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POSSIBLE ROLE OF CALMODULIN IN RENIN SECRETION FROM ISOLATED RAT KIDNEYS AND RENAL CELLS: STUDIES WITH TRIFLUOPERAZINE

BY J. C. S. FRAY, D. J. LUSH, D. S. SHARE AND A. N. D. VALENTINE

From the Department of Biological Sciences, Hunter College of the City University of New York, New York, NY 10021, U.S.A. and the Department of Physiology, University of Massachusetts Medical School, Worcester, MA 01605, U.S.A.

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SUMMARY

1. Trifluoperazine, an inhibitor of calmodulin and calmodulin-directed secretion, was used to examine a possible role of calmodulin in renin secretion from isolated perfused kidneys and renal cortical cells.

2. In isolated perfused kidneys trifluoperazine stimulated basal renin secretion in a dose-dependent manner, with 10 μM causing no stimulation and 50 μM causing 167 % increase.

3. Trifluoperazine potentiated the elevated renin secretion induced by isoprenaline and low Ca in isolated kidneys.

4. In renal cortical cells trifluoperazine increased basal renin secretion and potentiated the secretion induced by Ca omission.

5. Cells homogenized immediately after 1 h exposure to trifluoperazine had a substantial reduction in soluble renin without any effect on the change in granular renin. In the absence of trifluoperazine, soluble renin increased with 0 Ca and decreased with 1·5 mM-Ca.

6. It is concluded that trifluoperazine stimulates renin secretion by a cellular mechanism possibly at the level of the juxtaglomerular cell. It is suggested that the role of trifluoperazine, and by inference calmodulin, in the secretion of renin may be quite different from its role in secretion of several other substances.

INTRODUCTION

Renin secretion may occur by a process quite different from that postulated for most other secretory substances, especially with regard to the role played by Ca (Peart, 1977; Fray, 1980). Whereas the process for most substances is generally believed to be initiated by a rise in cytoplasmic Ca (Douglas, 1968; Rubin, 1970), for renin it may be by a fall in Ca (Fray, 1980). Recently, it was suggested that calmodulin acts as an intracellular Ca-receptor in mediating the role of Ca in secretion (DeLorenzo, 1982; DeLorenzo, Freedman, Vohe & Maurer, 1979; Douglas & Nemeth, 1982; Krausz,

Address correspondence to: Dr J. C. S. Fray, Department of Biological Sciences, Hunter College of CUNY, 695 Park Avenue, Box 582, New York, NY 10021, U.S.A.

Wollheim, Siegel & Sharp, 1980; Steinhardt & Alderton, 1982). This suggestion was prompted mainly by results of experiments showing that trifluoperazine, as well as other antipsychotic drugs that block calmodulin, cause powerful inhibition of secretion in all the systems studied. In fact, the trifluoperazine-sensitive stage – and by inference, that stage which is mediated by calmodulin – of secretion has been postulated to be the fusion of secretory granules with the plasma membrane (Burgoyne, Geisow & Barron, 1982). Although calmodulin has not been implicated in the process of renin secretion, its general mediator role in almost all Ca-mediated processes (Cheung, 1980) justifies some investigation, especially since the process of renin secretion has been suggested to be analogous to that of other substances (Lester & Rubin, 1977; Chen & Poisner, 1976). This paper presents studies showing that whereas trifluoperazine causes inhibition of secretion in other systems, it caused powerful stimulation of renin secretion, suggesting that perhaps calmodulin may play a different role in renin secretion.

METHODS

Male Sprague–Dawley rats (235–350 g) from Charles River Breeding Laboratories (Wilmington, MA) were fed a Na-deficient diet containing 2 m-equiv. Na kg^{-1} (BioServe, Frenchtown, NJ) and tap water. This regimen was followed for at least one week before the right kidney was prepared for perfusion.

