induced renin secretion and vasodilation. Conversely, forskolin reversed the high K^+-induced renin inhibition of renin secretion and vasoconstriction. These effects of forskolin and high K^+ were absent when Ca^{2+} was withheld from the perfusion medium. High renal perfusion pressure also reversed the forskolin-induced renin secretion.

4. Calmidazolium prevented the inhibition mediated by high K^+ and high perfusion pressure and thereby restored the forskolin-induced stimulation. Calmidazolium also caused a prompt and marked vasoconstriction.

5. The calmidazolium-induced stimulation of renin secretion was Ca^{2+}-dependent since the drug was ineffective in the absence of Ca^{2+}. On the other hand, the prompt and potent vasoconstriction was present even in the Ca^{2+}-free medium.

6. These results support the hypothesis that cyclic AMP stimulates renin secretion by a mechanism which involves a lowering of membrane permeability to Ca^{2+} in addition to lowering cytosolic Ca^{2+} concentration. High K^+ and high renal perfusion pressure inhibit renin secretion by raising the membrane permeability to Ca^{2+}, thereby raising the intracellular Ca^{2+} concentration which then inhibits renin secretion by a calmodulin-dependent process. A further general conclusion from these studies is that membrane permeability to Ca^{2+} and cellular Ca^{2+} concentration are of central importance in the control of renin secretion and renal blood flow.

INTRODUCTION

Although adenosine 3′,5′-phosphate (cyclic AMP) and Ca^{2+} have both been implicated in the control of renin secretion, the precise role of each has been uncertain. An abundant amount of evidence suggests that cyclic AMP stimulates whereas Ca^{2+} inhibits renin secretion (Peart, 1977; Fray, 1977; Harada & Rubin, 1978; Park & Malvin, 1978; Park, Han & Fray, 1981; Churchill & Churchill, 1982; Naftilan & Oparil, 1982; Ginesi, Munday & Noble, 1983; Fray, Lush & Valentine, 1983*b*; Hackenthal, Schwertschlag & Taugner, 1983; Kurtz, Pfeilschifter & Bauer, 1984; Fray & Lush, 1984; O'Dea, Hansen & Mirkin, 1984; Bondar, Cadnapaphornchai, McDonald & Taher, 1984). In an attempt to determine which of these two cellular signals play a possible primary role, Churchill & Churchill (1982) have suggested that Ca^{2+} is the final messenger which controls renin secretion and that cyclic AMP acts at some intermediate step along the Ca^{2+} cascade. This view was based on the observations showing that factors known to raise cytosolic Ca^{2+} concentration inhibited the renin secretion stimulated by cyclic AMP mechanisms (Vandongen & Peart, 1974; Churchill & Churchill, 1982; Schwertschlag & Hackenthal, 1982; Knepel, Reimann & Nutto, 1982). Bondar *et al.* (1984), on the other hand, have proposed that cyclic AMP is the second messenger and that Ca^{2+} may be of lesser importance. These conclusions were arrived at from experiments showing that dibutyryl cyclic AMP stimulated renin secretion in the presence of the Ca^{2+} ionophore A23187. In an attempt to resolve this controversy and to obtain insights into a potential functional coupling at the cellular level in the control of renin secretion and renal blood flow, we have used the isolated perfused kidney, a preparation known to respond to cyclic AMP- and Ca^{2+}-related factors, especially to changes in renal perfusion pressure. The results show that under certain circumstances Ca^{2+} inhibits the cyclic AMP stimulatory response whereas under others cyclic AMP reverses the Ca^{2+} inhibitory response. The inhibition appears to involve calmodulin.

The data presented here are in general agreement with the view of Churchill & Churchill (1982) that Ca^{2+} is a final common pathway whereby extracellular events are coupled to renin secretion; the results also agree with the recently advanced hypothesis that Ca^{2+} permeability of the juxtaglomerular cell membrane is of central importance in the mechanisms controlling renin secretion (Fray *et al.* 1983*b*). Since renal perfusate flow may also be monitored in this preparation, the interaction between cyclic AMP and Ca^{2+} in the control of flow has been examined and discussed in the context of a recently published hypothesis on the role of Ca^{2+} in the control of renal blood flow (Lush & Fray, 1984).

METHODS

Male Sprague–Dawley rats (185–330 g) from Charles River Breeding Laboratories (N. Wilmington, MA, U.S.A.) were fed a Na^+-deficient diet containing 2 mmol Na^+/kg rat chow (BioServe, Frenchtown, NJ, U.S.A.) and tap water. This feeding regimen was followed for at least 1 week before the right kidney was isolated and prepared for perfusion by a technique described previously (Fray, 1976). Briefly, each rat was anaesthetized with sodium pentobarbitone (40 mg/kg, I.P.) and placed on a surgical stand. Through an abdominal mid-line incision, the right kidney was exposed, cannulated and then removed. The kidney was transferred to the top of a perfusion reservoir in

a temperature-controlled box (37 °C) and perfused with a solution containing 20 g bovine serum albumin/l (*fraction* V. Miles Laboratories, Eckert, IN, U.S.A.).

Each kidney was perfused for several periods, each lasting 15 min. The first period was used for equilibration, during which renin secretion and perfusate flow were allowed to stabilize. Perfusion medium after this period was discarded whereas a 1 ml sample of medium was collected at the end of subsequent periods and saved for renin determination. The basic perfusion solution was a Krebs–Henseleit bicarbonate buffer with varying concentrations of Ca^{2+} and/or K^+. Medium with the addition or subtraction of Ca^{2+} lacked ionic substitution. In high-K^+ medium, however, KCl was substituted for NaCl in equimolar concentration. The medium was gassed with 95% O_2 and 5% CO_2. The kidneys were usually perfused at a pulsatile pressure (pulse 30 mmHg, mean 100 or 150 mmHg) delivered by a Watson–Marlow pump. The pressure was measured by a manometer placed into the perfusion line just before the kidney, and perfusate flow was measured by a timing syphon placed just beneath the kidney in the reservoir as described previously (Fray, 1976). Flow was measured and recorded at 5 min and again at the end of each period (15 min). The three basic experimental procedures followed are described below.

Forskolin dose–response relationship and the effect of Ca^{2+} concentration. To study the effects of forskolin on renin secretion and renal perfusate flow kidneys were perfused for four periods of 15 min each. The first period served as control during which the medium contained 5 mM-Ca^{2+}. In the subsequent three experimental periods the medium contained 10^{-7}, 10^{-6} and 10^{-5} M-forskolin, respectively. At the end of each perfusion period a 1 ml aliquot was collected for determination of renin activity and the kidney switched to a new reservoir containing fresh medium and a higher concentration of forskolin. In an additional set of kidneys these experiments were repeated except that Ca^{2+} in the medium was lowered to 1·25 mM. It may be of importance to note that each kidney served as its own control whereby experimental periods could be compared with the control period.

