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ROLE OF α-ADRENERGIC STIMULI IN THE CONTROL OF RAT RENAL AMMONIAGENESIS

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The effects of phenylephrine on renal ammoniagenesis and the involvement of Ca^{2+} in phenylephrine action were investigated in isolated proximal fragments of rat-kidney tubules. Phenylephrine stimulated renal ammoniagenesis from 1 and 2 mM glutamine whereas no significant changes took place at a higher concentration of glutamine (20 mM). Stimulation of ammonia synthesis by phenylephrine was found to be linear with time and dose-dependent between 10^{-9} and 10^{-4} M. Phenylephrine-stimulated ammoniagenesis was blocked by phentolamine (10 μM) but not by propranolol (10 μM) confirming that the effect is mediated by α-adrenergic stimuli. No stimulatory effect of phenylephrine was observed in Ca^{2+} depleted proximal tubule fragments, suggesting that Ca^{2+} is required in this adrenergic response.

Renal ammoniagenesis plays an essential role in the maintenance of the acid-base status of the animals and constitutes one of the most important adaptive mechanisms of renal cells during metabolic acidosis [1-3].

Nevertheless, the molecular mechanism of the initial adaptation after the onset of acidosis is poorly understood. To date, several different adaptive changes of renal ammoniagenesis during chronic and acute metabolic acidosis have been reported [2-7]. Some of these factors are related to variations in the intramitochondrial glutamine concentration [4] or to adaptive and/or permanent changes in the activity of the key enzymes of the ammonia synthesis pathway, namely phosphate-dependent glutaminase (PDG) and glutamate dehydrogenase (GDH) [2,5-8]. In this sense, we have found a 4-fold increase in PDG activity, assayed at low

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glutamine concentration, during acute acidosis [2,3] which is mediated through an α-adrenergic like mechanism [3]. This conformational adaptive change in PDG clearly precedes the increased enzyme synthesis which occurs during chronic acidosis [8] and which includes permanent changes in activity of GDH [9]. The activity of both enzymes is increased 3-5 fold.

Recent studies have identified and quantitated the different adrenergic receptors in the cortical nephron segments, showing that proximal tubules are enriched in α_1- and α_2-adrenergic receptors, whereas distal renal tubules are especially rich in β-adrenergic receptors [10].

In order to elucidate the short term factors regulating renal ammonia synthesis, these experiments were designed to test the effect of α-adrenergic agents on this metabolic pathway in isolated rat kidney proximal tubules. The results provide clear evidence that renal ammoniagenesis is significantly increased by α-adrenergic stimuli.

MATERIALS AND METHODS

Isolation of rat-kidney proximal tubules. Experiments were carried out on male Wistar rats weighing about 200g. The rats were housed under controlled temperature (22±2°C) and lighting (08:00 to 20:00h) conditions, and they were maintained on a commercial standard diet with free access to water. The animals were killed by cervical dislocation and the kidneys removed for preparation of renal tubules. Cortical tubules were prepared by collagenase digestion as described previously [11]. Proximal tubule fragments were obtained from a Percoll gradient [12,13]. Our study was carried out in the fraction enriched with proximal renal tubules (more than 95%).

Assay for renal ammonia synthesis. Proximal tubules were incubated at 37°C for 60 min in siliconized flasks with Krebs-Henseleit solution in a final volume of 4 ml with glutamine (Gln) at different concentrations as substrate, either alone or in combination with the different agonists or antagonists. The flasks were gassed with O_2:CO_2 (95:5) during all the incubation period. Incubations were stopped by the addition of perchloric acid (20% V/V). After neutralization ammonia was measured as described previously [2]. In the experiments in absence of Ca^{2+} the tubules were washed three times with 20 mM phosphate buffer pH 8 containing 1 mM EGTA. The tubules were rid of EGTA by washing twice with a calcium-free Krebs-Henseleit solution. The incubations in these cases were performed in this latter solution [14]. Results are expressed as μmol/hr/g dry wt. All values are

reported as means±SEM. Statistical comparisons were done using Student's t-test.

RESULTS AND DISCUSSION

To examine the regulation of renal ammoniagenesis by phenylephrine, isolated rat kidney proximal tubules prepared from renal cortex were assayed for renal ammonia production by measuring ammonia release from exogenously added glutamine. Phenylephrine 10^{-6} M stimulated almost 1.5 fold renal ammoniagenesis in these kidney-tubule fragments, at low glutamine concentrations. Control values for 1 and 2 mM glutamine were 195.0±6.4 (40) and 320.1±22.7 (9) whereas phenylephrine values were 286.1±10.1 (40) and 453.2±24.0 (9). No significant changes, however, were found at high glutamine concentration (20 mM), 1508.5±214.1 (3) vs 1480.8±123.0 (3).

