OUABAIN AND K^+ REMOVAL BLOCKS α-ADRENERGIC STIMULATION OF GLUCONEOGENESIS IN TUBULE FRAGMENTS FROM FED RATS

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Received 6 August 1979

1. Introduction

Gluconeogenesis in rat renal cortex is stimulated by catecholamine hormones through an α-type receptor [1,2]. Basal gluconeogenesis is stimulated by addition of extracellular Ca^{2+} [3–5] and Ca^{2+} has been assigned the possible role of an intracellular messenger in other systems responding to α-adrenergic stimuli [6–9]. To propose that the catecholamine hormones could stimulate renal gluconeogenesis simply by movement of Ca^{2+} across the plasma membrane is probably too simplistic. Removal of extracellular Ca^{2+} does not completely abolish adrenaline [2] or noradrenaline [10] stimulation of the process. On the other hand, stimulation by the synthetic imidazoline derivative oxymetazoline (a selective α-agonist) is abolished by removal of extracellular Ca^{2+} [2]. α-Adrenergic stimulation of some other systems is associated with changes in K^+ as well as Ca^{2+} flux [6,11–14]. Furthermore, movement of Ca^{2+} across biological membranes in exchange for Na^+ is now a well documented phenomenon in various tissues [15–21] including the proximal tubule of kidney [22,23], the site of renal gluconeogenesis [24].

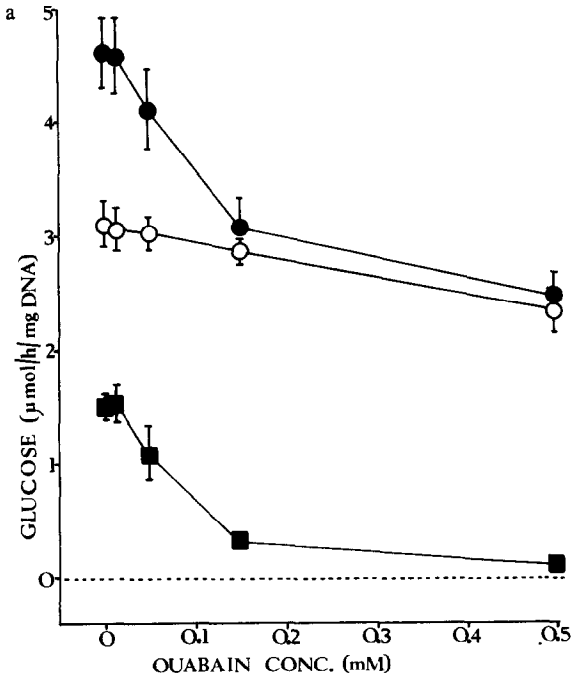
We have therefore investigated the effects of the Na^+/K^+ ATPase inhibitor ouabain and of K^+ removal on α-adrenergic stimulation of gluconeogenesis. Both treatments were found to greatly diminish the hormonal effect without appreciably perturbing the basal rate of the process. Although both ouabain and K^+ removal have been shown to diminish catecholamine stimulation of rat adipocyte lipolysis [25] (a β-adrenergic effect [26]) and to block β-adrenergic effects on contractility and K^+ flux in smooth muscle [27], we are unaware of reports of these treatments affecting α-adrenergic responses.