Interaction between forskolin and high K^+ and Ca^{2+}. To study the interaction between forskolin and high K^+ and a possible effect of Ca^{2+} three series of experiments were conducted. In the first series, kidneys were perfused for three periods. The first period served as control. During the second period the kidneys were exposed to forskolin (10^{-5} M) alone, whereas in the third period they were exposed to forskolin (10^{-5} M) plus high (50 mM) K^+. In the second series of experiments a similar three-period strategy was adopted. The first period served as control and the second the kidneys were exposed to high K^+ alone and the third to high K^+ plus forskolin (10^{-5} M). In the third series, one set of kidneys were exposed to control medium, 10^{-5} M-forskolin, and forskolin plus high K^+, in that sequence for three periods, whereas in another set they were exposed to a similar sequence but with the medium containing low Ca^{2+} (1·25 mM) or no Ca^{2+} at all.

Interaction between forskolin, high K^+, high pressure and calmodulin. To study the role of calmodulin in the stimulatory effect of forskolin and the inhibitory effect of high K^+ and high renal perfusion pressure four series of experiments were conducted. In the first series, kidneys were perfused for four periods with the initial period serving as control. During the second period they were exposed to forskolin (10^{-5} M) alone, the third to forskolin plus high K^+, and the fourth to forskolin plus high K^+ plus calmidazolium (50 μM). In the second series, they were also perfused for four periods, with the initial period serving as control. During the second period they were exposed to forskolin alone, during the third to forskolin plus high perfusion pressure (150 mmHg), and during the fourth to forskolin plus high pressure plus calmidazolium (50 μM). In a third series, kidneys were perfused for three periods, with the first serving as control, whereas the second had high K^+ alone and the third had high K^+ plus calmidazolium (50 μM). In the fourth series of experiments kidneys were perfused first for a control period and then for a period with calmidazolium (50 μM). Eight kidneys were perfused in this series, four kidneys with 5 mM-Ca^{2+} and four without Ca^{2+}.

Determination of renin secretion. At the end of each 15 min period a 1 ml sample of perfusion medium was removed from the perfusion reservoir and pipetted into a 5 ml plastic test-tube and frozen for later determination of perfusate renin concentration using excess nephrectomized-dog plasma for substrate as previously described (Fray, 1976). Renin secretion as averaged over the 15 min period was determined by multiplying the perfusate renin concentration by the perfusion volume and the reciprocal of the kidney wet weight (ng angiotensin I/h.g). Each kidney served as its own control. Statistical significance was assessed using paired and unpaired *t* tests.

Forskolin was purchased from Calbiochem, La Jolla, CA, U.S.A., and calmidazolium was purchased from Boehringer Mannheim, West Germany.

RESULTS

Forskolin dose–response relationship and the effect of Ca^{2+}

Forskolin stimulated renin secretion in a dose-dependent manner with the maximal effect at 10^{-5} M (Fig. 1*A*). Lowering extracellular Ca^{2+} from 5 to 1·25 mM increased basal renin secretion 3-fold from 498±141 to 1494±289 ng/h.g and shifted the forskolin dose–response curve upward by approximately 1000 ng/h.g, without changing the forskolin concentration required for 50% of maximal stimulation. Perfusate flow from the kidneys increased from 16±1 ml/min.g during control to 20±2 ml/min.g at the highest concentration of forskolin in 5 mM-Ca^{2+} ($P < 0·05$) and from 21±2 to 23±2 ml/min.g in 1·25 mM-Ca^{2+} (Fig. 1*B*). It appears, therefore, that

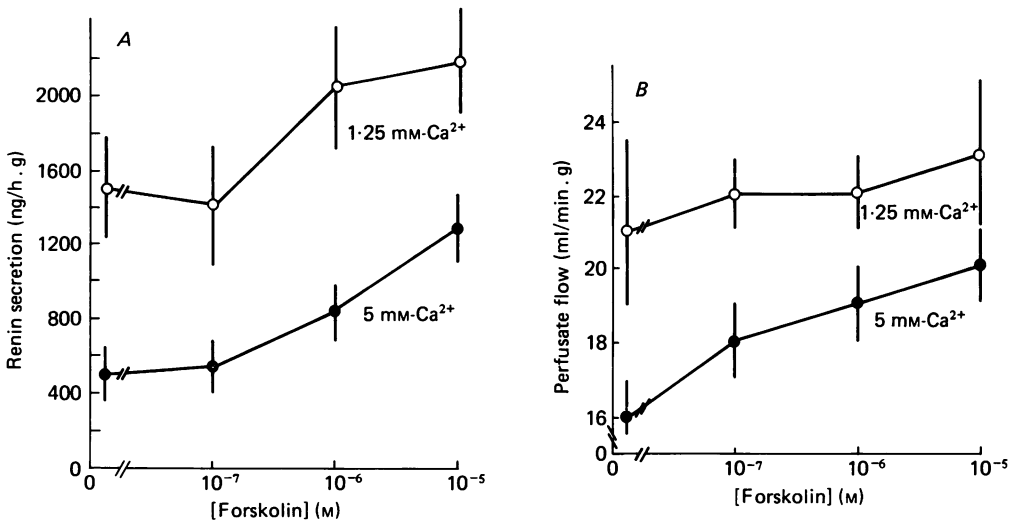

Fig. 1. Forskolin dose–response relationship and the effects of lowering extracellular concentration of Ca^{2+} from 5 mM ($n = 6$, ●) to 1·25 mM ($n = 5$, ○). *A* shows the renin secretion response whereas *B* shows perfusate flow. Points represents mean±S.E. of mean.

at the lower Ca^{2+} concentration forskolin retained its stimulatory effect on renin secretion but not the vasodilatory effect. In both the present study in isolated perfused kidneys and that of Kurtz *et al.* (1984) in isolated juxtaglomerular cells in culture forskolin at 10^{-5} M had a maximum stimulatory effect on renin secretion; therefore this concentration was used in all the relevant subsequent experiments.

To establish time-dependent changes in renin secretion and perfusate flow six kidneys were perfused with fresh control medium (5 mM-Ca^{2+}) for four successive periods each for 15 min. Renin secretion for each period was 127±36, 124±46, 150±67 and 136±61 ng/h.g, respectively. Perfusate flow was 13±2, 13±2, 13±1 and 14±2 ml/min.g for each of four successive periods.

