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The Effect of Papaverine on *In Vitro* Renin Secretion (40426)

H. JAY LYONS AND PAUL C. CHURCHILL

Department of Physiology, Wayne State University School of Medicine, Detroit, Michigan 48201

There are two generally accepted intrarenal mechanisms governing the secretion of renin by the kidney (1, 2). The afferent arteriole is hypothesized to be a baroreceptor which stimulates renin release in response to a fall in blood pressure (3, 4) and the macula densa is proposed to be a chemoreceptor which is sensitive to Na^+ load or concentration (5–7). The relative contribution of these two mechanisms has been difficult to determine since manipulations which affect one mechanism usually influence the other. Recently papaverine, a smooth muscle relaxant, has been used in an attempt to block the baroreceptive component of renin control (11–14). It is an important criterion of these studies that papaverine does not directly influence renin secretion. We have tested for such a direct effect by incubating renin-secreting rat cortical cell suspensions in a medium containing papaverine.

Methods. Sprague-Dawley rats (250–400 g) were bilaterally nephrectomized under sodium pentobarbital anesthesia (Nembutal, 50–60 mg/kg body wt). The kidneys were placed in ice-cold 0.15 *M* NaCl and the capsules and medullas removed. The cortex was minced into 1-mm pieces and the cortical tissue rinsed several times with a glucose, potassium, and sodium solution (GKN).[1] The tissue was then incubated in a collagenase solution (Worthington Biochemical Corp., 30 U/ml GKN) at 37° in an oscillating incubator to promote cell separation. At 30-min intervals, for three hours, the media were collected and centrifuged at low speed (200*g*). The supernatant was discarded and the pellet resuspended in Krebs-Ringer bicarbonate solution (KRB).[2] These fractions were combined, centrifuged again to facilitate the further removal of cell fragments, collagenase, and blood cells, and then the resultant pellet resuspended in KRB. This suspension of cortical cells was evenly distributed into tubes, centrifuged, and then resuspended in KRB containing 0, 0.13, or 0.26 m*M* papaverine hydrochloride (Amend Drug Co., Irvington, NJ). These suspensions (10 ml) were incubated at 37° and equilibrated with 95% O_2 and 5% CO_2. After one and three hours of incubation, 200 μl samples were removed, centrifuged, and 20 μl samples of the supernatant collected for renin determination.

The concentration of renin in the media samples was determined by standard radioimmunoassay techniques. Briefly, each 20 μl sample of incubation medium was added to 100 μl of rat renin substrate (15); dimercaprol, 8-OH quinoline sulfate, and EDTA were added to inhibit activities of converting enzyme and angiotensinases, and this mixture was incubated at 37° for 2 hr. Samples were removed at hourly intervals and analyzed for angiotensin I, using the angiotensin I radioimmunoassay kit of New England Nuclear Corp., Boston, MA. The results are presented as nanograms of angiotensin I (hr incubation of medium with renin substrate)$^{-1}$(ml incubation media)$^{-1}$, or (ng A-I)(hr ml)$^{-1}$.

The effect of papaverine on renin activity was also determined. Hog renin (2.0×10^{-4} units, Nutritional Biochemical, Cleveland, OH) containing 0, 0.13, and 0.26 m*M* papaverine was incubated with rat renin substrate containing inhibitors of converting enzyme and angiotensinases at 37° for 1 hr. The angiotensin I generated was determined by radioimmunoassay as described previously. The effect of papaverine on the radioimmunoassay was also determined, by comparing the antigen binding of several concentrations of synthetic angiotensin I (Calbiochem, San Diego, CA) either with or without papaverine (0.26 m*M*) addition.

The data are presented as mean values with

[1] Constituency of GKN (m*M*) = Na^+, 137; K^+, 5; Cl^-, 142; and glucose, 6.

[2] Constituency of KRB (m*M*) = Na^+, 144; K^+, 6; Ca^{++}, 3; Mg^{++}, 1; Cl^-, 142; HCO_3^-, 20; $H_2PO_4^-$, 1; and glucose, 11.

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variation expressed as standard error. The significance at each papaverine dose level, as compared with control values, was assessed by the Student's *t* test.

Results. The effect of papaverine on the release of renin from rat renal cortical cell suspensions is presented in Fig. 1. At least six cell suspensions, derived from four rats, were incubated at each papaverine dose level. The renin released from the suspensions containing 0.13 and 0.26 m*M* papaverine was significantly less than that released from the control suspensions at the one and three hr incubation period ($p < 0.01$). Although not significant, there was an inverse relationship between the concentration of papaverine in the media, and the rate of renin released by the renal cortical cell suspension.

We also examined the effect of papaverine on both renin activity and on angiotensin I-antibody binding to determine if the renin assay procedures were being affected by the drug. There was no effect of papaverine on the activity of hog renin (2.0×10^{-4} units) at the levels used in this study (Table I). Papaverine also has no effect on the binding of synthetic angiotensin I to rabbit serum antibody. The ratio of free to bound angiotensin I (F:B), was unaffected by papaverine (0.26 m*M*) addition (Fig. 2).

