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EVIDENCE FOR THE PRESENCE OF FUNCTIONAL BETA-ADRENOCEPTOR ALONG THE PROXIMAL TUBULE OF THE RAT KIDNEY

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Summary: Evidence for the presence of beta adrenoceptors on proximal tubules from the rat kidney has been obtained using enriched tubule suspensions prepared by Percoll centrifugation. Intact tubules demonstrated simultaneous enrichment of parathyroid hormone and isoproteronol sensitive cAMP production with no enrichment of antidiuretic hormone sensitive cAMP production. Both norepinephrine and epinephrine were less potent than isoproterenol and the stimulatory effect of catecholamines could be blocked with propranolol but not phentalamine. The stimulatory effect of norepinephrine on cellular pheylalamine uptake is blunted by co-addition of isoproterenol suggesting that the beta receptor may modulatory catecholamine stimulated transport.

Catecholamines are known to increase fluid and solute transport by the renal proximal tubule (1). However, the details by which adrenoceptors mediate the stimulatory action of catecholamines have remained elusive. Previous studies in rat proximal tubules have shown that norepinephrine (NE) increases fluid (2) HCO_3^- (3), and phenylalanine (Phe) absorption (4) via α adrenoceptor stimulation. In contrast both α and β receptors have been reported to increase fluid absorption in the rat proximal tubule (5) and the stimulatory effect of NE in rabbit proximal tubules appears to be mediated via β receptors (6). Previous studies using both microdissected nephron segments (7) and autoradiography (8) have failed to detect the presence of β receptors along the rat and rabbit proximal tubule. However, we have recently observed that the stimulatory effect of NE on *in vitro* Phe uptake by rat cortical tubule

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Abbreviations: NE, norepinephrine; EPI, epinephrine; ISO, isoproterenol; PTH, parathyroid hormone; ADH, antidiuretic hormone; Phe, phenylalanine; MEM, minimal essential medium (modified Eagle's); LAP, leucine aminopeptidase; F 1,6-βPase, fructose 1,6 Bisphosphatase; PFK, phosphofructokinase

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suspensions is prevented by co-addition of high, but not low, concentrations of isoproterenol (ISO). The present study was undertaken to determine whether this action of ISO could be mediated via β adrenoceptors present along the proximal tubule of the rat.

METHODS

Tubule Preparation: Outer cortical tubules from Nembutol anesthetized (35-40 mg/kg I.P.) male Sprague Dawley rats (200-300 g bw) were prepared by a modified collagenase separation technique. Briefly, kidneys were perfused in situ with 50 ml ice cold Minimal Essential Medium (MEM) containing 1% (W/V) BSA, 10 mM Hepes and 50 mg % collagenase and hyaluronidase, pH 7.4. Kidneys were then placed in ice cold MEM, decapsulated and the outer cortex, approximately 1 mm deep, was dissected and minced. The minced cortex was incubated in MEM containing 1% BSA, 10 mM Hepes, 100 mg % collagenase and hyaluronidase, 20 mg % DNAse and 2.0 mM $CaCl_2$ at 37°C for 30 min. Following incubation, suspensions were filtered and centrifuged. The final pellet was resuspended in MEM containing 1% BSA, 10 mM Hepes and 1 mM $CaCl_2$, pH 7.4, and preincubated for 30 min at 37°C. For density gradient separation BSA and $CaCl_2$ were excluded from the final suspension medium.

Percoll Gradient Centrifugation. Percoll enrichment of proximal tubules was performed by a modification of previously published methods (9). A 37.5% (v/v) percoll solution in MEM without BSA, Hepes or $CaCl_2$ was centrifuged at 11,000 x g for 20 min at 4°C in a Beckman SS-34 rotor, to preform the gradient, and then transferred to a HS-4 swing bucket rotor at 4°C. The cortical suspension was layered onto the gradient and centrifuged at 1,500 x g for 20 min at 4°C. For measurement of marker enzymes, collected bands were diluted in phosphate buffered saline, pelleted at 3,000 x g for 20 min, suspended in sucrose (250 mM sucrose, 30 mM imidazole, pH 7.4) at 4°C and homogenized. Samples were taken for determination of protein (10), DNA (11), and leucine aminopeptidase (LAP) (12). The homogenate was then diluted 50% in sucrose containing 0.1 mM EDTA and 0.01% (v/v) triton, rehomogenized and frucose 1,6-bisphosphatase (F 1,6-BPase) (13), and phosphofructokinase (PFK) (14) activities were determined.