The kidneys were perfused by a procedure described previously (Fray, 1976). Briefly, rats were anaesthetized with Na pentobarbitone (50 mg kg^{-1}, I.P.) and placed on a stand. Through a mid-line abdominal incision the right kidney was exposed, cannulated, and then removed and placed in a perfusion reservoir in a temperature-controlled box (37 °C) and perfused at 100 mmHg mean pressure (pulsatile) with Krebs–Henseleit bicarbonate solution containing 20 g bovine serum albumin l^{-1}. (Fraction V, Miles Laboratories, Eckert, IN). The ionic concentrations of the Krebs–Henseleit solution were (mM): Na^+, 155; K^+, 4; Ca^{2+}, 1·5; Mg^{2+}, 1; PO_4^{2-}, 1; HCO_3^-, 25. Medium was gassed with 95 % O_2 and 5 % CO_2. Before an experiment, the kidneys were perfused for an initial 15 min equilibration period, in which stabilization of perfusate flow and renin secretion were established, and after which the perfusion medium was discarded and the kidneys switched to a fresh reservoir containing the experimental medium. Each kidney was perfused for four 15 min periods. The first period served as control; in the second, isoprenaline (2·83 μM) was added; in the third, Ca was omitted from the medium; the fourth served as recovery, with medium the same as the first period. Three additional sets of identical experiments were conducted in which 10, 50, or 100 μM-trifluoperazine was added to the perfusion medium in all periods. Following each period, a 1 ml aliquot of perfusate was taken and frozen for later determination of renin concentration and the kidney switched to fresh medium. The sample was assayed for angiotensin I generation from nephrectomized-dog plasma for substrate. The renin radioimmunoassay procedure has been described previously (Fray, 1976). Renin secretion was calculated by multiplying cumulative perfusate renin activity over the 15 min period by the perfusate volume and dividing by the kidney wet weight (ng h^{-1} g^{-1} per fifteen minutes).

Cells were obtained by modification of a procedure described previously (Fray & Laurens, 1981). Briefly, kidneys were isolated as described above and perfused at 100 mmHg with Ca-free Krebs–Henseleit solution containing (mM): Na^+, 155; K^+, 4; Mg^{2+}, 1; PO_4^{2-}, 1; HCO_3^-, 25. Five minutes later Ca and collagenase (1A, Sigma) were added to the perfusion circuit (to 1·5 mM and 0·1 %, respectively) and allowed to recirculate in the kidney for 15 min. Kidneys were then removed from the perfusion apparatus and the cortex forced through a 106 μm sieve, washing with 15 ml cold, Ca-free Krebs–Henseleit solution. The suspension was centrifuged at 200 *g* for 4 min and the supernatant discarded. The pellet fraction was resuspended in 20 ml cold Ca-free solution, divided into 4 × 5 ml aliquots and centrifuged at 200 *g* for 5 min as before. The pellets were then resuspended in appropriate buffers. All buffers contained 8 mM-K and either 0 or 1·5 mM-Ca.

The 20 ml cell suspension was divided among 50 ml round-bottomed Nalgene centrifuge tubes

and 50 μM-trifluoperazine was added to half those with 0 Ca and half those with 1·5 mM-Ca. The air in each tube was displaced with 95% O_2 and 5% CO_2 and the tubes stoppered and incubated in a shaking water bath at 37 °C. 0·5 ml aliquots were removed at 0 and 60 min and centrifuged for 3–4 min at 200 g and 50 μl of the supernatant removed and frozen for later determination of renin activity. The sample was assayed for incubation renin activity by radioimmunoassay for angiotensin I generation from nephrectomized-dog plasma as substrate (Fray, 1976). Incubation renin activity represents net cumulative renin secreted from the cells; therefore renin secretion may be calculated by dividing the incubation renin activity by 60 min (ng ml^{-1} h^{-1} per sixty minutes).

To separate soluble renin from granular renin in the cell studies, 2 ml aliquots of the above cell suspension were treated by the method of Sagnella & Peart (1979) with minor modifications. Briefly, following centrifugation the cell pellet was resuspended in cold sucrose solution (0·3 M, 4 °C) and homogenized with eight full passes in a loose-fitting hand-operated homogenizer (A. H. Thomas, Type A). The homogenate was centrifuged at 300 *g* for 10 min and the pellet containing nuclear and cellular fractions discarded. The supernatant, containing soluble (cytoplasmic) renin and subcellular organelles (including renin granules), was centrifuged for 45 min at 81000 *g* in the presence of 5 mM-$LaCl_3$ to promote aggregation. The supernatant containing soluble renin was frozen for later determination of cytoplasmic renin. Granular renin was released by resuspending the pellet for 60 min in 20 mM-NaCl containing 0·1% Triton X-100 at 0 °C with intermittent vortexing. The suspension was then clarified by centrifugation at 44000 *g* for 20 min and the supernatant frozen for later determination of renin as described above.

Values are reported as mean ± S.E. of mean and statistical significance assessed using paired and unpaired *t* test. Trifluoperazine was a gift from Smith Kline and French Laboratories, Philadelphia, and isoprenaline was purchased from Breon Laboratories, New York. Lactate dehydrogenase was determined from a commercial kit (Sigma Chemical Co.).

