

Discussion

Aldosterone action: new answers, new questions

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Among those working on hormone action in the laboratory, the mineralocorticoid receptor (MR) has long been the Cinderella of the steroid/thyroid/retinoid/orphan receptor family. Among those working in hypertension or clinical endocrinology aldosterone has enjoyed a similar status, with attention largely confined to the occasional adenoma or pesky bilateral hyperplasia. Over the past few months there have been two reports—one basic, the other clinical—which merit the often abused term ‘breakthrough’, in terms of MR and the pathophysiology of aldosterone. The first is the characterization of *sgk-1*, a serine-threonine kinase, as a protein directly induced by aldosterone acting via mineralocorticoid receptors, and its role in increasing sodium transport by activation of epithelial sodium channels. Second is the demonstration, in the inevitably eponymous RALES trial, that very modest doses of the MR antagonist aldactone produce a remarkably beneficial effect in patients with moderately severe heart failure when given in addition to current standard therapy.

David Pearce and his colleagues (Chen et al., 1999) started off with A6 cells, a collecting duct-like cell line from *Xenopus laevis* kidney. As in the mammalian kidney, the effect of aldosterone on urinary electrolytes is mimicked by that of glucocorticoid acting via glucocorticoid receptors (GR: Funder et al., 1990; Naray-Fejes-Toth and Fejes-Toth, 1990). For this reason, and because GR are much more abundant than MR in A6 cells, David chose to use dexamethasone, as a perhaps counterintuitive ligand with which to probe A6 cells for MR-regulated genes.

When A6 cells were incubated with 10^{-7} M dexamethasone a number of RNA species were found to be upregulated compared with control, by the technique of suppression-subtraction hybridization. One of the clones so identified showed 92% identity and 96% similarity with rat *sgk*, previously cloned and characterized

across San Francisco Bay in Berkeley (Webster et al., 1993). Time course studies showed *sgk* mRNA in A6 cells to be rapidly steroid responsive, with a 3-fold increase in 15 min, reaching maximal levels at 45–60 min, and declining to 1.6-fold basal by 24 h despite the continued presence of ligand. The induction was not blocked by cycloheximide, at levels which reduce Na^+ transport by <90%, evidence for a direct transcriptional effect of the hormone. Both basal and steroid-induced levels of *sgk* mRNA in fact were elevated by cycloheximide, as previously described for other immediate early genes. Importantly, immunoreactive *sgk* protein also increased rapidly after steroid treatment, consistent with the known ≈ 45 min latency of aldosterone effects on measured Na^+ flux.

A6 cells and dexamethasone are one thing, the mammalian collecting duct and aldosterone are another. That *sgk* is aldosterone-induced in the appropriate mammalian nephron segments was shown by in situ hybridization studies in adrenalectomized rats. In such animals moderate levels of *sgk* expression were noted in the glomerular tufts, but none in the collecting tubules and collecting ducts. When such rats were treated with admittedly pre-emptive doses (50 $\mu\text{g}/100$ g bw) of aldosterone the glomerular signal remained unchanged, but the distal tubules/collecting ducts showed very marked *sgk* expression, exactly where MR and its protective enzyme, 11β -hydroxysteroid dehydrogenase, have been shown to be coexpressed.

David and his colleagues then closed the loop by coexpression studies in *Xenopus* oocytes. When *sgk* was coexpressed with a construct coding for the potassium channel ROMK2 no increase in amiloride-inhibitable current, as a surrogate for ion transport, was seen. In contrast, coexpression of *sgk* with the three sodium channel (ENaC) subunits resulted in a seven-fold increase in amiloride-sensitive current, indicating a marked stimulation of ion flux. Whether this represents

an increase in open probability of ENaC already in the membrane, or a rapid translocation of channels to the membrane, remains to be explored. While the present studies are consistent with a direct phosphorylation of the β - and γ -ENaC subunits by *sgk* as the mechanism of channel activation, they do not exclude additional components of a signalling cascade acting as obligate intermediates.

