A lack of correlation between rat kidney mitochondrial swelling and glutaminase activation in metabolic acidosis[1]

T. A. Rakowski, L. L. Vertuno and H. G. Preuss

Nephrology Division, Department of Medicine and the Department of Pathology, Georgetown University School of Medicine, Washington (D. C. 20007, USA), 18 May 1977

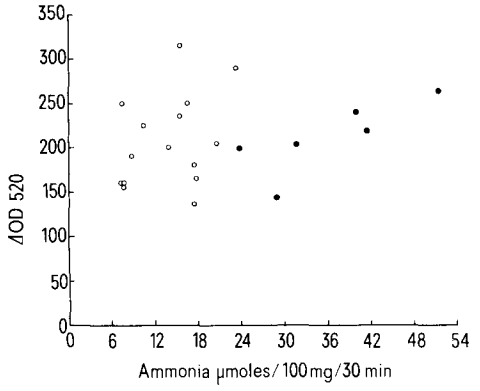
Summary. We found no overall correlation between mitochondrial swelling and PDG activity under many different conditions. We conclude that augmented PDG activity in acidosis is not related, at least to any great extent, to increased anion permeability produced by mitochondrial swelling.

We undertook the present investigation in order to determine whether the rate of swelling of rat kidney mitochondria correlates with the changes in phosphate dependent glutaminase (PDG) activity which occur during acidosis. NiFhaolain and O'Donovan[2] postulated that an accelerated swelling rate of isolated kidney mitochondria in acidosis could explain, at least in part, the increased phosphate activation of renal PDG seen in the rat. Earlier, Guha and Chakravarti[3] showed that swelling of mitochondria was a prerequisite for anion activation of PDG in guinea-pig liver mitochondria.

Table 1. ΔOD and ammoniagenesis by mitochondria from control and acidotic rats in the presence of different concentrations of phosphate

Phosphate concentrations	No. rats for C and A	ΔOD (30 min)	Ammonia (μm/30 min)
0	9C	198 ± 18	1.4 ± 0.3
	7A	182 ± 53	1.6 ± 0.7
0.02 M	8C	166 ± 17	4.9 ± 0.8
	6A	201 ± 21	7.9 ± 0.9*
0.05 M	9C	165 ± 16	9.1 ± 0.8
	7A	196 ± 25	13.8 ± 1.5**
0.15 M	9C	193 ± 17	15.1 ± 1.6
	7A	228 ± 22	28.0 ± 3.8**
0.25 M	8C	220 ± 17	17.8 ± 2.0
	5A	252 ± 36	36.5 ± 6.8**
0.30 M	9C	227 ± 20	19.3 ± 1.6
	7A	247 ± 32	37.9 ± 4.5***

C, mitochondria from control rats; A, mitochondria from acidotic rats. * $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$, acidotic mitochondria compared to control.



Plot showing lack of correlation between ΔOD and PDG activity in mitochondria from control (○) and acidotic (●) rats incubating in 0.3 M phosphate.

Methods. Male rats (Sprague-Dawley), 200–400 g, were used. Metabolic acidosis was produced by replacing drinking water with 1% NH_4Cl solution (w/v) for 5–7 days. Mitochondria were prepared by the method described by Tapley[4] in accordance with NiFhaolain and O'Donovan[2]. Stringent attempts were made not to vary this procedure. The washed mitochondrial pellet was suspended in 0.3 M sucrose brought to pH 7.4 with 0.2 Tris (hydroxymethyl) aminomethane (Tris) buffer. Mitochondria from 200 mg of kidney cortex were incubated at 37 °C for 30 min with 1 ml of 0.2 M glutamine and 4.8 ml of 0.3 M sucrose buffered to pH 7.4 with 0.02 M Tris. When phosphate activation of glutaminase was studied, 0.3 M phosphate pH 7.4 replaced some of the sucrose in the incubation medium in graded amounts as indicated in table 1. After incubation, ammonia was estimated on the supernate according to the method of Preuss et al.[5]. Appropriate tissue and substrate blanks were run simultaneously in all experiments, and ammonia results were corrected for these blanks.

The technique used for measuring mitochondrial swelling is described by Tapley[4]. The rate of swelling was estimated by the decrease in OD at 520 nm. In some experiments, we added EDTA, a substance known to affect mitochondrial swelling[2–4].

Results and discussion. Increasing medium phosphate in graded amounts (table 1) enhanced swelling of mitochondria from control and acidotic rats only at the greater concentrations of phosphate (.25 M or above). At no given concentration of phosphate did swelling of mitochondria from acidotic rats prove statistically different from control.

Despite no marked changes in swelling, ammonia production by mitochondria from acidotic rats increased markedly over ammonia production of control mitochondria, even at the lower concentrations of phosphate (table 1). Ammonia production was related to phosphate concentration in the medium with maximal ammonia production

1 Acknowledgments. Supported by NIH Grant AM 15458. The authors wish to express their appreciation to Betty Mendelson, Susan Dreux, and Patti Werr for secreterial assistance.

2 I. NiFhaolain and D. J. O'Donovan, Am. J. Physiol. *221*, 58 (1971).
3 S. R. Guha and H. S. Chakravarti, Experientia *16*, 451 (1960).
4 D. F. Tapley, J. biol. Chem. *222*, 325 (1956).
5 H. G. Preuss, B. W. Bise and G. E. Schreiner. Clin. Chem. *12*, 329 (1966).

Table 2. ΔOD and ammoniagenesis in the presence of EDTA (10 mM)

Condition	No.	ΔOD (30 min)	Ammonia (μm/30 min)
Control	4	0.208 ± 0.017*	10.0 ± 0.2
Test	6	0.170 ± 0.002	9.9 ± 0.2
		$p < 0.025$	p NS

* = SEM; NS = not significant.

occurring at the highest phosphate concentration tested (0.3 M).