Interaction between forskolin stimulation and inhibition of renin secretion by 50 mM-K^+

Figs. 2 and 3 show the antagonistic effect of forskolin and 50 mM-K^+ on renin secretion. When kidneys were first exposed to forskolin alone, renin secretion increased and then decreased back to control level when exposed to forskolin plus

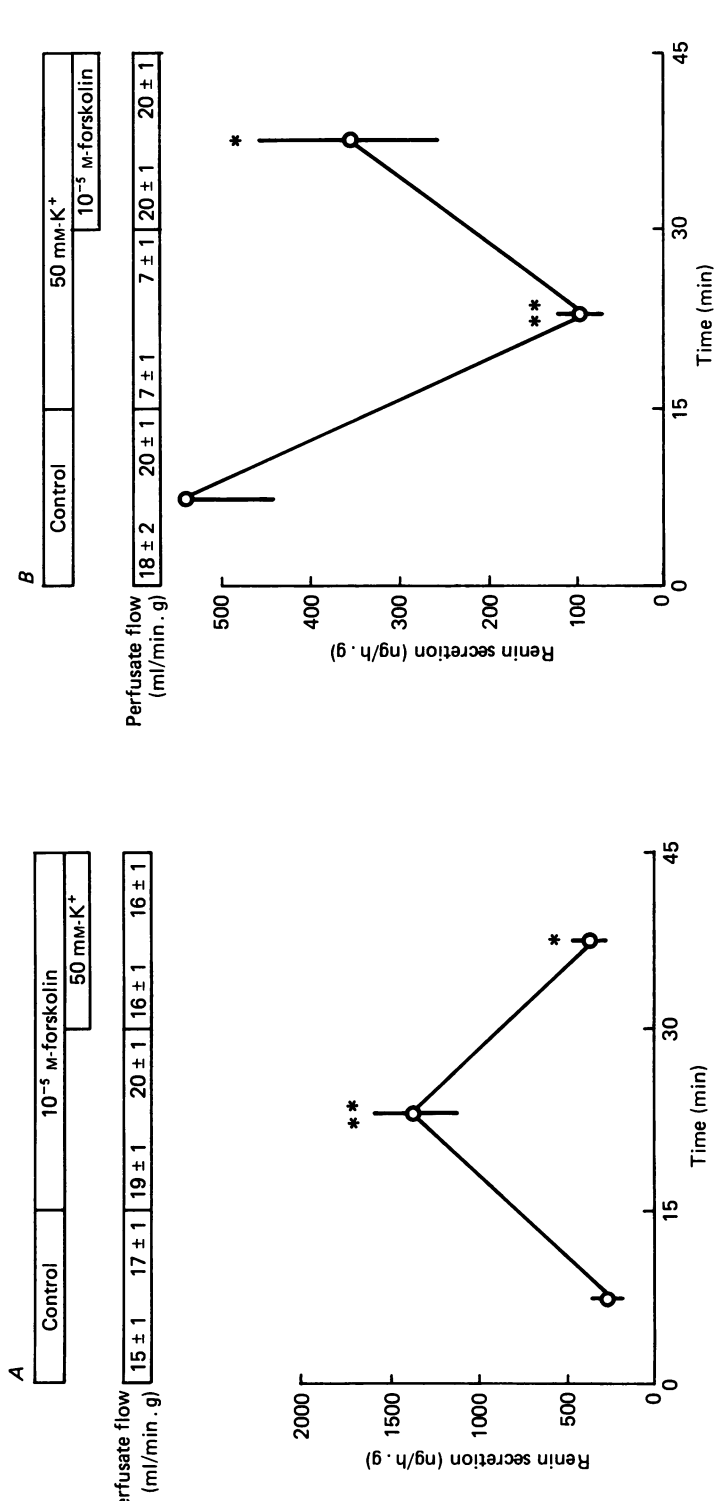

Fig. 2. The interaction between 10^{-5} M-forskolin and 50 mM-K^+ in controlling renal perfusate flow and renin secretion. *A* shows the effect of forskolin alone and then forskolin plus K^+ ($n = 11$). *B* shows the effect of K^+ alone and then K^+ and forskolin ($n = 5$). The medium Ca^{2+} concentration was 5 mM in these experiments. * and ** indicate $P < 0{\cdot}05$ and $< 0{\cdot}025$ compared to the preceding period, respectively. Since all periods were observed in the same kidney each kidney served as its own control in paired analyses. This applies to all experiments in the subsequent Figures.

50 mM-K^+ (Fig. 2*A*). On the other hand, kidneys first exposed to high K^+ and then to both high K^+ and forskolin showed a decrease and then an increase in renin secretion (Fig. 2*B*). Renin secretion during high K^+ and forskolin (Fig. 2*B*) was higher than high K^+ alone ($P < 0.05$) but not significantly different from control. Similarly, secretion during forskolin and high K^+ (Fig. 2*A*) was lower than during forskolin alone ($P < 0.05$) but not significantly different from control.

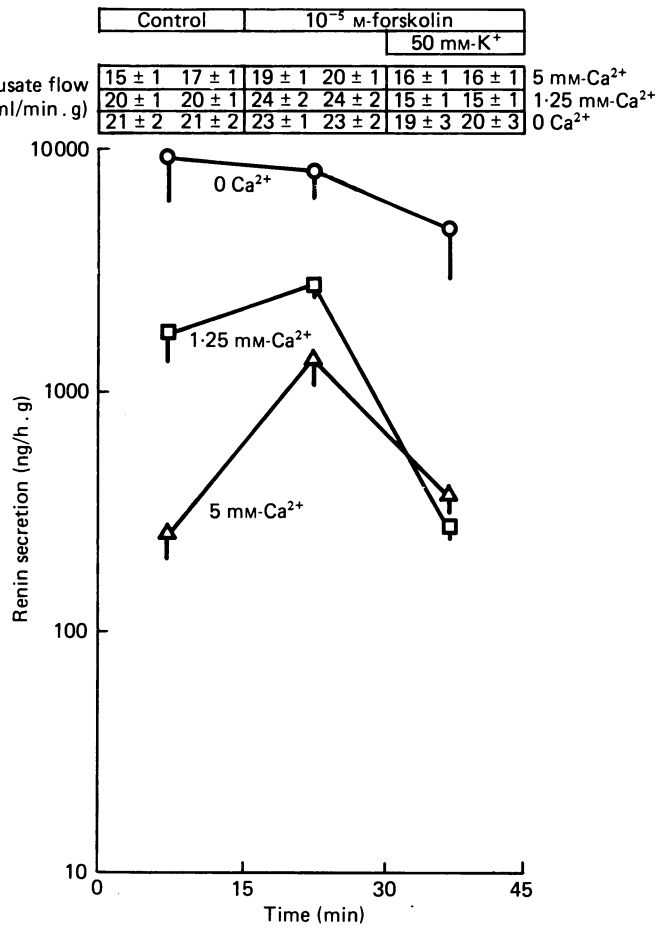

Fig. 3. Effect of lowering Ca^{2+} on the interaction between 10^{-5} M-forskolin and 50 mM-K^+ in controlling renal perfusate flow and renin secretion. Perfusion medium contained 5 mM-Ca^{2+} ($n = 11$), 1·25 mM-Ca^{2+} ($n = 6$), or 0 Ca^{2+} ($n = 4$). Renin secretion is shown on a logarithmic scale.