It's a complex and finely structured paper, in wine terms a Richebourg or Romanée-Conti: given its Californian provenance, and that it's the first unequivocal result of over thirty very frustrating years of work, it might be better thought of as Opus One. There are, of course, questions; some of these, like the role of *sgk* in the glomerulus, are addressed in the paper while others are not. In the original *sgk* cloning paper, highest levels of expression were found in ovary, thymus and lung, hardly classical aldosterone target tissues, with low but detectable levels in most other tissues. Like the MR, which is widely distributed with its highest levels in the hippocampus, *sgk* may have been pressed into service in epithelia, with the specificity in the system vested squarely in 11β HSD2-expression of which itself is not totally confined to epithelia (Roland et al., 1995). Secondly, while ENaC is the clear target (direct or indirect) for aldosterone induction in the kidney, it may well not be expressed in response to aldosterone activation of MR in nonepithelial tissues, which probably account for many of the pathophysiologic and clinical effect of aldosterone/salt imbalance. Finally, the IEG profile of mRNA levels is intriguing at one level, and satisfying at another. It is intriguing given the very similar pattern of aldosterone synthase expression in response to ACTH, where levels also return essentially to basal within 24 h of constant stimulus. It is satisfying in an additional, and perhaps more important, account of the phenomenon of mineralocorticoid escape, long held to be the province of counter-regulation by atrial natriuretic peptide.

The RALES trial (Packer and Cohn, 1999) similarly raises a number of questions, having provided a very clear cut answer in terms of lowering mortality, as the primary end point, and a series of morbidity associated indices as secondary end points. The trial took New York Heart Association class III/class IV patients with heart failure, treated with angiotensin converting enzyme inhibitors, loop diuretics and (in $\approx 70\%$ of patients) digitalis, and randomized them into two groups. One group received Aldactone[®] 25 mg/day in addition, and the other placebo. On clinical grounds, or at the physician's discretion, the dose of either could be doubled or halved, a change observed more frequently with placebo than with spironolactone. The trial was meant to run for 3 years, but at the insistence of the monitoring commit-

tee the code was broken just after half way through, on ethical grounds. Patients receiving spironolactone, at this very modest dose, showed 25–30% improvement in terms of primary and secondary end points, and the monitoring committee very appropriately took the line that there was no ethical basis to continue the placebo arm.

Over a range of indices there were no differences between the two groups. The randomization was essentially perfect, with no differences between placebo and spironolactone groups, each of which numbered 800+ patients. No differences in compliance or drop-out rate in blood pressure over the period of the study, or in the incidence of marked (>6 meq/l) hyperkalemia were recorded. There were two salient differences, one small, the other large. First, spironolactone-treated patients showed a small (≈ 0.3 meq/l) but significant elevation of plasma K^+ after chronic administration. Secondly, they showed an 8.5% incidence of gynecomastia, even on the very modest dose of drug, compared with the control value of just over 1%.

The therapeutic, and indeed public health, implications of the trial are profound. Addition of aldosterone—or its candidate replacement, the selective aldosterone receptor antagonist eplerenone, which should not produce gynecomastia—to current 'best practice' can be predicted to radically improve morbidity and mortality. Addition of the non-selective β adrenoreceptor antagonist carvedilol to current 'best practice' treatment regimes similarly appears to improve mortality figures, particularly when patients are stratified on the basis of BNP levels; together they may provide a real key to improving the neuro- as well as the humoral status of patients with severe heart failure, now an epidemic disease. The mechanism of the efficacy of spironolactone in the RALES trial is not immediately apparent; the rapidity with which the two groups diverged would need a very rapid response in terms of ameliorating cardiac fibrosis, and it may be that its action at least to some extent involves the small but significant differences noted in plasma potassium: as the French say, on verra.

The Western world expects great things from the Year 2000 AD—fireworks, jubilees in Italy, the collapse of civilization as we know it on 1/1/00, thanks to the long-sightedness of our computer programmers. For those happy few for whom aldosterone is the main game it would be impossible to imagine a better year than 1999. To David Pearce and his colleagues, and to those who persuaded Searle/Monsanto to invest the resources for RALES using an out-of-patent drug, the happy few—and the millions of patients with heart failure—owe a debt of very sincere thanks.

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