Pergamon

0960-0760(95)00021-6

J. Steroid Biochem. Molec. Biol. Vol. 53, No. 1–6, pp. 53–55, 1995

0960-0760/95 $9.50 + 0.00

Mineralocorticoid Receptors and Hypertension

John W. Funder

Baker Medical Research Institute, Prahan, Victoria 3181, Australia

Mineralocorticoid receptors (MR) have equal affinity for the mineralocorticoid aldosterone, and the physiological glucocorticoids cortisol and corticosterone. In epithelial tissues *in vivo*, MR are protected against glucocorticoid occupancy by the enzyme 11β-hydroxysteroid dehydrogenase, allowing access by the lower circulating levels of the physiological mineralocorticoid aldosterone. In non-epithelial tissues, including the heart and most areas of the central nervous system, MR are not so protected, and their physiological ligand is cortisol/corticosterone. Intracerebroventricular infusion studies have shown that aldosterone occupancy of such unprotected circumventricular MR is necessary for mineralocorticoid hypertension, and the hypertensinogenic effects of peripherally infused aldosterone can be blocked by intracerebroventricular infusion of the MR antagonist RU28318. Prolonged (8 weeks) administration of mineralocorticoids to salt-loaded rats has been shown to be followed by hypertension, cardiac hypertrophy and cardiac fibrosis. Whether the hypertrophy and fibrosis reflect primary effects of aldosterone via cardiac MR, or effects secondary to occupancy of protected, epithelial MR, remains to be determined, as does the mechanism of action of salt loading in this model of mineralocorticoid hypertension.

J. Steroid Biochem. Molec. Biol., Vol. 53, No. 1–6, pp. 53–55, 1995

MR IN EPITHELIAL AND NON-EPITHELIAL TISSUES

Although mineralocorticoid receptors (MR) have similar high affinity for aldosterone, cortisol/corticosterone and progesterone in all tissues studied, they appear to operate very differently in epithelial and non-epithelial tissues. In epithelia, they appear to be protected from occupancy by the higher circulating levels of glucocorticoids under physiological conditions by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11HSD2), which has recently been cloned and expressed in the human [1] and sheep [2]. The first difference between epithelial and non-epithelial tissues would thus appear to be in terms of physiological ligand—aldosterone in Na^+ transporting epithelia (and in the hypothalamic area subserving salt appetite) and cortisol/corticosterone elsewhere.

This is clearly, however, not the only difference. MR can be activated by physiological glucocorticoids as well as aldosterone not only *in vitro*, in cotransfection systems [3], but also in the rat kidney *in vivo* [4]. Similarly, both *in vivo* [4] and *in vitro* [5], the selective glucocorticoid agonist RU28362—which has negligible affinity for MR—produces effects on Na^+ flux indistinguishable from those of aldosterone via MR, providing that the RU28362 can access the tubular glucocorticoid receptors (GR). From these data it would appear that transcriptional regulation by MR in kidney and other epithelial tissues is probably regulated via canonical pentadecamer hormone response elements, which are known to be activated by both GR or MR, and by MR whether activated by aldosterone or physiological glucocorticoids [3].

In non-epithelial tissues, however, there is clear evidence that MR and GR produce quite different effects; in addition, in a variety of systems, there is clear evidence that effects of aldosterone via non-epithelial MR can be blocked by corticosterone, and vice versa [6–8]. The difference between MR- and GR-mediated effects in the hippocampus [5, 6] or in the A3V3 region of the brain [8] may thus reflect transcriptional regulation via composite response elements, at which binding of activated GR but not MR has been shown to block AP-1 induced transcription [9]. The mechanism underlying the agonist/antagonist difference between MR in epithelial and non-epithelial tissues remains to be determined; of possible relevance is the recent report that the oestrogen agonist effects of tamoxifen are

Proceedings of the IX International Congress on Hormonal Steroids, Dallas, Texas, U.S.A., 24–29 September 1994.

uniquely mediated via composite response elements, whereas at classical oestrogen response elements it always acts as an antagonist [10].

MR AND HYPERTENSION

There is now abundant evidence that occupancy of circumventricular MR by aldosterone is necessary for mineralocorticoid hypertension. A modest salt intake is also required, and the elevation of blood pressure can be accelerated and exacerbated by increased salt and reduction of renal mass by uninephrectomy. Intracerebroventricular (i.c.v.) infusion of very low doses of aldosterone raises blood pressure in uninephrectomized rats drinking 1% NaCl solution with a time course of 14–20 days; central infusion of the same dose of corticosterone or the selective GR agonist RU26988, or peripheral infusion of the same dose of aldosterone, did not elevate blood pressure [8]. When higher doses of aldosterone are infused peripherally blood pressure rises; concomitant i.c.v. infusion of the selective MR antagonist RU28318 completely blocks the hypertensive response, at doses which do not affect the systemic salt-and-water effects of aldosterone, and which do not block the blood pressure rise if peripherally infused with aldosterone [11]. Finally, the rise in blood pressure in response to i.c.v. aldosterone can be substantially decreased by the concomitant i.c.v. infusion of corticosterone, at doses similar to those of aldosterone [8], suggesting that the effects of steroids on blood pressure are via MR which are unprotected by 11-HSD or other glucocorticoid-excluding mechanisms.