Fig. 3 shows that 50 mM-K^+ reversed the forskolin-stimulated renin secretion to the same fixed level providing Ca^{2+} was present in the perfusion medium. Thus, although the basal and forskolin-induced renin secretion were greater in the 1·25 mM-Ca^{2+} medium (2700 ± 199 compared to 1363 ± 242 ng/h.g in 5 mM-Ca^{2+}), 50 mM-K^+ brought the secretory rate down to a value not statistically different from that in the 5·0 mM-Ca^{2+} medium. Removal of Ca^{2+} from the perfusion medium produced a very different effect (Fig. 3). Removing Ca^{2+} alone increased basal renin

secretion to an exceedingly high level and forskolin was no longer able to stimulate secretion further. High K^+ was ineffective in inhibiting renin secretion in the Ca^{2+}-free perfusate.

Renal perfusate flow showed similar directional changes as renin secretion. Fig. 2*A* shows that forskolin caused a slight increase in perfusate flow which was promptly reversed by high K^+ ($P < 0{\cdot}05$). Conversely, Fig. 2*B* shows that high K^+ caused a significant decrease in perfusate flow ($P < 0{\cdot}05$), the inhibitory effect of which was reversed by forskolin. Fig. 3 shows that this general reversal of flow was independent of the extracellular concentration of Ca^{2+}, at least in the range of 1·25–5·0 mM. Fig. 3 also demonstrates a similar pattern in renin secretion, suggesting a possible interaction or common mechanisms controlling renal perfusate flow and those controlling renin secretion. It seems important to note that the vasodilation induced by forskolin and the vasoconstriction induced by high K^+ were absent in the Ca^{2+}-free medium (Fig. 3). It appears, therefore, that removing Ca^{2+} from the extracellular fluid renders forskolin completely ineffective in stimulating renin secretion or causing vasodilation. It might be well to note, however, that in the absence of extracellular Ca^{2+} renin secretion proceeded at substantially high rates and the kidneys were vasodilated. Thus the effects of forskolin on both renin secretion and vasodilation might be no longer apparent.

Effect of high K^+ and high perfusion pressure on forskolin-induced renin secretion and the role of calmodulin

Fig. 4 shows that either high perfusate K^+ (Fig. 4*A*) or high perfusion pressure (Fig. 4*B*) inhibited the forskolin-induced elevation of renin secretion and that calmidazolium, a blocker of calmodulin, reversed the inhibitory effect. Forskolin stimulated renin secretion but either 50 mM-K^+ (Fig. 4*A*) or 150 mmHg (Fig. 4*B*) inhibited the stimulation. To test for calmodulin involvement in the inhibition of renin secretion, calmidazolium was infused during a fourth period in the presence of forskolin and 50 mM-K^+ or 150 mmHg. Calmidazolium restored renin secretion to a level not statistically significantly different from that with forskolin alone. Thus, these results suggest that calmidazolium completely blocked the inhibitory effect of high K^+ and high pressure, suggesting that calmodulin is involved at some step of the inhibitory action of high K^+ and high pressure. On the other hand, the stimulatory effect of forskolin on renin secretion appears to be calmodulin-independent.

Fig. 4 also shows striking changes in renal perfusate flow worthy of mention. High K^+ promptly lowered the flow during forskolin infusion (Fig. 4*A*) and calmidazolium lowered it further. Conversely, high perfusion pressure increased flow over the forskolin levels (Fig. 4*B*) though calmidazolium reversed the rise in flow. These results suggest that calmidazolium lowered flow when given to kidneys exposed to forskolin and high K^+ or high perfusion pressure. The effect of calmidazolium was time-dependent. After just 5 min of calmidazolium, flow decreased to 65% of pre-calmidazolium values and after 15 min it decreased further to 43% (Fig. 4*A*). Fig. 5 shows that calmidazolium not only reversed the inhibitory effect of 50 mM-K^+ but further stimulated renin secretion; calmidazolium had no effect on the intense vasoconstriction.