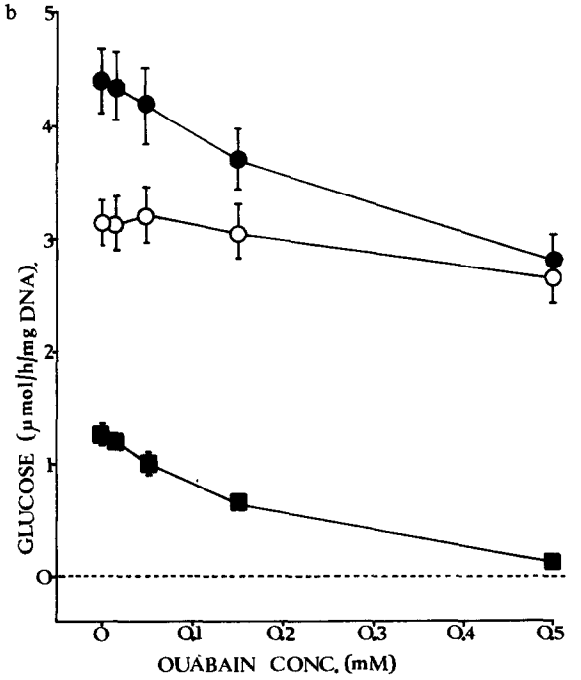
2. Materials and methods

Chemicals were obtained as in [2]. In addition, noradrenaline bitartrate was from Sigma, ouabain from Boehringer and oxymetazoline was a gift from Merck. Renal cortical tubule fragments were isolated by collagenase treatment of cortex pieces obtained from fed male Sprague-Dawley rats (160–180 g body wt) in [2]. Portions of tubules were continuously gased with O_2 + CO_2 (95:5%) and shaken for 1 h at 37°C in Krebs-Ringer bicarbonate buffer containing, unless otherwise stated, 6 mM K^+, 1.27 mM Ca^{2+}, fatty acid-poor albumin (10 mg/ml) and 5 mM sodium L-lactate. Incubations were deproteinised and glucose assayed as in [2]. DNA was estimated by the Burton method [28]. Statistical significance was determined by Student's *t*-test on a paired basis.

3. Results and discussion

Noradrenaline at 1 μM maximally stimulates gluconeogenesis in rat tubule fragments ([1,2] and see fig.2). Ouabain at 150 μm abolished the effect of the hormone without appreciably altering the basal rate of the process (fig.1a). On the other hand, this concentration of ouabain was not sufficient to abolish the effect of oxymetazoline (fig.1b), which was also added at its optimal concentration of 10 nM [2]. The





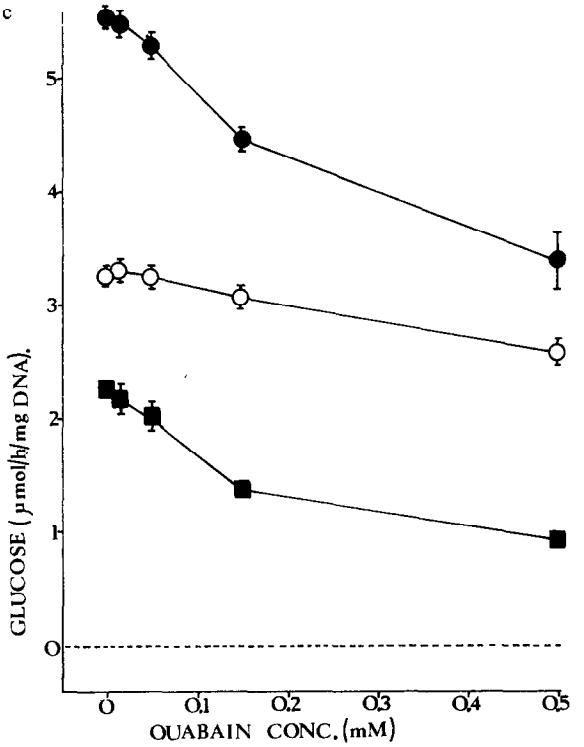


Fig.1. Effect of ouabain on gluconeogenesis and its stimulation by noradrenaline (1 μM), oxymetazoline (10 nM) and 3′,5′-cyclic AMP (0.1 mM). The values are means ± SEM for the indicated number of separate tubule preparations. Where not shown, error bars lie within the symbols. (○) Basal rate; (●) with agonist; (■) increase due to agonist. (a) Agonist = noradrenaline; 5 measurements; 193 ± 2 μg tubule DNA/ml flask contents. (b) Agonist = oxymetazoline; 4 measurements; 184 ± 6 μg tubule DNA/ml flask contents. (c) Agonist = 3′,5′-cyclic AMP; 4 measurements; 173 ± 3 μg tubule DNA/ml flask contents.

reason for this difference between these agonists is unclear at present, but their actions differ in other respects; i.e., oxymetazoline is ineffective in the absence of extracellular Ca^{2+} [2] and, unlike noradrenaline, does not stimulate release of ^{45}Ca from tubular tissue preloaded with this isotope [10]. Stimulation of gluconeogenesis by added 3′,5′-cyclic AMP presumably is not mediated by a cell-surface receptor linked to some intracellular messenger system. Ouabain was considerably less effective in opposing the stimulatory action of 100 μM 3′,5′-cyclic AMP (fig.1c). It is unlikely that ouabain is acting as an α-receptor competitive antagonist since