In agreement with Tapley[4], we found that there was difficulty in reproducing mitochondrial swelling results found by others, Our data on mitochondrial swelling, obtained using the same methods described by NiFhaolain and O'Donovan were not in concert with theirs[2] – agreeing more closely with Tapley's results[4]. While NiFhaolain and O'Donovan report very little swelling with 0.3 M sucrose, we found, that at this sucrose concentration, extensive swelling of rat kidney mitochondria occurred even to the point that swelling in 0.3 M sucrose was not significantly different from the swelling obtained at 0.3 M phosphate ($p > 0.05$). Why there was a difference in swelling between experiments is less important than finding no correlation between mitochondrial swelling and ammonia production. Our data do not negate the hypothesis of Guha and Chakravarti[3] that some swelling of mitochondria is needed for phosphate activation of PDG, for under every condition that we studied PDG, there was some mitochondrial swelling.

To further disassociate mitochondrial swelling from glutamine ammoniagenesis, the ΔOD's for control and acidotic rat kidney mitochondrial suspensions at 0.3 M phosphate concentrations from all experiments were plotted against ammonia production (figure). No correlation could be found between ΔOD and ammonia production when the concentration of phosphate was unchanged. In the presence of 0.3 M phosphate and 10 mM EDTA (6 flasks) mitochondrial swelling was inhibited compared to mitochondria incubated in the phosphate alone (table 2) ($p < 0.025$). Despite decreased mitochondrial swelling, no inhibition of ammoniagenesis was demonstrated.

These data do support the suggestion of NiFhaolain and O'Donovan[2] that increased permeability of mitochondrial membranes to glutamine is not the rate limiting factor for PDG activity, again because at any given phosphate concentration ammonia production from glutamine is not related to swelling (figure). We conclude that the major increase in PDG activity in acidosis is not related to increased anion permeability produced by mitochondrial swelling.

Colchicine inhibition of ADH effect on frog skin permeability*

M. Svelto and C. Lippe

Institute of General Physiology, University of Bari, via Amendola 165A, I-70126 Bari (Italy), 8 August 1977

Summary. ADH and AMPc enhance both thiourea unidirectional fluxes in frog skin. This effect is completely abolished by colchicine pretreatment. The ADH increase of thiourea discharge with or without colchicine led us to suppose that colchicine does not directly affect ADH action on outer membrane permeability, but exerts its effects on a site which is limiting for the ADH action on transepithelial permeability.

It is well known that some antimitotic agents such as colchicine inhibits certain cytoplasmic phenomena such as intracellular movement[1] and exocytotic transport[2]. These effects are due to a microtubule disruption wich occurs after a long time-lag.

Recently Taylor et al.[3] have reported that colchicine treatment strongly inhibits the action of vasopressin on osmotic water movement across the toad bladder, without affecting Na active transport. In a previous paper[4], we have demonstrated that noradrenaline-induced secretion of nonelectrolytes through the frog skin is suppressed by colchicine treatment.

This work deals with the effects of colchicine on ADH-activated thiourea permeability across the frog skin (*Rana esculenta*). Table 1 reports the effects of ADH on thiourea transepithelial fluxes. It can be seen that ADH treatment results in a symmetrical increase of the thiourea fluxes. 4 h preincubation in the presence of colchicine completely abolishes the ADH effect on both fluxes. Colchicine added immediately after the equilibration period (i.e. without preincubation) fails to influence the ADH effect on thiourea permeability.

Thus we can exclude the possibility of the inhibitory effect of colchicine being due to its interaction with the ADH receptor. The presence of a wide time-span seems to confirm that the colchicine effect is due to a true interaction with microtubule protein[5]. Lumicolchicine, a colchicine derivative which does not bind microtubule protein, is generally used as a control of the specificity of colchicine effect[5].

In frog skin, 4 h exposure to lumicolchicine does not affect the ADH action on thiourea permeability. Thus the presence of a wide time-span for the colchicine effect, and the failure of lumicolchicine to affect ADH action, strongly suggest that colchicine effect is quite specific, i.e. it is due to interaction with microtubule protein. Dibutyryl-AMPc 10^{-3} M mimics the ADH effect on thiourea permeability (table 2), this effect being completely abolished by colchicine pretreatment. Cyclic AMP is generally considered the cellular mediator of ADH actions. Thus, the inhibition induced by colchicine appears to be subsequent to cAMP production. Finally the effect of colchicine on ADH action cannot be due to tissue damage[4]. In fact thiourea control values (table 1 and 2) are virtually the same with and without colchicine.

All these considerations strongly support the idea that colchicine inhibition of ADH action on thiourea permeability is related to the disruptive effect of the antimitotic on microtubules. It is very difficult to propose a model for this ADH action, as the existence of a symmetric ADH effect, colchicine-sensitive, seems to exclude a secretory process mediated by exocytotic vesicles. However, it is improbable that ADH effect is mediated by a microtubule assembly only, because the hormone effect is very selective.

* The present work has been supported by C.N.R., Rome.

1 D. Mazia, J. gen. Physiol. *50*, suppl., 221 (1967).
2 T. S. Orr, D. E. Hall and A. C. Allison, Nature *236*, 350 (1972).
3 A. Taylor, M. Mamelak, E. Reaven and R. Maffly, Science *181*, 347 (1973).
4 M. Svelto, M. Perrini and C. Lippe, J. Membrane Biol., 36, 1 (1977).
5 L. Wilson and M. Friedkin, Biochemistry 6, 3126 (1967).