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EDITORIAL

The case against aldosterone: not proven

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In their paper, in the current issue [1], Fu et al. adduce three lines of evidence for aldosterone-induced osteopontin (OPN) expression in vascular smooth muscle cells (VSMCs). First, they show that spironolactone reduces vascular injury-induced neointimal hyperplasia in rat vessels in vivo. Second, aldosterone at 100 nM produces a doubling of OPN expression after 24 h (but not 6 or 12 h) exposure; OPN dose–response curves climb steadily (if disconcertingly) in a straight line for aldosterone concentrations from 1 to 100 nM. Finally, they show that MAPK, but not JUNK, pathways are involved in the acute effects of aldosterone (again at 100 nM) to elevate OPN levels.

There are several minor points of detail in these findings that deserve comment, and one major issue of interpretation, as foreshadowed by the title of this commentary. The affinity of aldosterone for mineralocorticoid receptors (MR) is in the mid-to-high picomolar range: progressively increasing responses from 1 to 100 nM suggest mechanisms in addition to MR in producing the effect observed. Second, despite repeated assertions, neither spironolactone nor RU486 are specific antagonists for MR/glucocorticoid receptors (GR): spironolactone is an antagonist at androgen receptors (AR), and a progesterone receptor (PR) agonist, and RU486 is a PR antagonist. Finally, and very surprisingly, given its fivefold lower affinity for MR, spironolactone at 1 μM completely reversed the acute effect of aldosterone (100 nM) on OPN levels.

The major assumption/inference is that aldosterone is the responsible pathophysiologic actor in vivo. The authors are not alone in this position: the opening sentence of the abstract reads "Osteopontin (OPN) is known to be one of the cytokines that is involved in the vascular inflammation caused by aldosterone." The final sentence of paragraph 3 of the Discussion summarizes the findings thus: "These results suggest that aldosterone in its physiological and/or pathophysiological concentrations regulates OPN expression through MR in VSMCs."

To question both the opening sentence (… caused by aldosterone…) and the interpretation of the study findings requires a broader consideration of the promiscuity of MR, aldosterone selectivity-conferring mechanisms in epithelial cells (and VSMC), and the bivalent actions of the physiologic glucocorticoids at MR under normal conditions (antagonist) and in conditions of tissue damage (agonist). MR were the first of the MR/GR/AR/PR subfamily of nuclear receptors to branch off a common ancestor [2], are found in bony and cartilaginous fish, and antedate by millions of years the appearance of aldosterone synthase in terrestrial vertebrates. They are also spectacularly promiscuous, binding with equivalent high affinity aldosterone and deoxycorticosterone as agonists, progesterone as an antagonist, and cortisol and corticosterone as antagonist or agonist depending upon the circumstances.

In epithelia, the vessel wall and the nucleus tractus solitarius MR are "protected" by the enzyme 11βhydroxysteroid dehydrogenase type 2 (11βHSD2), which converts cortisol and corticosterone to their receptor-inactive 11-keto analogs cortisone and 11-dehydrocorticosterone [3]. It is still commonly assumed that the enzyme protects MR from being occupied by glucocorticoids, which would require >999 of every 1,000 cortisol molecules being metabolized to cortisone in an organ (the kidney) which receives 20–25 % of the cardiac output.

This was shown almost 20 years ago not to be the case [4]. What the enzyme does is debulk intracellular glucocorticoid levels of aldosterone from 100-fold to 10-fold,

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and somehow to prevent the ~90 % of MR thus occupied by cortisol to be activated by the hormone. From indirect evidence, this would appear to involve the redox change attending the conversion of NAD to NADH, molecule for molecule for the conversion of cortisol to cortisone. In the Kagawa bioassay, for example, the dose–response curve for aldosterone is shifted an order of magnitude to the left in adrenalectomized versus sodium-loaded rats, reflecting the ~10-fold higher availability of renal MR.

It is widely accepted that when 11βHSD2 is deficient or blocked, as in apparent mineralocorticoid excess or licorice abuse, glucocorticoids not only occupy but activate MR. What is less well known is that the same thing—MR activation by normal levels of physiologic glucocorticoids—occurs in both epithelial and non-epithelial tissues in the context of tissue damage, reactive oxygen species generation, and the resultant intracellular redox change. Normally the physiologic glucocorticoids block the MR-mediated effects of aldosterone; in tissue damage, however, they mimic them, as recently shown to be the case in cardiac ischemia-reperfusion studies [5].

What the in vivo study by Fu et al. shows is that spironolactone reduces the vascular injury-induced neointima formation and elaboration of OPN, not that aldosterone is responsible. It may be, but it is in fact more likely to represent activation of vascular wall MR by corticosterone in the damaged tissue. Rats given deoxycorticosterone acetate (DOCA) plus salt show a doubling of coronary perivascular fibrosis after 4 weeks, and a trebling after 8 weeks. If DOCA is discontinued after 4 weeks, the extent of perivascular fibrosis stays at double; if the selective MR antagonist eplerenone is added to the DOCA for weeks 5–8, perivascular fibrosis returns to control, baseline levels [6]. The inference from these studies, in adrenal intact animals, is that the inflammatory state initiated by DOCA/salt can be maintained by corticosterone after DOCA withdrawal.

In vitro experiments classically show what can happen, not what does happen in vivo; nowhere is this case more than for aldosterone. It is not difficult to see how this has happened: the physiology of aldosterone was taught as neatly epithelial, a nephrologists' hormone, then enthusiastically (but uncritically) adopted by the cardiologists in the wake of the RALES trial [7]. Aldosterone is a near magical feat of evolution, in that cyclization of the hydroxyl at carbon 11 with the unique and very reactive aldehyde group at carbon 18 of the steroid skeleton renders it impervious to attack by 11βHSD2, in cells in which the enzyme is coexpressed. The hormone took centre stage, having been characterized 2 decades before the receptor, despite being millions of years behind in evolution. That MR are the cognate receptor for aldosterone is incorrect, not only in terms of evolution but also for human biology, in that 90–99 % of MR in the human body are occupied by cortisol, with a range of physiologic roles unfortunately only sketchily documented to date.

Grossly elevated aldosterone levels in chronic sodium deficiency are homeostatic not pro-inflammatory, so an inflammatory response does not necessarily follow MR activation by aldosterone, in epithelia or the vessel wall. The enigma is that the inflammatory response to MR activation occurs only in the presence of sodium excess, which will drive endogenous aldosterone levels down. Given the aldosterone-mimicking effect of glucocorticoids in tissue damage, and their orders of magnitude higher circulating free levels than those of aldosterone, it is most likely that they are the culprits; it does not prove that aldosterone is not involved in the OPN response via MR in damaged tissue, but it makes it highly unlikely. In the words of the old and admirable verdict in Scottish law, the verdict must be (at least) "not proven."

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Conflicts of interest The author has no conflicts of interest.

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