For measurement of intact cell function, bands were resuspended in MEM containing BSA, Hepes and $CaCl_2$ and preincubated at 37°C for 30 min. Oxygen consumption was determined manometrically in a Gilson differential respirometer (15) while intracellular Phe concentration was determined 15 min after [^{3}H] Phe and [^{14}C] inulin addition (16).

For measurement of cAMP production cells were incubated at 37°C for 15 min in MEM containing BSA, Hepes, $CaCl_2$, 1mM methylisobutylxanthine and various adrenergic agonists and antagonists as described in the text. Incubation was terminated by transferring the flasks to an ice cold bath and adding (final concentrations) 0.4N $HClO_4$ and 20% (v/v) methanol. Following protein removal and pH neutralization with KOH the clarified supernatants were applied to separate Dowex AG 1x8 columns (0.5 x 1 cm). Cyclic AMP was eluted with 2 N formic acid, lypholized and then quantitated by RIA (New England Nuclear).

All data are presented as mean ± SEM. For multiple group analysis an analysis of variance followed by the Student-Newmans-Keuls test was used to determine significant differences between means. When only one variable was examined paired student-t test was used. Differences between means were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Addition of ISO to rat proximal tubule suspensions has no appreciable effect on cellular Phe uptake in contrast to the stimulatory effect of NE (Table 1), epinephrine (EPI), and clonidine (16). However when both NE and ISO were added at 10^{-5}M, the stimulatory effect of NE alone was no longer observed (Table 1).

To determine whether the action of ISO was mediated via β receptors, the effect of adrenergic agonists on proximal tubule cAMP production was examined. Percoll centrifugation yielded two dominant bands: an upper (F_1) consisting of glomeruli, short tubule fragments and cell debris, and a lower (F_2) containing proximal tubules with open lumens. The distributions of several proximal and distal tubule markers are shown in Tables 2 and 3. The reduction in DNA/protein in F_2 is consistent with removal of glomeruli (17) as confirmed by microscopic examination. The proximal markers F 1,6-BPase and PTH sensitive cAMP production were both increased in F_2 relative to the starting suspension. In preliminary studies similar enrichment of the brush border enzyme LAP was also observed. In contrast, the distal marker PFK was unchanged in F_2 and ADH sensitive cAMP production was reduced. Both cellular oxygen consumption and ouabain inhibitable Phe uptake were enriched and well-preserved in the F_2

Table 1

Effect of Isoproterenol on Norepinephrine Stimulated Phenylalanine Uptake

Treatment	30 sec.	10 min.	n
Control	62.4 ± 4.2	92.4 ± 7.2	8
NE 10^{-5}	105.0 ± 13.2*	122.0 ± 11.6	6
ISO 10^{-5}	78.8 ± 4.3	104.8 ± 3.3	4
NE 10^{-5} + ISO 10^{-7}	120.6 ± 19.8*	131.1 ± 17.0	4
NE 10^{-5} + ISO 10^{-5}	66.2 ± 2.0	87.7 ± 1.5	3

Suspensions were preincubated for 25 min at 37°C afterwhich norepinephrine (NE) or isoproterenol (ISO) were added and the incubation continued for an additional 5 min. At time zero an aliquot of ^{3}H Phe and ^{14}C inulin was added and samples collected, in duplicate, at 30 sec and 10 min. Samples were diluted in ice cold buffer and pelleted by rapid centrifugation. Pellet ^{3}H activity was corrected for ^{14}C space and is expressed as DPM/μg DNA. ^{3}H Phe specific activity was 2.28 mCi/mmol.

Table 2

Density Gradient Marker Enzymes

Marker	IN	F_1	F_2	F_2/IN	n
DNA/protein	40.0 ± 5.8	49.3 ± 6.5	25.0 ± 1.5*	0.63	4
F 1,6-BPase	0.8 ± 0.1	0.69 ± 0.08	1.35 ± 0.25*	1.67	3
LAP	6.0	4.26	9.87	1.65	2
QO_2	2.00 ± 0.05	ND	2.99 ± 0.22*	1.50	4
IC Phe	878 ± 20	ND	1780 ± 300*	2.03	3
+ Ouabain	540 ± 72	ND	532 ± 36	0.99	3
PFK	0.84 ± 0.38	ND	0.87 ± 0.40	1.04	3

Tubule markers measured in the initial suspension (IN), and in the upper (F_1) and lower (F_2) bands by Percoll centrifugation. DNA/protein, μg DNA/mg protein; F 1,6-BPase, nmoles NADP/min/μg DNA at 25°C; LAP, nmoles β-napthylamine/min/μg DNA at 37°C; QO_2 nmole O_2/min/μg DNA; IC Phe, intracellular Phe concentration, μM, in the presence of 0.2mM Phe in the medium; Ouabain, (1mM) added 30 min prior; PFK, nmoles NADP/min/μg DNA at 25°C.