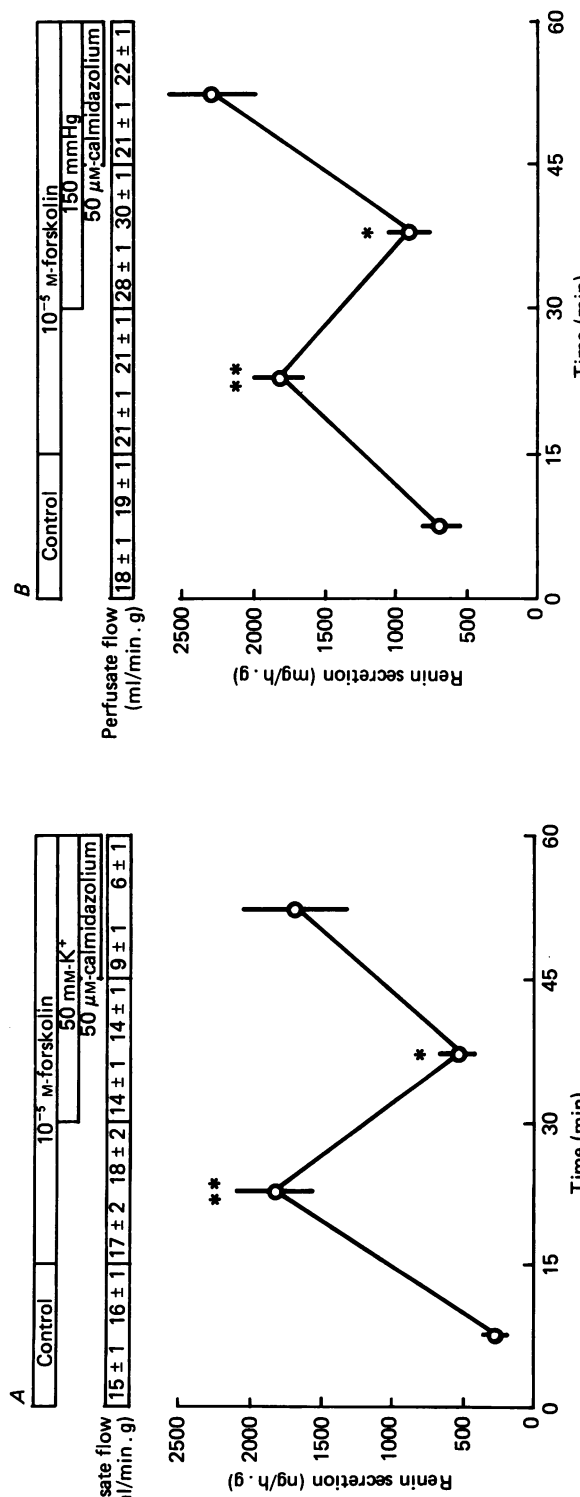

Fig. 4. The effect of 50 μM-calmidazolium on the interaction between 10^{-5} M-forskolin and 50 mM-K^+ (*A*) or 150 mmHg (*B*) on perfusate flow and renin secretion. Five kidneys were perfused for each series of experiments. * indicates $P < 0.05$ compared to forskolin alone and ** indicates $P < 0.05$ compared to control.

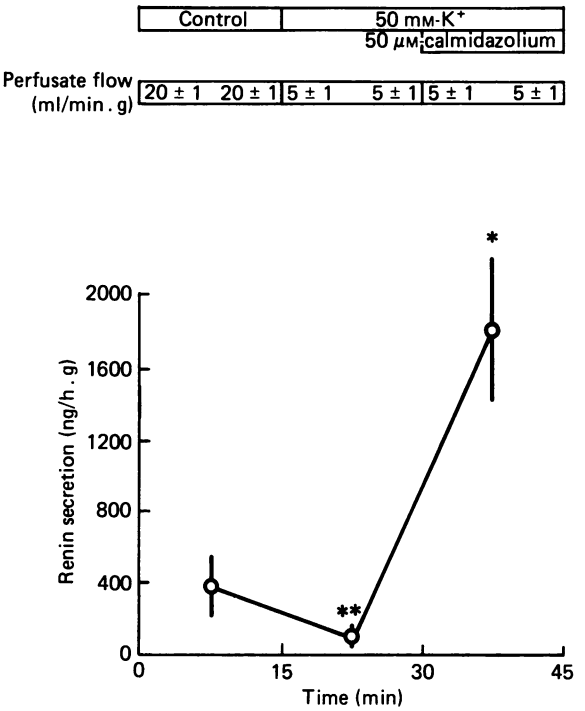

Fig. 5. The effect of 50 μM-calmidazolium on the inhibition of renin secretion by 50 mM-K^+ perfusate ($n = 5$). * indicates $P < 0{\cdot}05$ compared to 50 mM-K^+ and ** indicates $P < 0{\cdot}05$ compared to control.

Additional experiments were designed to examine the interaction between calmidazolium and Ca^{2+} in controlling renin secretion and perfusate flow. Fig. 6 shows that in the presence of Ca^{2+} calmidazolium caused a 10-fold increase in renin secretion but a prompt reduction in perfusate flow. On the other hand, in the absence of Ca^{2+} basal renin secretion increased over 30-fold and calmidazolium caused no further increase, though it still caused a reduction in perfusate flow. Thus, calmidazolium consistently decreased renal perfusate flow but stimulated or had no effect on renin secretion, thereby dissociating changes in renin secretion from perfusate flow.

DISCUSSION

The data presented here deal primarily with the interaction between cyclic AMP and Ca^{2+} in controlling renin secretion, along with concurrent observations on the association and dissociation between changes in renin secretion and perfusate flow through the kidney. High extracellular K^+ concentration or high renal perfusion pressure inhibited, albeit not completely, renin secretion stimulated by forskolin. Conversely, forskolin reversed the inhibition of renin secretion caused by high-K^+ perfusate. Both the stimulatory and inhibitory effects were remarkably independent of extracellular Ca^{2+} concentration (at least in the range of 1·25–5·0 mM-Ca^{2+}) though Ca^{2+} has been suggested to be an important factor in the cellular mechanisms

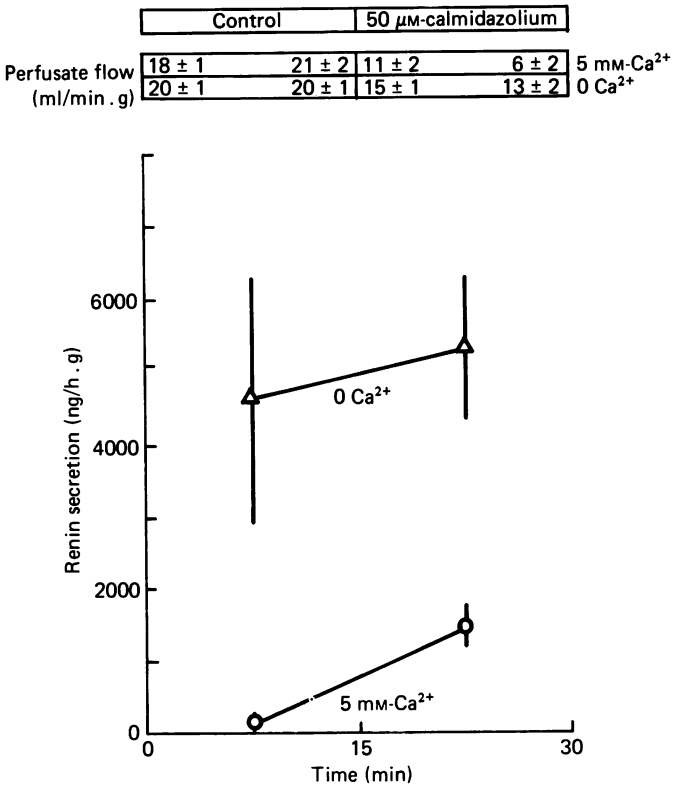

Fig. 6. The effect of calmidazolium on perfusate flow and renin secretion in the presence (5 mM-Ca^{2+}, $n = 4$) or absence (0 Ca^{2+}, $n = 4$) of Ca^{2+}.