If this is the case, then, such receptors would presumably be physiologically occupied by glucocorticoids rather than by aldosterone, except perhaps at the nadir of circadian glucocorticoid levels in states of severe sodium deficiency. Although it may be tempting to speculate that in such a system corticosterone is the physiological agonist, maintaining normotension reflecting its occupying the bulk of the MR involved, there are a number of indications that this may not be the case. For example, adrenalectomized rats maintained on 1% NaCl do not become hypertensive even when infused i.c.v. with aldosterone, which is evidence against a tonic inhibitory role for corticosterone in the central control of blood pressure. Secondly, given the equivalent effects of i.c.v. corticosterone and RU28318 in blocking the hypertensive effect of aldosterone, an agonist role for corticosterone would entail a similar agonist role for RU28318—a formal possibility, but one for which there is no supporting data.

It would also seem that despite their being apparently "unprotected", occupancy of a relatively small percentage of circumventricular MR by aldosterone can lead to elevated blood pressure. Evidence for such an interpretation is the ability of i.c.v. RU28318 to block the hypertensive effect of endogenous aldosterone in jr/SS rats in response to 6% NaCl solution to drink [12]. Similarly, the demonstration that i.c.v. carbenoxolone raises blood pressure [13] is very difficult to reconcile with its peripheral effect, of blocking 11-HSD and thus allowing glucocorticoids to access otherwise protected MR; it may thus be that in this instance carbenoxolone at the locally infused dose is acting as an aldosterone mimetic, as it has been shown to do at high doses in the periphery [14].

ALDOSTERONE, SALT AND CARDIAC FIBROSIS

In addition to developing hypertension, uninephrectomized rats on 1% NaCl drinking solution infused s.c. with 0.75 μg/h aldosterone for 8 weeks show a considerable degree of cardiomegaly and both interstitial and perivascular cardiac fibrosis [15, 16]. Infusion of aldosterone at the same dose to animals on a low salt intake neither elevates blood pressure nor causes cardiac fibrosis, underlining the crucial role of a mineralocorticoid:salt imbalance in this model [15]. In both *in vivo* studies published to date, deoxycorticosterone (20 mg/week s.c.) appeared to cause an increased level of perivascular fibrosis, but lesser interstitial fibrosis, compared with aldosterone [15, 16]. This has been interpreted as reflecting the mineralocorticoid agonist/glucocorticoid antagonist activity of deoxycorticosterone, as administration of the antiprogestin, antiglucocorticoid RU486 (2 mg/day s.c.), produced very marked perivascular fibrosis and only marginally elevated levels of interstitial fibrosis [16].

The pathogenesis of cardiac fibrosis in response to an excess mineralocorticoid-for-salt status remains to be determined. Although MR can be detected by ligand binding assay in rat heart, cardiac 11-HSD activity largely has the characteristics of the reductase 11-HSD1 [17], and no mRNA for 11-HSD2 can be detected by Northern blots [1]. In addition, high dose corticosterone (2 mg/day s.c.) to uninephrectomized rats on 1% NaCl does not produce cardiac fibrosis, supporting evidence for the cardiac MR being not only unprotected, but—to the extent that they are involved in the genesis of cardiac fibrosis—clearly differentiating between aldosterone and corticosterone in terms of agonist/antagonist effects.

There are, however, conflicting reports on the ability of aldosterone to increase collagen synthesis by cardiac fibroblasts *in vitro*, which would constitute strong evidence for a direct effect. In an initial study, [^{3}H]hydroxyproline incorporation into collagen was reported to be lowered by dexamethasone, but elevated by angiotensin II and aldosterone when added to cultures of rat cardiac fibroblasts [18]. This study posed certain problems in interpretation, as the doubling over control seen with 10^{-9} M aldosterone was completely abolished by an equal concentration of spironolactone, but not by a 3000-fold excess. In subsequent studies from our laboratory, glucocorti-

coids were shown to lower collagen synthesis by cardiac fibroblasts *in vitro*, and angiotensin II to elevate it; under a variety of experimental conditions (neonatal vs adult fibroblasts; Sprague–Dawley, SHR or WKY rats; cultured alone or cocultured with cardiomyocytes) we have been unable to demonstrate any effect of aldosterone on collagen synthesis *in vitro* [19]. These studies clearly do not exclude a direct effect of aldosterone on cardiac collagen synthesis *in vitro*; they do, however, leave open the possibility that the effect may be secondary to aldosterone-induced changes elsewhere in the body.

