Editorial Focus. Trilostane, FAD286, and the role of aldosterone in the central regulation of blood pressure: focus on “Role of central nervous system aldosterone synthase and mineralocorticoid receptors in salt-induced hypertension in Dahl salt-sensitive rats”

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In a previous issue of this journal, Elise Gomez-Sanchez et al. (4) presented unequivocal evidence that intracerebroventricular (ICV) infusion of the 3β-hydroxy steroid dehydrogenase inhibitor trilostane prevented the development of hypertension in response to high-salt intake in Dahl salt-sensitive (Dahl S) rats; it also reversed the blood pressure elevation in rats with established hypertension. The effects were indisputable; the inference that they constituted evidence for local production of aldosterone being responsible for the salt-induced blood pressure rise was disputed in an accompanying commentary (2). As trilostane is an early stage (conversion of pregnenolone to progesterone) inhibitor of steriodogenesis, not only would levels of putatively produced aldosterone be predicted to fall, but also those of corticosterone, previously shown by the same authors to be a stoichometric blocker of the elevation of blood pressure when infused with aldosterone (7). The commentary did not dispute the findings but urged caution on their interpretation and proposed that a more direct study would involve using the relatively specific CYP11B2 (aldosterone synthesis) blocker FAD286. Huang et al. (8) from the Ottawa Heart Institute and Novartis, have now done this study.

The blood pressure findings in the Huang’s study are equally unequivocal, albeit less marked than those with trilostane. From the day 3 of a high-salt diet, mean arterial pressure (MAP) was significantly different between Dahl S rats infused with FAD286 100 μg·kg⁻¹·day⁻¹ and those infused with artificial cerebrospinal fluid as vehicle. At the end of week 2, MAP had reached plateau levels 35% lower than vehicle. In contrast, spironolactone at 10 μg·kg⁻¹·day⁻¹ reduced the elevated MAP by > 90% to levels not different from control. A similar extent of MAP reduction was seen with either 10 or 80 μg·kg⁻¹·day⁻¹ doses of FAD286, suggesting that the more modest effect than seen with spironolactone was not a dosing phenomenon. On the basis of the fall in blood pressure, which as noted above is unequivocally demonstrated, and a measured rise in aldosterone levels in the hypothalamus (but not other brain areas) of Dahl S rats, the authors open their discussion with the statement that “The present study provides direct evidence for a biologically relevant role of aldosterone produced locally in the CNS in the regulation of cardiovascular homeostasis in Dahl rats fed a high-salt diet. . . ” (8).

Two major lines of evidence are produced in support of this claim. The first is that high-salt intake decreases hypothalamic aldosterone content in Dahl salt-resistant (Dahl R) rats but caused increases in Dahl S rats. The measured fall in hypothalamic aldosterone concentration in Dahl R rats is to levels one-third of those in rats on a regular salt intake; similar falls in hippocampal aldosterone levels and hypothalamic corticosterone levels are reported, with no change in plasma levels of either steroid. The implications of these remarkable changes are not discussed. The measured tissue levels of aldosterone on regular salt are around 1 ng/g, compared with the measured plasma levels on either diet, which are around 0.5 ng/ml. Aldosterone has a very high reflection coefficient at the blood-brain barrier, ~8 times higher than corticosterone (1). Tissue-free levels, therefore, would be expected to be considerably lower than those in plasma.

The implications are 1) that most of the measured aldosterone in the two tissues is sequestered in lipid and thus not available for receptor binding and 2) that somehow high salt lowers to one-third the levels of sequestered aldosterone, with no change in plasma levels. The lack of change in plasma levels between regular and a high-salt diet presumably reflects the relatively high (though not stated) salt content of regular chow. An alternative possibility for the Dahl R findings is the notorious unreliability of the aldosterone radioimmunoassay, even in very experienced hands. Whatever the ultimate explanation, the major fall in reported aldosterone levels in the two tissues, one of which is putatively a site for ectopic biosynthesis and the other not, while plasma levels remain unchanged, should introduce a note of caution into interpretation of the data.

The measured increase of hypothalamic aldosterone in Dahl S rats in response to high salt is 35% to levels equivalent to those seen in Dahl R rats on the regular diet, and hypothalamic levels are lowered by 10 or 80 μg·100 g⁻¹·day⁻¹ FAD286 or 10 μg·kg⁻¹·day⁻¹ spironolactone to levels seen in Dahl S rats on regular chow. The corresponding plasma levels in Dahl S rats show no change, with one salient exception; spironolactone at a dose too low for systemic mineralocorticoid receptor (MR) effects, nonetheless, lowered plasma aldosterone levels to 35% of control and corticosterone levels to > 50%. The commentary offered by Huang et al. (8) for this observation is unconvincing: “The lower plasma aldosterone may then lead to less uptake of aldosterone into the hypothalamus, resulting in lower hypothalamic content.” is undeniable, except that the hypothalamic content was not lowered. “Alternatively, independent of MR, as one of its nonspecific actions, spironolactone may directly inhibit enzymes involved in steroid biosynthesis (32), thereby preventing increases in hypothalamic aldosterone by high salt intake.” is very unlikely, given the much lower dose of spironolactone compared with FAD286 and its much more potent effect on MAP. Whatever the ultimate explanation, the
disconnect between plasma and hypothalamic levels of both steroids in the Dahl S rats infused ICV with spironolactone should again introduce a note of caution into interpretation of the data.