controlling renin secretion. This indicates that the chemical gradient for Ca^{2+} may not be a primary determining factor controlling renin secretion under these conditions. Although there is much evidence that factors which increase cyclic AMP also stimulate renin secretion, there is no clear correlation between cyclic AMP and renin secretion in juxtaglomerular cells (Kurtz *et al.* 1984). This observation led Kurtz *et al.* (1984) to conclude that renin secretion is controlled by Ca^{2+} and not by cyclic AMP. The following arguments are submitted as possible explanations for these discrepancies in the context of current thinking on renin secretion and on the stretch receptor hypothesis for renin secretion. The current thinking as embodied in the stretch receptor hypothesis holds that cytosolic Ca^{2+} is a final common pathway through which stimulatory and inhibitory factors act to control renin secretion, in such a way that factors which tend to lower cytosolic Ca^{2+} concentration increase renin secretion whereas those which tend to raise Ca^{2+} decrease it. Although intracellular stores of Ca^{2+} may be of importance in the final regulation, as recently shown with TMB-8 (Fray & Lush, 1984), Ca^{2+} flux at the level of the plasma membrane of the juxtaglomerular cell may be of primary importance (Baumbach & Leyssac, 1977) since, in general, factors which increase Ca^{2+} influx decrease secretion, whereas those which promote efflux increase secretion (Park *et al.* 1981; Fray, 1980*a*; Fray *et al.*

1983*b*). Further compelling evidence for Ca^{2+} flux at the cellular level has been provided recently by Kurtz *et al.* (1984). It may be important to note that a great deal of our conceptual understanding of the role of Ca^{2+} in renin secretion has come from the proposition that renin secretion is analagous to smooth muscle contraction and relaxation regulated by cytosolic Ca^{2+} concentration since the renin-secreting juxtaglomerular cell is a modified smooth muscle cell (Peart, 1977). When flow through the kidney is also considered, the analogy is even more interesting since it points to a certain coupling between renin secretion and blood flow, as recently predicted on theoretical grounds (Lush & Fray, 1984).

Renin secretion

Forskolin stimulated renin secretion by a mechanism independent of extracellular Ca^{2+} (in the range of 1·25–5 mM). The general stimulatory effect shown in these studies is in agreement with that shown by other workers (Kurtz *et al.* 1984; Schwertschlag & Hackenthal, 1982). Furthermore, Kurtz *et al.* (1984) have shown that forskolin stimulates renin secretion by increasing cyclic AMP production in juxtaglomerular cells and that it decreases Ca^{2+} influx and Ca^{2+} permeability of juxtaglomerular cells. Figs. 1*A* and 3 show the characteristics of the forskolin stimulation of renin secretion when the extracellular Ca^{2+} concentration was in the range of 1·25–5·0 mM; the forskolin concentration for 50% of maximal stimulation was the same and the absolute magnitude of the forskolin-induced renin secretion was almost identical, despite a 7-fold higher basal renin secretion in the lower-Ca^{2+} medium (1·25 mM). These findings are suggestive of a mechanism of forskolin stimulatory action independent of or non-competitive with extracellular Ca^{2+} concentration. What is also striking is that raising the intracellular Ca^{2+} concentration (by depolarizing cells with high K^+) reverses the stimulatory effect of forskolin to the same absolute level regardless of whether the kidneys were exposed to 1·25 or 5 mM-Ca^{2+}. These observations and the general conclusion that raising intracellular Ca^{2+} blocks the renin secretory response induced by cyclic AMP-mediated agents (or by an analogue of cyclic AMP) are in agreement with several reports (Vandongen & Peart, 1974; Logan & Chatzilias, 1980; Churchill & Churchill, 1982; Knepel *et al.* 1982; Kurtz *et al.* 1984). They disagree with the conclusions of a recently published study showing that cyclic AMP-mediated renin secretion is unaffected by A23187, the Ca^{2+} ionophore which is believed to raise cellular Ca^{2+} (Bondar *et al.* 1984). An obvious explanation for the discrepancy may be that the concentration of A23187 used in the study of Bondar *et al.* (1984) was insufficient to raise the level of cytosolic Ca^{2+} to a level required for inhibition, as suggested by the authors. This explanation is strengthened further by the lack of effect of the ionophore on reducing renal blood flow which should be a consequence of the increase in cytosolic Ca^{2+} in renal vascular smooth muscle cells. Indeed, Fynn, Onomakpome & Peart (1977) have shown that A23187 caused a prompt renal vasoconstriction and marked renin inhibition. Thus, the conclusions of Bondar *et al.* (1984) can only be tentative at the present time until additional studies are provided to clarify this point. Kurtz *et al.* (1984) have postulated that forskolin increases cellular levels of cyclic AMP, which subsequently stimulates renin secretion by decreasing Ca^{2+} influx through decreasing Ca^{2+} permeability of the juxtaglomerular cells. This postulated change in Ca^{2+} permeability can account for

some of the present findings. For example, the renin secretion stimulated by forskolin was readily inhibited by experimental manipulations which increase Ca^{2+} influx (that is, high K^+ concentration and high perfusion pressure) or vice versa (Figs. 2–4). These cyclic AMP-mediated changes in Ca^{2+} permeability have been demonstrated in many cell types (Rinaldi, Capony & Demaille, 1982; Reuter, 1983; Bean, Nowycky & Tsien, 1984; Cachelin, de Peyer, Kokubum & Reuter, 1983; Siegelbaum & Tsien, 1983; Mauger, Poggioli, Guesdon & Claret, 1984). Recent evidence has also suggested another mechanism whereby forskolin (and thereby cyclic AMP) may stimulate renin secretion. According to the available evidence, the renin secretion stimulated by isoprenaline and adrenaline is completely blocked by ouabain (Park *et al.* 1981; Churchill & Churchill, 1982; Fray, 1980*a*), suggesting that cyclic AMP (raised by forskolin or β-adrenergic receptor activation) stimulates renin secretion by increasing Ca^{2+} efflux by a Na^+–Ca^{2+} exchange mechanism or by the Ca^{2+}-ATPase (Fray *et al.* 1983*b*). A moderate but significant stimulation of renin secretion by forskolin in high-K^+ medium (Fig. 2*B*) might also be suggestive of an independent mechanism of stimulation by cyclic AMP. In this case Ca^{2+} and cyclic AMP may respectively inhibit and stimulate renin secretion without direct interaction, but the rate of secretion may be dependent upon the net effect of the strength of the Ca^{2+} inhibitory and cyclic AMP stimulatory signals.