In a series of recent studies (Young, Head and Funder, submitted for publication) a clear distinction between the mechanisms of hypertension, cardiomegaly and cardiac fibrosis in response to salt/mineralocorticoid excess has emerged. Rats infused peripherally with aldosterone and i.c.v. with RU28318 do not develop hypertension, but are indistinguishable in terms of cardiomegaly and cardiac fibrosis from peripheral aldosterone : i.c.v. vehicle infused hypertenstive rats. Secondly, rats infused with 9α-fluorocortisol do not develop cardiac hypertrophy, but show blood pressure elevation and a degree of cardiac fibrosis similar to aldosterone infused animals. It would thus seem that the hypertensinogenic, hypertrophic and fibrinogenic activity of mineralocorticoids in the presence of excess salt can be separated; whether the two latter effects are direct cardiac effects, or secondary to aldosterone-induced effects on epithelia, remains to be explored. Similarly, remaining to be explored, at the conceptual as well as the experimental level, is how the crucial role of salt loading for all three processes is mediated, and the physiological roles, if any, of aldosterone in terms of the central control of blood pressure, and in terms of cardiac hypertrophy and collagen deposition.

REFERENCES

1. Albiston A., Obeyesekere V., Smith R. and Krozowski Z.: Cloning and tissue distribution of the human 11-beta hydroxysteroid dehydrogenase type II enzyme. *Molec. Cell. Endocr.* **105** (1994) R11–R17.
2. Agarwal A. K., Mune T., Monder C. and White P. C.: NAD^+-dependent isoform of 11 β-hydroxysteroid dehydrogenase: cloning and characterization of cDNA from sheep kidney. *J. Biol. Chem.* **269** (1994) 25,959–25,962.
3. Arriza J., Simerly R. B., Swanson L. W. and Evans R. M.: Neuronal mineralocorticoid receptor as a mediator of glucocorticoid response. *Neuron* **1** (1988) 887–900.
4. Funder J. W., Pearce P., Myles K. and Roy L. P.: Apparent mineralocorticoid excess, pseudohypoaldosteronism and urinary electrolyte excretion: towards a redefinition of "mineralocorticoid" action. *FASEB J.* **4** (1990) 3234–3238.
5. Naray-Fejes-Toth A. and Fejes-Toth G.: Glucocorticoid receptors mediate mineralocorticoid-like effects in cultured collecting ducts cells. *Am. J. Physiol.* **259** (1990) F672–F678.
6. Nestler E. J., Rainbow T. C., McEwen B. S. and Greengard P.: Corticosterone increases the level of protein 1, a neuron-specific protein in rat hippocampus. *Science* **212** (1981) 1162–1164.
7. de Kloet E. R., Sybesma H. and Reul J. M. H. M.: Selective control by corticosterone of serotonin$_1$ receptor capacity in raphe-hippocampal system. *Neuroendocrinology* **42** (1986) 513–521.
8. Gomez-Sanchez E. P., Venkataraman M. T. and Thwaites D.: ICV infusion of corticosterone antagonizes ICV-aldosterone hypertension. *Am. J. Physiol.* **258** (1990) E649–E653.
9. Pearce D. and Yamamoto K. R.: Mineralocorticoid and glucocorticoid receptor activities distinguished by nonreceptor factors at a composite response element. *Science* **259** (1993) 1661–1665.
10. Webb P., Lopez G. N. and Kushner P. J.: Gene activation by anti-estrogens via a complex of estrogen receptor and AP-1. *J. Cell. Biochem.* **18B** (1994) 374.
11. Gomez-Sanchez E. P., Fort C. M. and Gomez-Sanchez C. E.: Intracerebroventricular infusion of RU28318 blocks aldosterone-salt hypertension. *Am. J. Physiol.* **258** (1990) E482–E484.
12. Gomez-Sanchez E. P., Fort C. and Thwaites D.: Central mineralocorticoid receptor antagonism blocks hypertension in Dahl S.JR rats. *Am. J. Physiol.* **262** (1992) E96–E99.
13. Gomez-Sanchez E. P. and Gomez-Sanchez C. E.: Central hypertensinogenic effects of glycyrrhizic acid and carbenoxolone. *Am. J. Physiol.* **263** (1992) E1125–E1130.
14. Armanini D., Karbowiak I., Krozowski Z., Funder J. W. and Adam W. R.: The mechanism of mineralocorticoid action of carbenoxolone. *Endocrinology* **11** (1982) 1683–1686.
15. Brilla C. G. and Weber K. T.: Mineralocorticoid excess, dietary sodium and myocardial fibrosis. *J. Lab. Clin. Med.* **120** (1992) 893–901.
16. Young M., Fullerton M., Dilley R. and Funder J. W.: Mineralocorticoids, hypertension and cardiac fibrosis. *J. Clin. Invest.* **93** (1994) 2578–2583.
17. Slight S., Ganjam V. K., Nonneman D. and Weber K. T.: Hydroxysteroid dehydrogenase activity in cultured cardiac fibroblasts versus aortic endothelial cells. *Clin. Res.* **40** (1992) 683A.
18. Guarda E., Myers P., Brilla C., Tyagi S. and Weber K. T.: Endothelial cell induced modulation of cardiac fibroblast collagen metabolism. *Cardiovasc. Res.* **27** (1993) 1004–1008.
19. Fullerton M. and Funder J. W.: Aldosterone and cardiac fibrosis: *in vitro* studies. *Cardiovasc. Res.* **28** (1994) 1863–1867.