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Rapid actions of aldosterone revisited: Receptors in the limelight

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ABSTRACT

Steroid hormones like aldosterone have been conclusively shown to elicit both late genomic and rapid, nongenomically initiated responses. Aldosterone was among the first for which rapid, clinically relevant effects were even shown in humans. Yet, after over 30 years of research, the nature of receptors involved in rapid actions of aldosterone is still unclear. Such effects may be assigned to the classical, intracellular steroid receptors, in this case mineralocorticoid receptors (MR, class IIa action Mannheim classification). They typically disappear in knockout models and are blocked by MR-antagonists such as spironolactone, as shown for several cellular and physiological, e.g. renal or cardiovascular effects. In contrast, there is also consistent evidence suggesting type IIb effects involving structurally different receptors ("membrane receptors") being insensitive to classic antagonists and persistent in knockout models; IIb effects have lately even been confirmed by atomic force detection of surface receptors which bind aldosterone but not spironolactone. Type IIa and b may coexist in the same cell with IIa often augmenting early IIb effects. So far cloning of IIb receptors was unsuccessful; therefore results on G-protein coupled estrogen receptor 1 (GPER1) being potentially involved in rapid aldosterone action raised considerable interest. Surprisingly, GPER1 does not bind aldosterone. Though under these circumstances GPER1 should not yet be considered as IIb-receptor, it might be an intermediary signaling enhancer of mineralocorticoid action as shown for epithelial growth factor receptors reconciling those results. We still seem to be left without IIb-receptors whose identification would however be highly desirable and essential for clinical translation.

1. Introduction and background

Mission accomplished – it is now no longer a matter of debate whether rapid actions of steroid hormones do exist or do not exist. Although such observations were reported as early as in the 30ies and 40ies of the last century [1,2] they remained in the backyard of research under the dogmatic occlusion by the classic theory of steroid action. In that world only slow genomic responses would exist as they were explained by the Karlson theory [3]. Some 50 years later this dogma was increasingly challenged by a multitude of reproducible findings of steroid effects starting in seconds or minutes after addition; they were reported for all steroid hormones including the secosteroid vitamin D and steroid-like hormones such as thyroid hormone [4]. While at 1980 about 10–20 papers in this area could be identified, in 2015 there were over 7000 Medline entries relating to this topic [5]. In reflection of the surge in observations it became clear even in the 90ies that those rapid responses to steroid hormones (RRSH, often also termed nongenomic or membrane-initiated steroid actions) were a mixed bag of entities characterized by concentration requirements, co-agonists and presumed receptors as suggested by nonclassical pharmacology. In 2000, the Mannheim classification of non-genomically initiated steroid effects [6] was published as a proceeding of the 1st international RRSH meeting in that German city in 1998. It still covers all major classes of rapid steroid actions; in its section II, there are two subclassifications: IIa describes the involvement of classic steroid hormones receptors (superfamily of intracellular steroid receptors, [7]), an option which has been proven to exist for all steroid hormones in between. Class IIb was the more revolutionary entry describing the involvement of receptors unrelated to the classic receptors that were thought at that time to reside in the plasma membranes of cells thus often also termed "membrane receptors" (In between we know that classic receptors may reside in the plasma membrane as well, and, conversely, intracellular receptors could induce rapid signaling as steroids may easily enter cells by their lipophilicity and/or

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transporters). While the cloning of classic steroid receptors was almost complete in the 90ies, IIb receptors are still a matter of controversial debates, and only few have been identified, cloned and biologically characterized so far, e.g. $GABA_A$-receptors for neurosteroids [8], integrin αvβ3 for thyroid hormone/nanotetrac [9], the TRPM8 channel for testosterone [10,11], vomeronasal receptors V1R for steroidal pheromones [12] and GPER1 for estrogen [13,14]. Latter receptor will be of particular interest here as it has not only been thoroughly studied and clearly established as class IIb receptor for estrogens, but is also under consideration as a potential IIb aldosterone receptor.

This brief review focusses on rapid actions of aldosterone and the postulated receptors and pathways; it is not an encyclopedically complete review, but aims at summarizing related research that is pivotal and pinpoints the current conceptual problems in the plot.

2. Rapid actions of aldosterone: Type IIa versus type IIb actions

Mineralocorticoids were among those steroid hormone classes whose rapid actions were studied relatively early in history; first reports may be found around 1960 [15–17], not long after the discovery of aldosterone in 1953 [18]. Though several rapid effects of aldosterone have been demonstrated in different tissues and cell systems it is still the steroid for which the knowledge about the mechanisms of rapid actions is quite limited compared to other steroids, such as estrogens. Several second messengers like cAMP, DAG (diacylglycerol), IP_3 (inositol trisphosphate) and Ca^{2+}, as well as protein kinases and mitogen activated protein kinases (MAPK) are affected by aldosterone [for review of early observations see 4] within seconds to minutes. In addition, such rapid aldosterone effects have been found for intracellular electrolyte concentrations and cell volume, for the activity of the sodium-proton exchanger in different cells like human mononuclear leukocytes, vascular smooth muscle cells and kidney cells. As a key feature of nongenomically initiated steroid actions these effects are resistant to inhibitors of transcription or protein synthesis; a number of them were found to be insensitive against classical anti-mineralocorticoids such as spironolactone, e.g. those on the sodium-proton exchanger in vascular smooth muscle cells [19,20]. These effects were also characterized by an EC50 value of 0.1 nM and thus not only reflecting the free physiological concentration of aldosterone, but also at least one order of magnitude lower than the Kd-value of the MR for aldosterone. As a further pharmacological feature a high selectivity for aldosterone over hydrocortisone was observed, again being incompatible with the involvement of MR.