Discussion. Papaverine is an opium alkaloid used classically as a nonspecific smooth muscle relaxant. Recent evidence indicates that this effect may be due to a papaverine-induced inhibition of both calcium and sodium translocation across cell membranes (16, 17). Also this drug inhibits phosphodiesterase activity thereby increasing intracellular CAMP (18, 19). Calcium (20, 21), sodium (22, 23) and CAMP (24, 25), have been demonstrated to directly alter the rate of renin released from juxtaglomerular cells (JG cells). Therefore, it is reasonable to expect a direct effect of papaverine on the rate of renin release from renal cortical tissue. Additionally, papaverine has been reported to alter the rate of release of other hormones *in vitro,* such as corticosterone (26).

Although there is reason to expect papaverine to alter renin release, it would be difficult to suggest the direction of this effect. CAMP has been demonstrated to increase the rate of renin secretion by several investigators (24, 25), yet others have found no effect of CAMP or dibutyryl CAMP on renin release (27, 28). Theophylline, another inhibitor of phosphodiesterase activity, has been shown to both increase renin release (28), and to have no effect (29). While the majority of investigators believe that renin is inversely related to the tubular sodium load (1), the majority of *in vitro* studies report the opposite, or a direct relationship between these modalities (30). Finally, there is also much confusion over the role of calcium in the control of renin release (31). An understanding of

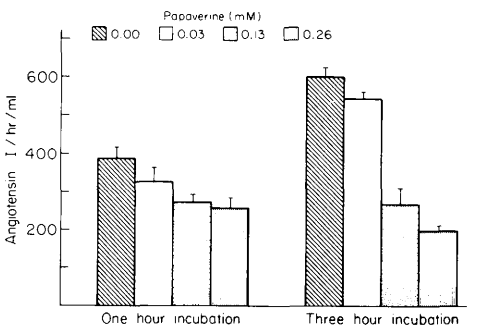

FIG. 1. The effect of papaverine on renin secretion from rat renal cortical cell suspensions. The incubation media containing papaverine in the concentrations indicated were sampled at 1 and 3 hr ($n = 6$). * $P < 0.01$.

TABLE I. EFFECT OF PAPAVERINE ON HOG RENIN ACTIVITY ng A-I (UNIT-HR)$^{-1} \times 10^{-4}$.[a]

Control	Papaverine		
	0.03m*M*	.13 m*M*	.26 m*M*
5.83 ± 0.05	5.89 ± 0.71	5.78 ± 0.16	5.78 ± 0.22
(6)	(6)	(6)	(6)

[a] Nanograms angiotensin I ($\times 10^{-4}$) (Unit of hog renin)$^{-1}$ (hr substrate incubation)$^{-1}$.

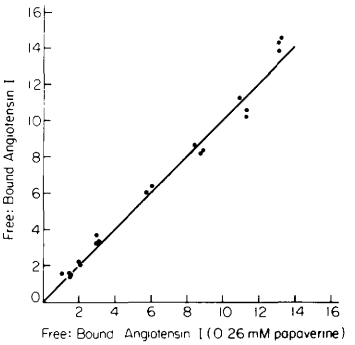

FIG. 2. The effect of papaverine (0.26 m*M*) on the binding of angiotensin I to rabbit serum antibody.

any papaverine effect on renin secretion must await a clearer understanding of the pharmacology of papaverine, as well as the molecular mechanisms governing the release of renin.

We have found papaverine to inhibit the release of renin from renal cortical cell suspensions. This inhibition was significant at media concentrations of 0.13 and 0.26 m*M* papaverine. These levels of this alkaloid are similar to the expected plasma concentrations of this drug *in vivo* studies, where it has been utilized to investigate the control of renin secretion. The renal plasma concentration of papaverine, assuming no recirculation and using the prescribed renal arterial infusion rates and determined renal plasma flows, would range from 0.093 to 0.428 m*M* (11–13). In all but one of the experiments cited in these *in vivo* reports, the average levels of papaverine exceeded 0.13 m*M*, the level at which we found a significant papaverine-induced inhibition.

It is possible that papaverine was inhibiting the enzymatic activity of renin or reducing the sensitivity of our radioimmunoassay. However, we were unable to demonstrate any effect of this drug on our assay procedures at levels which would be similar to those present in this study.

Witty *et al.* (11, 12) and Gotshall *et al.* (13) reported that papaverine did not effect the level of renin secretion in dogs during control experiments. However it is difficult to control the many factors which could alter renin secretion *in vivo*. The direct effect of this opium alkaloid may have been masked in these experiments by other coincident influences. On the other hand, it is possible that the drug may be species specific or that papaverine receptor sites are more available in renal cortical cell suspensions to the drug. Then the response would be accentuated in this *in vitro* system.

Summary. The influence of papaverine-HCl, a smooth muscle relaxant, on *in vitro* renin release was investigated. We demonstrated a papaverine-induced inhibition of renin release from renal cortical cell suspensions. This inhibition was significant when compared with the release of renin into control medium, at papaverine concentrations of 0.13 and 0.26 m*M*/l. No effect of papaverine on our radioimmunoassay procedures was found.

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