RESULTS

Fig. 1 shows that trifluoperazine potentiated the stimulatory effect of isoprenaline. 10 μM-trifluoperazine had no effect on renin secretion, but 50 μM increased secretion by 167% ($P < 0.025$) and 100 μM increased secretion by 633% ($P < 0.0005$). Perfusate flow during control and the three levels of trifluoperazine was 15±1, 20±2, 17±2, and 16±2 ml min^{-1} g^{-1}, respectively.

Fig. 1 shows that trifluoperazine potentiated the stimulatory effect of isoprenaline. In isoprenaline periods the usual increase in renin secretion was observed in the absence of trifluoperazine; 10 and 50 μM-trifluoperazine increased this isoprenaline-induced secretion further. At 100 μM-trifluoperazine the isoprenaline-induced renin secretion was slightly lower than at 50 μM. Perfusate flow during the four isoprenaline periods was 13±1, 18±1, 16±2, and 14±2 ml min^{-1} g^{-1}.

Fig. 2 shows that trifluoperazine potentiated the renin secretion induced by 0 Ca. At 0 trifluoperazine, 0 Ca induced the usual significant stimulation of renin secretion ($P < 0.025$), and at just 10 μM this stimulation increased 726% ($P < 0.025$). At 100 μM-trifluoperazine the 0 Ca-induced renin secretion was lower than at 10 and 50 μM. Perfusate flow during 0, 10, 50, and 100 μM-trifluoperazine and 0 Ca was 13±2, 10±1, 16±3, 19±1 ml min^{-1} g^{-1}, respectively.

Fig. 3 shows that trifluoperazine stimulated renin secretion from isolated renal cortical cells both in the presence ($P < 0.05$) and absence ($P < 0.001$) of Ca. Omitting Ca from the incubation medium stimulated renin secretion significantly ($P < 0.001$) and trifluoperazine caused a two-fold potentiation ($P < 0.001$). Lactate dehydrogenase was also secreted into the incubation medium, but unlike renin, its secretion was unaffected by Ca or trifluoperazine. For example, lactate dehydrogenase activity (as

μmol pyruvate consumed min^{-1} ml^{-1} cell suspension at 37 °C) was $0{\cdot}83 \pm 0{\cdot}17$ and $17{\cdot}45 \pm 0{\cdot}28$ at 0 and 60 min in the 0 Ca medium, and $1{\cdot}25 \pm 0{\cdot}18$ and $17{\cdot}47 \pm 0{\cdot}57$ at 0 and 60 min in the 1·5 mM-Ca medium. Calculations indicate that these values are comparable to those of other studies in kidney slices (Churchill, 1979; Lyons, 1980).

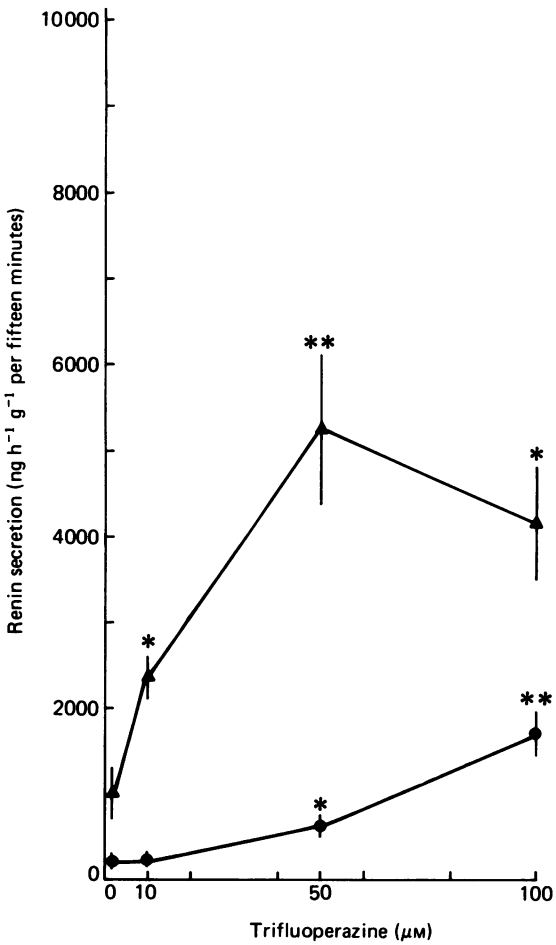

Fig. 1. Effects of trifluoperazine on renin secretion in isolated perfused kidneys without (●, control, $n = 4$) and with isoprenaline (▲, 2·83 μM, $n = 4$). Each point represents mean ±S.E. of mean of renin secreted over a 15 min period. *$P < 0{\cdot}05$ compared to 0 trifluoperazine; **$P < 0{\cdot}025$.