Dose and time-response curves for phenylephrine stimulation of renal ammoniagenesis are shown in Fig. 1. Half-maximal stimulation was obtained at 800 nM phenylephrine. Maximal stimulation was achieved at 10μM. Also, included in Fig. 1 is the effect of increasing concentrations of phenylephrine (10^{-7}, 10^{-6} and 10^{-5} M) on ammoniagenic activity at different time. Ammonia production was dose dependent and nearly linear with time. The role of renal adrenergic receptors in modulating many of the metabolic and physiologic functions of the kidney is being increasingly considered [10], and both α- and β-adrenergic receptors have been characterized and implicated in the mediation of the sympathetic nervous system effects on the kidney [15], although the precise contribution of these receptors to some renal functions is controversial.

In order to examine the involvement of adrenergic stimuli in phenylephrine action on isolated rat renal tubules, specific α

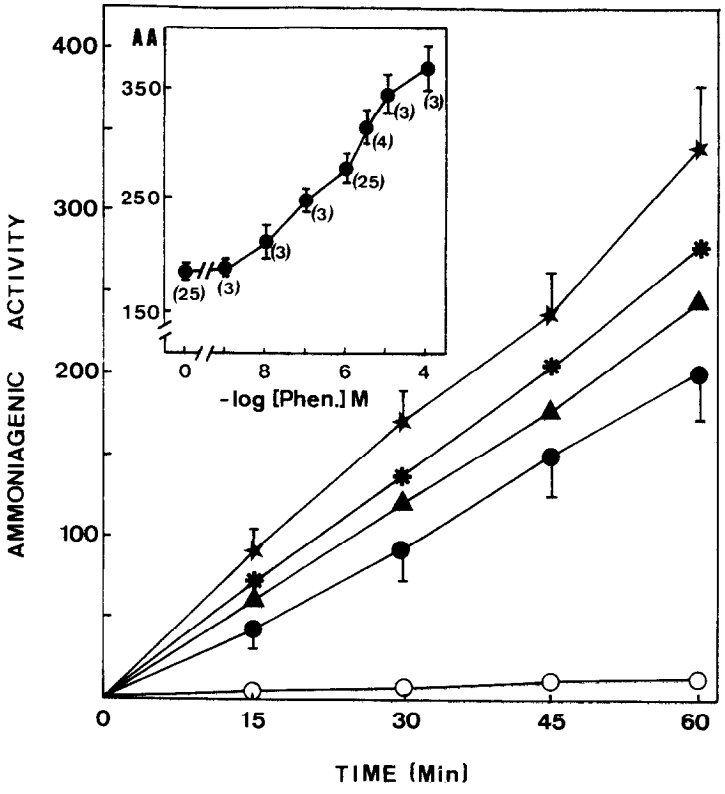

Figure 1. Time and dose-response curves for the stimulation of renal ammoniagenesis by phenylephrine. Isolated rat proximal renal tubules have been used as indicated in the Materials and Methods section. Incubations were carried out in the absence of substrate (○) and in the presence of glutamine 1mM (●) with 10^{-7} M (▲), 10^{-6}M (✱) and 10^{-5} (★) of phenylephrine. Ammoniagenic activity is expressed as µmol NH_3 x g^{-1} dry weight. Results are means ± SEM of at least 4 observations. Insert plot shows the dose-response curve, AA indicates the ammoniagenic activity expressed as µmol NH_3 x h^{-1} x g^{-1}dry wt. The results are means ± SEM with the number of observations in brackets.

and β antagonists were used (fig. 2). The phenylephrine-mediated increase in ammonia production was completely blocked by the α-adrenergic antagonist phentolamine, whereas no blockade was observed with the β-adrenergic antagonist propranolol. Both antagonists alone did not cause any change in renal ammoniagenesis.

It is widely accepted that several metabolic processes mediated through α-adrenergic stimuli require Ca^{2+} for maximal stimulation [14,16] and that depletion of this cation in the