fraction. The enrichment of F 1,6-BPase and PTH sensitive cAMP as well as reduced distal markers in the F_2 fraction are consistent with previous studies (9). In contrast, the enrichment in LAP activity and sustained high intracellular Phe concentration suggest that low speed centrifugation was less traumatic than the originally described method in which brush border enzymes were not enriched (9). ISO sensitive cAMP production was also enriched in F_2 to the same degree as F 1,6-BPase and PTH sensitive cAMP production. The lack of significant enrichment of distal markers and absence of glomeruli in the F_2

Table 3

cAMP Producton (pmol/15min/μg DNA)

Hormone	IN	F_1	F_2	F_2/IN	n
PTH	12.0 ± 3.9	ND	29.5 ± 2.6*	2.45	6
ADH	0.59 ± 0.1	0.57 ± 0.12	0.42 ± 0.05	0.71	5
ISO	1.43 ± 0.27	0.81 ± 0.13	3.75 ± 0.76*	2.62	5

Cyclic AMP production in the presence of 10^{-6}M parathyroid hormone (PTH); 100 mU/ml antidiuretic hormone (ADH); and 10^{-6}M isoproterenol (ISO).

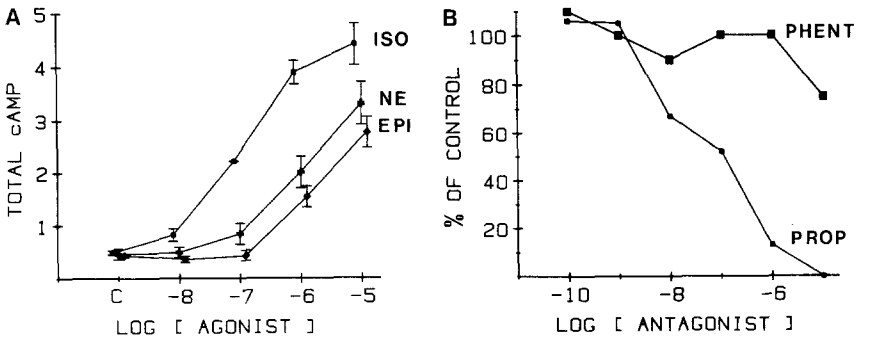

Figure 1.

A. Effect of isoproterenol (ISO), norepinephrine (NE) and epinephrine (EPI) on cAMP production (pmol/15 min/μg DNA) in enriched proximal tubule suspensions. Tubules were incubated for 15 min in the presence of catecholomine and methylisobutyl xanthine.

B. Effect of propranolol (PROP) and phentolamine (PHENT) on isoprotenenol (10^{-5}M) stimulated cAMP production. Similar results were obtained using either norepinephrine or epinephrine as agonists (data not shown).

fraction demonstrates that the predominant source of ISO stimulated cAMP production was the proximal tubule.

When enriched proximal tubules were incubated with various concentrations of catecholamines a dose-dependent increase in cAMP production was clearly observed with ISO being more potent then either NE or EPI (Figure 1). Propranolol, but not phentolamine, blocked ISO stimulated cAMP production consistent with a β adrenoceptor mediated action of ISO. Neither antagonist altered PTH stimulated cAMP production. The stimulatory effect of ISO was additive to that of PTH while EPI reduced PTH stimulated cAMP production consistent with the presence of inhibitory α_2 receptors (18) (data not shown).

While the presence of β receptors along the proximal tubule can be demonstrated by this method the magnitude of cAMP production is only 13% of that observed for PTH and may account for the lack of significant detection in microdissected nephron segments (7) and radioligand autography studies (8). Previous studies using rabbit renal cells separated by free flow electrophoresis have suggested the presence of β receptors in proximal tubules cells (19) and double antibody fluorescent examination of alpreolol binding has demonstrated the presence of β receptors along the rat proximal tubule (20). Finally, recent studies in microdisected nephron segments from the dog have demonstrated β receptors along the proximal straight segment (21). The

present data would suggest that bulk preparations of intact proximal tubules and fluorescent antibody studies may be more sensitive methods for detecting low levels of β receptors in rat proxmal tubules than labeled radioligands or microdissection methods.

While relatively low levels of β adrenoceptor mediated cAMP production were observed in proximal tubules, the physiological role for these receptors may be significant as evidenced by the present observation that ISO effectively blocked NE stimulated Phe uptake. The present results indicate that multiple receptor interactions may play an important modulatory role for the actions of catecholamines on proximal tubule transport.

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