Trifluoperazine also caused a substantial decrease in soluble renin in the isolated cell studies after 60 min incubation (a $54{\cdot}8 \pm 7{\cdot}2$ ng ml^{-1} h^{-1} decrease in the presence of trifluoperazine (50 μM) and 0 Ca compared to a $3{\cdot}3 \pm 1{\cdot}3$ ng ml^{-1} h^{-1} increase without trifluoperazine ($P < 0{\cdot}005$); a $32{\cdot}3 \pm 10{\cdot}3$ ng ml^{-1} h^{-1} decrease with trifluoperazine and 1·5 mM-Ca compared to a $5{\cdot}9 \pm 1{\cdot}9$ ng ml^{-1} h^{-1} without ($P < 0{\cdot}05$)). An interesting observation in experiments without trifluoperazine was that whereas soluble renin increased in the absence of Ca ($+3{\cdot}3$ ng ml^{-1} h^{-1}) it decreased in the presence of Ca ($-5{\cdot}9$ ng ml^{-1} h^{-1}, $P < 0{\cdot}025$). Granular renin decreased to the same extent with or without Ca.

DISCUSSION

These studies show that trifluoperazine stimulated renin secretion from isolated perfused kidneys and isolated cells. This suggests that trifluoperazine may affect renin secretion by direct cellular mechanisms. Indeed, it is at the cellular level that the mechanism of action of trifluoperazine, and by inference calmodulin, has been

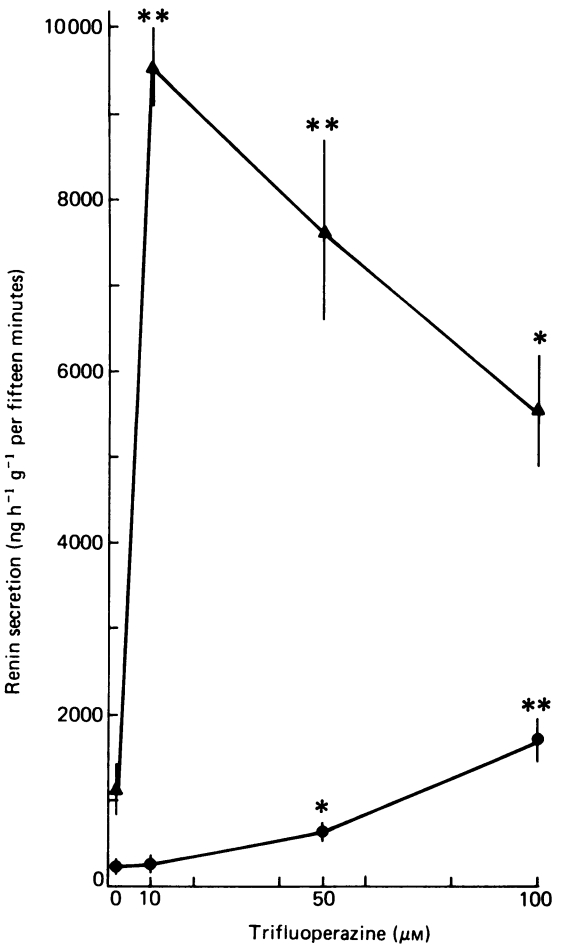

Fig. 2. Effects of trifluoperazine on renin secretion in isolated perfused kidneys without (▲, 0 Ca, $n = 4$) and with 1·5 mM-Ca (●, control, $n = 4$). *$P < 0.05$ compared to 0 trifluoperazine; **$P < 0.025$.

discussed in other secretory systems. The striking difference, however, is that whereas trifluoperazine inhibited secretion in other systems, it stimulated renin secretion at concentrations comparable to those used in other systems (DeLorenzo, 1982; Douglas & Nemeth, 1982; Krausz *et al.* 1980).