If spironolactone or eplerenone were active the classical MR is thought to be involved in rapid aldosterone actions. In 2004 about 50% of these effects were counted as MR antagonist-insensitive [21]. With a surge of papers on rapid effects of aldosterone at the beginning of this century it seemed for a while that more recent studies predominantly identified rapid effects sensitive to MR antagonists such as a recent observation on the aldosterone-induced sensitization of the glomerular feedback mechanism [22]. However, Ashton et al. [23] clearly demonstrated an aldosterone-induced, rapid ERK1/2 phosphorylation in cardiac myocytes which was insensitive to spironolactone, but also detected MR-dependent actions of aldosterone in the same model. Grossmann et al. demonstrated both MR-dependent and −independent effects of aldosterone in Chinese hamster ovary and human embryonic kidney cells [24]. Similarly, Le Moellic et al. [25] showed that nongenomically initiated effects of aldosterone on Isc in rat cortical collecting duct cells turn into spironolactone-sensitive effects after about 2 h. These studies show that nongenomic and genomic effects of aldosterone may be present in the same cells, and rapid actions may be spironolactone-sensitive and insensitive depending on the signals measured in the same biological system.

It has to be kept in mind that spironolactone itself is inactive at MR and has to be metabolized to its active form canrenone which may or may not be achieved by the in vitro systems studied. However, eplerenone which is – unlike spironolactone – highly specific for MR and directly binds to MR was inactive to block rapid aldostrone effects in some studies as well, e.g. in that of Miyata et al. [26], and only partially active in the system studied by Gros et al. [27]. In addition, the other peculiarity of the pharmacological profile of some rapid actions of aldosterone, its 10^5 fold selectivity over hydrocortisone, remains unexplained as well.

As a matter of clinical translation, rapid effects of aldosterone could be demonstrated in humans as acute vascular reponses resulting in changes of blood pressure and vascular tone [28,29].

An alternative receptor for spironolactone-insensitive rapid actions was postulated 25 years ago [20], a view that was also supported by MR-knockout experiments: in skin cells of MR knockout mice the intracellular Ca^{2+} and cAMP levels rapidly increase after aldosterone stimulation similarly as in cells from wildtype mice [30]. These findings are important as the differential antagonist activity alone may also be interpreted as a result of different conformations of MR including one which does bind aldosterone, but not spironolactone. However, it should be taken into account that even these experiments may be inconclusive as unliganded steroid hormone receptors may control transcription of multiple genes (e.g. glucocorticoid receptors [31]) resulting in phenotypic changes with impact on signalling and hormone responsiveness.

The wide spectrum of rapid aldosterone effects is highly likely to involve MR as a type IIa receptor, but the evidence for alternative receptors – type IIb receptors – is compelling by a multitude of analogous, reproducible observations with the initial evidence dating back to the late 80ies. Obviously, the existence of both IIa and IIb receptors would be similar to the situation found in the estrogen arena: while there is clear evidence for the involvement of classic estrogen receptors in some rapid estrogen action, there is evidence IIb receptors, in particular in the brain (POMC-neurons) as well in that in a similar approach classic antagonists are inactive and a model compound (STX) acts as nongenomic agonist not producing any of the genomic responses of estrogens [32]. The putative receptor was identified as GQ-coupled membrane receptor for estrogens [33].

3. Receptors for Type IIb actions: GPER1 in focus

The pharmacological profile of some rapid actions of aldosterone being so different from that of MR necessitated the search for alternative receptor structures beginning in the 90ies. In traditional binding studies, a membrane protein was detected on human mononuclear leukocytes that had a 10-fold higher affinity to aldosterone than MR and did not bind cortisol, unlike the classical MR [34]. Binding of aldosterone to membranes has been shown also in porcine liver microsomes. The protein responsible for the binding could not be identified, but showed features typical of a GTP-binding protein [35]. Wildling et al. [36] detected aldosterone binding sites in the membrane of human endothelial cells by atomic force microscopy. Aldosterone binding to these sites was not blocked by spironolactone and dexamethasone. If the limitations of spironolactone mentioned above would render this piece of evidence inconclusive, we are still left with the dexamethasone finding like the hydrocortisone conundrum mentioned above.

Thus, these studies identified binding sites with pharmacological properties incompatible with those of MR, but no biological function could be assigned to them; this, however, is – amongst

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