Calmidazolium, a calmodulin antagonist far more potent than the more commonly used trifluoperazine (Gietzen, Sadorf & Bader, 1982; Anderson, Coll & Murphy, 1984), stimulated basal renin secretion (Fig. 6) and completely reversed the inhibitory effect of high-K^+ perfusate (Figs. 4*A* and 5) and high perfusion pressure (Fig. 4*B*). These findings are in good agreement with several previous reports (Hackenthal *et al.* 1983; Schwertschlag, Hackenthal, 1983; Matsumura, Miyawaki & Morimoto, 1984; Churchill & Churchill, 1983; Kawamura & Inagami, 1983; Fray *et al.* 1983*a*). It is well documented that high-K^+ perfusate and high perfusion pressure inhibit renin secretion as a result of an increased intracellular Ca^{2+} concentration subsequent to an increased Ca^{2+} influx (Park *et al.* 1981; Fray, 1980*a*). Thus, the stimulatory action of calmidazolium is most likely through blocking the inhibitory step of Ca^{2+} via calmodulin on the renin secretory process. One important note on this point may be that the Ca^{2+}–calmodulin complex is an inhibitory signal for renin secretion, in contrast to the stimulatory signal for many other glandular secretions, and the molecular mechanism(s) of the apparent opposite secretory response to the same cellular signal may be at the step of signal transduction by the Ca^{2+}–calmodulin complex.

Flow

Although the effect of forskolin on renal perfusate flow was not as striking as on renin secretion, the interaction between forskolin and other factors is quite telling and may provide clues as to the control of renal blood flow. Compared to the secretory response of renin, renal perfusate flow was much less responsive to changes in perfusate Ca^{2+} concentration and to forskolin. By lowering perfusate Ca^{2+} from 5 to 1·25 mM renal perfusate flow increased by only 31 % compared to a 200 % increase in renin secretion (Fig. 1*A vs. B*). Similarly, in response to forskolin at 10^{-5} M, renal perfusate flow showed no change at 1·25 mM-Ca^{2+} and an increase of 25 % at 5 mM-Ca^{2+} compared to 47 % and 140 % increases in renin secretion at the respective Ca^{2+}

concentrations (Fig. 1*A vs. B*). Such disparate responses of the two were revealed throughout the present experiments. The reactivity of renal vascular smooth muscles seems intact, in that a high-K^+ perfusate caused severe vasoconstriction which was then completely reversed by forskolin (Fig. 2*B*). These findings indicate that the low flow response compared to that of renin secretion seems attributable to a low sensitivity of renal vascular smooth muscles to this range of Ca^{2+} concentration. That is, much higher intracellular Ca^{2+} concentration seems necessary to modulate vascular tone than to modulate renin secretion. For example, the intracellular Ca^{2+} concentration at 5 mM-extracellular Ca^{2+} seems sufficiently high to inhibit most of the renin secretion but caused only moderate vasoconstriction. Of course, the disparate response of renin secretion and vasconstriction could be due to difference in sensitivity of the juxtaglomerular cell and the other vascular smooth muscle cells. This point will be discussed below with respect to the effect of calmidazolium on renal perfusate flow and renin secretion.

The consistent vasoconstrictor response to calmidazolium was unexpected on the basis of the current theory that contraction of the smooth muscle is initiated by the phosphorylation of a pair of myosin light chains by myosin kinase activated by Ca^{2+}–calmodulin complex (Adelstein, 1980). The underlying mechanism for vasoconstriction by calmidazolium is not clear at present. Calmidazolium reduced renal perfusate flow with and without perfusate Ca^{2+}, albeit the magnitude of reduction of flow was less without Ca^{2+}. This suggests that the site of vasoconstrictor action of calmidazolium is not directly dependent upon extracellular Ca^{2+} concentration. Furthermore, when the intracellular Ca^{2+} was supposed to be high as in a high-K^+ perfusate (Fig. 5), calmidazolium did not reduce perfusate flow.

Interaction between renin secretion and flow

Although in some instances there was an inverse relationship between renin secretion and renal vascular resistance, in other instances closer examination showed that there was also a direct relationship. Forskolin caused a substantial increase in renin secretion and this was associated with a small but significant increase in flow (Figs. 1*A*, 3 and 4*A*). On the other hand, high K^+ caused a marked suppression of renin secretion and this was associated with a potent vasoconstriction (Fig. 2*B*). However, when calmidazolium was given by itself (Fig. 6) or combined with forskolin and high K^+ there was a marked stimulation of renin secretion but an attended vasoconstriction (Fig. 4*A*). In addition, when Ca^{2+} was present in the perfusion medium calmidazolium caused a potent stimulation of renin secretion and a prompt vasoconstriction; but when Ca^{2+} was absent calmidazolium did not affect renin secretion though it still caused the vasoconstriction (Fig. 6). Thus a clear demonstration of a dissociation between flow through the kidney and renin secretion may be achieved under a wide variety of circumstances. This may be of importance since it has become customary to associate increased renin secretion with vasodilation and decreased secretion with vasoconstriction. The chief motivation for this association is the observation that decreased cytosolic Ca^{2+} concentration correlates with vasodilation and increased renin secretion, and increased Ca^{2+} with vasoconstriction and decreased renin secretion (Peart, 1977). The present study strongly indicates a difference in the control mechanism of renin secretion and renal vascular tone at the level of the dependence on Ca^{2+} concentration and calmodulin activity. Recent

theoretical arguments have been advanced which also suggest that this simplistic view may not be sufficient for a proper understanding of the interaction of renin secretion and flow at the cellular level of the renal afferent arteriole (Lush & Fray, 1984).

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REFERENCES

ADELSTEIN, R. S. (1980). Regulation and kinetics of the actin–myosin–ATP interaction. *Annual Review of Biochemistry* **49**, 921–956.

ANDERSON, K. W., COLL, R. J. & MURPHY, A. J. (1984). Inhibition of skeletal muscle sarcoplasmic reticulum CaATPase activity by calmidazolium. *Journal of Biological Chemistry* **259**, 11487–11490.

BAUMBACH, L. & LEYSSAC, P. P. (1977). Studies on the mechanism of renin release from isolated superfused rat glomeruli: effects of calcium, calcium ionophore and lanthanum. *Journal of Physiology* **273**, 745–764.

BEAN, B. P., NOWYCKY, M. C. & TSIEN, R. W. (1984). β-adrenergic modulation of calcium channels in frog ventricular heart cells. *Nature* **307**, 371–375.

BONDAR, N., CADNAPAPHORNCHAI, P., MCDONALD, F. D. & TAHER, S. (1984). Mechanism of effect of dibutyryl cyclic adenosine 3′,5′-monophosphate on canine renal renin release. *Journal of Physiology* **355**, 33–41.

CACHELIN, A. B., DEPEYER, J. E., KOKUBUN, S. & REUTER, H. (1983). Ca^{2+} channel modulation by 8-bromo-cyclic-AMP in cultured heart cells. *Nature* **304 4**, 462–464.