Trifluoperazine is representative of a class of antipsychotic agents that have been shown to bind specifically to calmodulin (Levin & Weiss, 1977; Reynolds & Claxton, 1982) and thereby inhibit the activity of various calmodulin-sensitive enzymes and physiological processes, some of which are related to secretion (DeLorenzo, 1982;

Cheung, 1982; Levin & Weiss, 1976). Perhaps it is for these reasons, and for others besides, that trifluoperazine has been used so successfully in studying the process of exocytotic secretion, in that it predictably causes a marked inhibition in all secretory systems notwithstanding the possibility that trifluoperazine may have other cellular effects unrelated to the inhibition of calmodulin and the additional caution which must be explicitly exercised when interpreting results from only one drug.

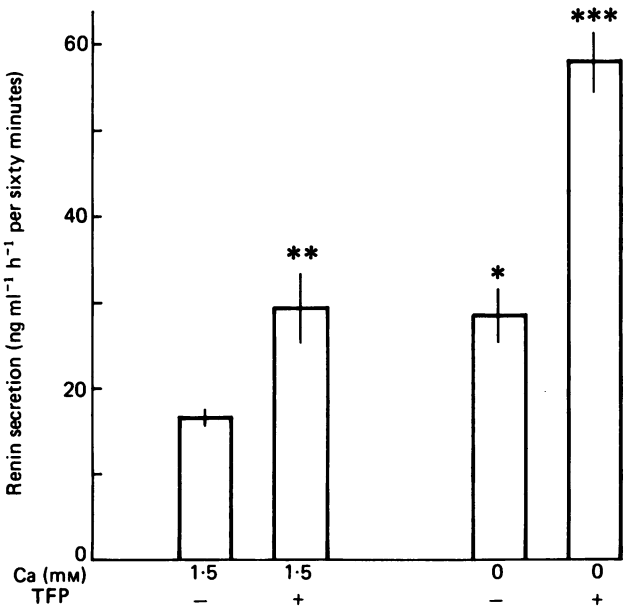

Fig. 3. Effects of trifluoperazine (TFP) (50 μM) on renin secretion in isolated renal cortical cells without (0 Ca, $n = 10$) and with 1·5 mM-Ca ($n = 10$). Each bar represents mean ± S.E. of mean cumulative renin secreted over the 60 min incubation period. *$P < 0{\cdot}01$ compared to 1·5 mM-Ca without trifluoperazine; **$P < 0{\cdot}05$ compared to 1·5 mM-Ca without trifluoperazine; and ***$P < 0{\cdot}001$ compared to 0 Ca without trifluoperazine.

Isoprenaline, as well as other β-adrenergic agonists, is believed to stimulate renin secretion by a cyclic AMP mechanism (Keeton & Campbell, 1981; Churchill & Churchill, 1982). Recently it has been shown that trifluoperazine binds to calmodulin and prevents it from activating phosphodiesterase, the enzyme which degrades cyclic AMP (Reynolds & Claxton, 1982). Thus, by preventing the degradation of cyclic AMP, trifluoperazine may potentiate the stimulatory effect of isoprenaline, but other mechanisms may be possible.

Ca omission has been one of the most powerful and consistent activators of renin secretion, but the mechanisms responsible are still unknown. It has been postulated that low Ca increases a cytoplasmic pool of readily releasable renin (Fray, Lush & Valentine, 1983). The present paper presents evidence to support this view. That is to say, whereas soluble renin increased $3{\cdot}25 \pm 1{\cdot}33$ ng ml^{-1} h^{-1} in 0 Ca medium, it decreased $5{\cdot}88 \pm 1{\cdot}88$ ng ml^{-1} h^{-1} in 1·5 mM-Ca ($P < 0{\cdot}025$). Granular renin decreased to a similar extent with or without Ca. It may be argued that the renin found in the soluble fraction may represent renin liberated from granules during the homogen-

ization procedure, as may be expected, and that the increased renin in 0 Ca experiments reflected enhanced liberation from the 0 Ca-induced fragility of the granules. Although this may in part be the case, the observation that the granular fraction liberated similar amounts of renin with or without Ca makes this possibility unlikely, though not excluded, for some of the renin in the soluble fraction may undoubtedly have originated from the granular fraction during homogenization. An additional possibility is that changes in granular and soluble renin with time and in response to Ca may have reflected changes in the efficiency of homogenization. This was discounted by the observation that the percentage release of cellular lactate dehydrogenase remained stable throughout the experiment. Thus, if soluble renin may in part be assumed to reflect cytoplasmic readily releasable renin as determined in these studies, then these studies support the view that lowering Ca increases this pool of renin (Fray *et al.* 1983).

In conclusion, the present studies add to the growing body of evidence which has demonstrated that agents, such as trifluoperazine, which have similar effects on the secretion of several substances, have a very different effect on the secretion of renin.

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