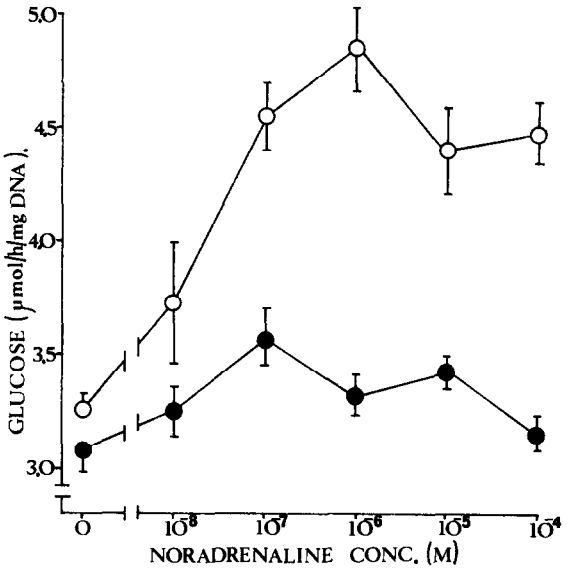


Fig.2. Effect of ouabain (150 μM) on noradrenaline dose curve. The values are mean ± SEM for 4 separate tubule preparations. The mean tubule DNA was 173 ± 3 μg/ml flask contents. (○) Without ouabain; (●) with ouabain.

ouabain diminished the stimulatory effect of noradrenaline at all tested concentrations of the catecholamine (fig.2).

Table 1 shows that omission of K^+ from the incubation medium did not alter the basal rate of gluconeogenesis from that found with the normal K^+ concentration of 6 mM. K^+ removal did however abolish the effects of both noradrenaline and oxymetazoline. With the intermediate K^+ concentration of 1.5 mM, basal gluconeogenesis was greater than at 6 mM K^+. A similar effect has been reported in [29]. Both noradrenaline and oxymetazoline stimulated gluconeogenesis in 1.5 mM K^+, but the effect of noradrenaline was slightly blunted, whereas that of oxymetazoline was slightly enhanced.

During this study ouabain was reported to abolish a stimulatory effect of angiotensin II on gluconeogenesis in tubule fragments from 24 h fasted rats [29]. This may imply some similarity in the mechanisms of α-adrenergic and angiotensin stimulation of this process, although the dose of ouabain required to abolish angiotensin action was considerably higher (1 mM) [29]. Small increases in renal gluconeogenesis have been observed when tissue from fasted rats is incubated with ouabain [29,30]. Whether our inability to observe a stimulation of basal gluconeogenesis with ouabain reflects differences in dietary status between this study and [29,30] is unclear.

In conclusion, it is suggested that the effect of noradrenaline is dependent on 'normal' cellular levels of K^+ and/or Na^+. Alternatively, or additionally, movements of one or both of these ions (possibly in association with Ca^{2+}) is involved in message transfer from the cell surface when the tubular cell is stimulated by α-adrenergic agonists. These possibilities are the subject of further study.

Table 1
Effect of K^+ concentration on gluconeogenesis and its stimulation by noradrenaline (1 μM) and oxymetazoline (10 nM)

K^+ (mM)	Glucose formation (μmol .h^{-1} .mg DNA^{-1})		
	Basal	+ Noradrenaline	+ Oxymatazoline
0	2.71 ± 0.20	2.84 ± 0.25	2.87 ± 0.31
1.5	3.26 ± 0.16[b]	4.00 ± 0.25[a,c]	4.37 ± 0.24[a,d]
6	2.88 ± 0.16[g]	3.89 ± 0.20[a,f]	3.68 ± 0.21[a,e,g]

[a] Indicates $P < 0.01$ for effects of agonists versus the apropriate basal value
[b–d] Indicate $P < 0.05$, 0.01, 0.001, respectively, for comparison of 0 and 1.5 mM K^+
[e,f] Indicate $P < 0.05$, 0.01, respectively, for comparison of 0 and 6 mM K^+
[g] Indicates $P < 0.01$ for comparison of 1.5 and 6 mM K^+

The values are means ± SEM for 5 separate tubule preparations. The mean tubule DNA was 151 ± 2 μg/ml flask contents

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