CHURCHILL, P. C. & CHURCHILL, M. C. (1982). Isoproterenol-stimulated renin secretion in the rat: second messenger roles of Ca and cyclic AMP. *Life Sciences* **30**, 1313–1319.

CHURCHILL, P. C. & CHURCHILL, M. C. (1983). Effects of trifluoperazine on renin secretion of rat kidney slices. *Journal of Pharmacology and Experimental Therapeutics* **224**, 68–72.

FRAY, J. C. S. (1976). Stretch receptor model for renin release with evidence from perfused rat kidney. *American Journal of Physiology* **231**, 936–944.

FRAY, J. C. S. (1977). Stimulation to renin release in perfused kidney by low calcium and high magnesium. *American Journal of Physiology* **232**, F377–382.

FRAY, J. C. S. (1980*a*). Mechanism by which renin secretion from perfused rat kidneys is stimulated by isoprenaline and inhibited by high perfusion pressure. *Journal of Physiology* **308**, 1–13.

FRAY, J. C. S. (1980*b*). Stimulus-secretion coupling of renin: role of hemodynamic and other factors. *Circulation Research* **47**, 485–492.

FRAY, J. C. S. & LUSH, D. J. (1984). Stretch receptor hypothesis for renin secretion: the role of calcium. *Journal of Hypertension* **2**, suppl. 1, 19–23.

FRAY, J. C. S., LUSH, D. J., SHARE, D. S. & VALENTINE, A. N. D. (1983*a*). Possible role of calmodulin in renin secretion from isolated kidneys and renal cells: studies with trifluoperazine. *Journal of Physiology* **343**, 447–454.

FRAY, J. C. S., LUSH, D. J. & VALENTINE, A. N. D. (1983*b*). Cellular mechanisms of renin secretion. *Federation Proceedings* **42**, 3150–3154.

FYNN, M., ONOMAKPOME, N. & PEART, W. S. (1977). The effects of ionophores (A23187 and R02-2985) on renin secretion and renal vasoconstriction. *Proceedings of The Royal Society* **B 199**, 199–212.

GIETZEN, K., SADORF, I. & BADER, H. (1982). A model for the regulation of the calmodulin-dependent enzymes erythrocyte Ca^{2+} transport and brain phosphodiesterase by activators and inhibitors. *Biochemical Journal* **207**, 541–548.

GINESI, L. M., MUNDAY, K. A. & NOBLE, A. R. (1983). Secretion control for active and inactive renin: effects of calcium and potassium on rabbit kidney cortex slices. *Journal of Physiology* **344**, 453–463.

HACKENTHAL, E., SCHWERTSCHLAG, U. & TAUGNER, R. (1983). Cellular mechanisms of renin release. *Clinical and Experimental Hypertension, B, Theory and Practice* **A5 (7&8)**, 975–993.

HARADA, E. & RUBIN, R. P. (1978). Stimulation of renin secretion and calcium efflux from the isolated perfused cat kidney by noradrenaline after prolonged calcium deprivation. *Journal of Physiology* **274**, 367–379.

KAWAMURA, M. & INAGAMI, T. (1983). Calmodulin antagonists stimulate renin release from isolated rat glomeruli. *Endocrinology* **112**, 1857–1859.

KNEPEL, W., REIMANN, W. & NUTTO, D. (1982). On the mechanism of the vasopressin-induced inhibition of renin release. *Hormone and Metabolic Research* **14**, 157–160.

KURTZ, A., PFEILSCHIFTER, J. & BAUER, C. (1984). Is renin secretion governed by the calcium permeability of the juxtaglomerular cell membrane? *Biochemical and Biophysical Research Communications* **124**, 359–366.

LOGAN, A. G. & CHATZILIAS, A. (1980). The role of calcium in the control of renin release from the isolated rat kidney. *Canadian Journal of Physiology and Pharmacology* **58**, 60–66.

LUSH, D. J. & FRAY, J. C. S. (1984). Steady-state autoregulation of renal blood flow: a myogenic model. *American Journal of Physiology* **247**, R89–R99.

MATSUMURA, Y., MIYAWAKI, N. & MORIMOTO, S. (1984). Effects of W-7 and W-5 on renin release from rat kidney cortical slices. *Japanese Journal of Pharmacology* **36**, 268.

MAUGER, J. P., POGGIOLI, J., GUESDON, F. & CLARET, M. (1984). Noradrenaline, vasopressin, and angiotensin increase Ca^{2+} influx by opening a common pool of Ca^{2+} channels in isolated rat liver cells. *Biochemical Journal* **221**, 121–127.

NAFTILAN, A. J. & OPARIL, S. (1982). The role of calcium in the control of renin release. *Hypertension* **4**, 670–675.

O'DEA, R. F., HANSEN, J. A. & MIRKIN, B. L. (1984). Effect of calcium, sofium, and isoproterenol on renin secretion from disaggregated rat renal cortical cells. *Research Communications in Chemical Pathology and Pharmacology* **46**, 187–205.

PARK, C. S., HAN, D. S. & FRAY, J. C. S. (1981). Calcium in the control of renin secretion: Ca^{2+} influx as an inhibitory signal. *American Journal of Physiology* **240**, F70–74.

PARK, C. S. & MALVIN, R. L. (1978). The role of calcium in the control of renin release. *American Journal of Physiology* **235**, F22–25.

PEART, W. S. (1977). The kidney as an endocrine organ. *Lancet* **ii**, 543–548.

REUTER, H. (1983). Calcium channel modulation by neurotransmitters, enzymes and drugs. *Nature* **301**, 569–574.

RINALDI, M. L., CAPONY, J. P. & DEMAILLE, J. G. (1982). The cyclic AMP-dependent modulation of cardiac sarcolemmal slow calcium channels. *Molecular and Cellular Cardiology* **14**, 279–289.

SCHWERTSCHLAG, U. & HACKENTHAL, E. (1982). Forskolin stimulates renin release from the isolated perfused rat kidney. *European Journal of Pharmacology* **84**, 111–112.

SCHWERTSCHLAG, U. & HACKENTHAL, E. (1983). Trifluoperazine antagonizes inhibition of renin release by angiotensin II. *Clinical and Experimental Pharmacology and Physiology* **10**, 605–608.

SIEGELBAUM, S. A. & TSIEN, R. W. (1983). Modulation of gated ion channels as a mode of transmitter action. *Trends in Neuroscience* **6**, 307–313.

VANDONGEN, R. & PEART, W. S. (1974). Calcium dependence of the inhibitory effect of angiotensin on renin secretion in the isolated perfused kidney of the rat. *British Journal of Pharmacology* **50**, 125–129.