The second plank in the authors’ argument is that ICV FAD286 prevents a major part of this increase in blood pressure and heart rate, whereas MR blockade fully prevents the hypertension. What is not discussed are the differences in blood pressure responses between the two series of studies on Dahl S rats. In the first, rats were infused ICV for 4 wk with FAD286 at 10 or 80 μg·kg−1·day−1, and MAP changes at the end of the study are of the order of 50–70%. In the second study, in which MAP was measured daily for 17 days of high salt, the fall reached plateau levels by day 14 and was 35%; which is highly significant, but not a major part of the increase in blood pressure. Variation between experiments, all of which show concordant results, are commonplace in every laboratory; why the single time point observations are preferred to the much more detailed and convincing second study is unclear, particularly given the doses used.

There is, on the other hand, very strong evidence against a biologically relevant role for aldosterone, produced locally in the hypothalamus or from the adrenal, in blood pressure control. The first is that corticosterone has as high or higher affinity for MR than aldosterone, circulates at free concentrations 100–200 times that of aldosterone (and was measured in the hypothalamus at levels 300 times higher), and is a stoichiometric blocker of activation of MR when coinfused with aldosterone. This was demonstrated by Elise Gomez-Sanchez et al. (7) over 15 years ago, who showed that an equal concentration of ICV infused corticosterone significantly reduced the blood pressure elevation seen with aldosterone, and double the concentration reduced the blood pressure rise to half. The MR involved in raising blood pressure in response to infused aldosterone are thus not protected by 11β-hydroxy steroid dehydrogenase-2, and consequently are not aldosterone-selective. Additional evidence comes from studies of rats on a high, normal, or low-salt intake, with correspondingly different circulating aldosterone levels (9). Administration of an ICV bolus dose of the MR antagonist RU28318 produced 20–30 mmHg blood pressure falls in all three groups, regardless of circulating (or any locally produced in response to sodium status) aldosterone levels. There is no question that aldosterone-infused ICV can raise blood pressure, or that MR blockade can lower blood pressure in Dahl S rats given high salt (6, 8). There is, however, overwhelming evidence against aldosterone, from whatever endogenous source, having any biologically relevant role in the hypothalamic inputs to blood pressure regulation (3).

There are, in addition, other more theoretical considerations, as is also the case for the heart, long proposed but now no longer considered a site of ectopic aldosterone synthesis (5, 10). When levels of the enzymes required for steroid biosynthesis are determined in sites of putulated ectopic aldosterone biosynthesis, they are commonly a thousand times lower than in whole adrenal gland (of which the zona glomerulosa is only a few percent). If this distribution is equivalent across all cells in the tissue sample studied, every enzymatic step becomes rate limiting in terms of sequential substrate concentrations, and throughput to final product becomes minuscule at best. It may be that putative aldosterone biosynthesis is confined to one cell in a thousand (or ten thousand, given the difference between whole adrenal and zona glomerulosa), which thus represents a single cell surrounded, on average, by 10–20 cells in every dimension. If these hot-spots were confined to a single nucleus, they should be able to be visualized by immunohistochemistry or in situ hybridization; and even then, the issues raised in the previous paragraph remain to be dealt with.

If a physiological role for aldosterone in hypothalamic input to blood pressure control thus appears improbable, how is it that triostane and FAD286 raise blood pressure when infused ICV? Under Limitations of the Study, Huang et al. (8) acknowledge that drugs, such as FAD286 or triostane, have not yet been extensively studied and may have actions other than inhibition of steroidogenesis. In the present study, there is no question that FAD286 lowers blood pressure, but the data on plasma and tissue measured levels of steroids deserve to be interpreted with considerable caution. For ease of infusion, the FAD286 was administered as the hydrogen tartrate in the studies of Huang et al. (8). Perhaps it would be wise to exclude the hydrogen tartrate moiety as having activity under such circumstances.

The brain is a multiplex organ, and in many ways nothing, if proven, need come as a surprise. In this present case, what is adequately proven is that ICV infusion of FAD286 hydrogen tartrate modestly but significantly lowers MAP, just as ICV infusion of aldosterone raises it. Just as the latter has proven not to constitute evidence for a biologically relevant role for aldosterone in central nervous system control of blood pressure for reasons of prudence, neither should the former.

REFERENCES