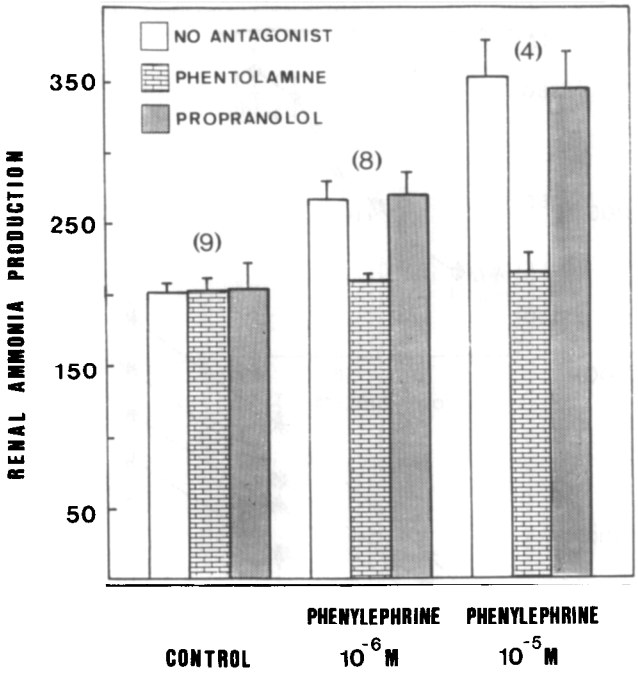

Figure 2. Effects of α- and β- adrenergic antagonists on phenylephrine-stimulated ammoniagenesis from glutamine (1mM) in isolated rat renal proximal tubules. General features of the experiments are described in the Materials and Methods section. Adrenergic blockers (phentolamine and propranolol) were added at 10 μM concentration. Results are expressed as μmol NH_3 x h^{-1} x g^{-1} dry weight and are means ± SEM with the number of observations in brackets.

cells brings about an unresponsiveness to α-agonist stimuli [17]. Moreover, increased levels of cytosolic free Ca^{2+}, strongly consistent with phenylephrine [18] and other hormone mediated effects, have been shown [19].

This is why we wished to study the effect of this cation on renal ammoniagenesis stimulation brought about by several α-adrenergic agonists. Table 1 shows that adrenaline and noradrenaline, as well as phenylephrine, increased renal ammoniagenesis at low glutamine concentration, whereas these adrenergic agents did not cause any effect in Ca^{2+}-depleted proximal kidney tubules. In no case significant differences were found when high glutamine concentrations were used.

Table 1. Effects of various adrenergic agonists on renal ammoniagenesis in normal and calcium-depleted isolated rat kidney-proximal tubules

Addition	Substrate	Normal	Calcium-depleted
Control	Gln 1mM	210.0±12.6 (7)	186.5±8.9 (7)
	Gln 20mM	1507.9±214.0 (3)	1619.2±146.1 (3)
Phenylephrine 10^{-6} M	Gln 1mM	308.9±19.8 (7)**	190.1±11.3 (7)§§
	Gln 20mM	1622.6±123.7 (3)	1706.2±179.8 (3)
Adrenaline 10^{-6} M	Gln 1mM	303.5±27.6 (4)*	188.9±8.7 (4)§
	Gln 20mM	1724.8±203.5 (3)	1636.7±225.1 (3)
Noradrenaline 10^{-6} M	Gln 1mM	286.0±15.4 (4)**	184.9±9.0 (4)§
	Gln 20mM	1645.2±291.1 (3)	1623.0±164.2 (3)

General features of the experiments are described in the Materials and Methods section. Results are expressed as μmol of ammonia x hr^{-1} x g^{-1} dry weight and are means±S.E.M. with the number of observations in brackets. Significant differences between control and adrenergic agents:* P<0.01; ** P<0.005, and between normal and calcium depleted cells: § P<0.01, §§ P<0.001.

Several authors have demonstrated that an α-adrenergic response is involved in the increase of renal gluconeogenesis by catecholamines. Noradrenaline stimulates gluconeogenesis in various renal systems [20] and this effect appears to be mediated by an α_1-receptor. Moreover this effect is inhibited by the Ca^{2+} channel blocking agent methoxyverapamil [20]. On the other hand, Pettinger et al. [21] have suggested that renal α_1-adrenoceptor responds to renal nerve noradrenaline, whereas α_2-adrenergic receptor responds to circulating adrenaline.

Our results seem to exclude any increase in plasma and/or mitochondrial membrane permeability to glutamine in response to α-adrenergic stimuli, as neither the α-agent phenylephrine nor catecholamines, stimulate renal ammoniagenesis when proximal tubules are incubated in the presence of high glutamine concentrations (Table 1).

Moreover, Ca^{2+} at a concentration of 0.2-1.0 mM activates PDG [22], and it is also remarkable that PDG responds in different

ways to metabolic acidosis. During chronic acidosis the increased PDG activity is due to an increased amount of the enzyme [8] whereas during acute acidosis PDG activity increases only at cellular substrate concentrations [2,3] with no induction/ repression phenomena.

If any of these aspects are related to the results presented here, further work is needed for the complete understanding of the intracellular molecular response originated during the α-adrenergic control of renal ammoniagenesis.

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