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Position paper: Rapid responses to steroids: current status and future prospects.

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COMMENTARY

Position Paper: Rapid responses to steroids: current status and future prospects

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Abstract

Steroids exert their actions through several pathways. The classical genomic pathway, which involves binding of steroids to receptors and subsequent modulation of gene expression, is well characterized. Besides this, rapid actions of steroids have been shown to exist. Since 30 years, research on rapid actions of steroids is an emerging field of science. Today, rapid effects of steroids are well established, and are shown to exist for every type of steroid. The classical steroid receptors have been shown to be involved in rapid actions, but there is also strong evidence that unrelated structures mediate these rapid effects. Despite increasing knowledge about the mechanisms and structures which mediate these actions, there is still no unanimous acceptance of this category. This article briefly reviews the history of the field including current controversies and challenges. It is not meant as a broad review of literature, but should increase the awareness of the endocrinology society for rapid responses to steroids. As members of the organizing committee of the VI International Meeting on Rapid Responses to Steroid Hormones 2009, we propose a research agenda focusing on the identification of new receptor structures and the identification of mechanisms of actions at physiological steroid concentrations. Additionally, efforts for the propagation of translational studies, which should finally lead to clinical benefit in the area of rapid steroid action research, should be intensified.

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Introduction

Steroids exert their biological functions through several mechanisms. The classical genomic action of steroids is well established and characterized since decades. Besides this, rapid or nonclassical actions of steroids have been described as early as 1942 when Hans Selye discovered instant anesthetic effects of i.p. injected progesterone in rats (1). These and few other findings especially those originating from Pietras & Szego (2) in the 1970s remained dormant and in the depository of science until the middle of the 1980s. At that time, related findings were more widely recognized, and the dogma of genomic steroid action as the only available hypothesis was increasingly challenged by the scientific community. Subsequently, from the 1970s up to the time of writing this article, literature on rapid steroid actions developed from about ten papers to a current archive of about 2000 citations.

In the past 10 years, it was shown that classical receptors are involved in rapid signaling involving almost all steroid hormones including thyroid hormone and vitamin D (reviewed in (3)). Membrane-located

forms of estrogen and progesterone receptors were identified, and probably exist for other steroid hormone receptors such as glucocorticoid and androgen receptors, as the membrane location may be due to palmitoylation, which also occurs in these receptors (4, 5). Several signaling cascades, for example, those involving phosphoinositide-3 kinase, MAPKs, tyrosine kinases, or the JAK/STAT pathways, have been identified (reviewed in (3)).

In the 1990s, the interest in this field increased by findings which did not fit to the involvement of classical receptors. For example, it was demonstrated that rapid effects of aldosterone are not sensitive toward mineralocorticoid receptor inhibitors such as spironolactone. In addition, rapid actions of aldosterone have been shown in cells lacking the classical mineralocorticoid receptor (6, 7). These and other data resulted in claims for novel, alternative receptors. Meanwhile, this topic is discussed in a series of international meetings on rapid response to steroid hormones (RRSH) and in specialized section meetings embedded in large congresses, e.g. for endocrinology or neurosciences.

Table 1 Physiologically relevant rapid actions of steroids.

Steroid	Physiologically relevant effect	Involved receptor	References
Progesterone	Effects on motility and chemotaxis of human spermatozoa	Unknown	Reviewed in Baldi <i>et al.</i> (2009) (16)
	Increase of intracellular calcium in human sperm	Unknown	Baldi <i>et al.</i> (1991) (24)
	Acrosome reaction in human sperm	Unknown	Morales <i>et al.</i> (1992) (25) reviewed in Baldi <i>et al.</i> (2009) (16)
Aldosterone	Regulation of cell volume in human mononuclear leukocytes	Unknown	Wehling <i>et al.</i> (1991) (26)
	Vasoconstriction of resistance arteries in male humans	Unknown	Romagni <i>et al.</i> (2003) (27)
	Alteration in pH in MDCK cells	Unknown	Gekle <i>et al.</i> (1996) (28)
	Inflammation, hypertrophy, fibrosis in rat heart	Classical mineralocorticoid receptor	Rocha <i>et al.</i> (2002) (29), Young <i>et al.</i> (1994) (30) and Sun <i>et al.</i> (2002) (31)
	ENaC trafficking in renal CCD cells	Classical mineralocorticoid receptor	McEaney <i>et al.</i> (2008) (32)
	PKD signaling in renal cell proliferation	Classical mineralocorticoid receptor	McEaney <i>et al.</i> (2009) (33)
Estrogen	Rapid vasodilation in postmenopausal women	Classical estrogen receptor?	Gilligan <i>et al.</i> (1994) (34)
	Activation of ERK in uterine arterial endothelial cells from pregnant ewes	Classical estrogen receptor	Chen <i>et al.</i> (2004) (35)
	Activation of nitric oxide synthase in endothelial cells of rat adipocytes	Classical estrogen receptor	Jaubert <i>et al.</i> (2007) (36)
	Increase in [Ca] _i in chicken and pig granulosa cells, triggered by inositol 1,4,5-trisphosphate	Unknown	Morley <i>et al.</i> (1998) (37), Shears (1991) (38) and Eppig (1991) (39)
	Activation of ERK and increase in insulin biosynthesis	Estrogen receptor α	Alonso-Magdalena <i>et al.</i> (2008) (40)
	Insulinotropic action	Estrogen receptor β	Nadal <i>et al.</i> (1998) (41) and Soriano <i>et al.</i> (2009) (42)
	Female sex-specific antisecretory responses in intestine	Membrane estrogen receptor α	O'Mahony <i>et al.</i> (2009) (43, 44)
Vitamin D	Effects on membrane-gated calcium channels, phospholipase C activity, and the sodium/hydrogen antiport in osteoblasts	Vitamin D receptor	Norman <i>et al.</i> (2002) (45) and Huhtakangas <i>et al.</i> (2004) (46)
	Opening of chloride channels in osteoblasts requires intact VDR	Vitamin D receptor	Zanello <i>et al.</i> (2004) (47)
	In keratinocytes and <i>in vivo</i> in skin: protection against u.v.-induced DNA damage	Vitamin D receptor	Dixon <i>et al.</i> (2007) (48)
	VDR is present in T-tubule membranes of heart muscle cells, and is associated with myocyte contraction	Vitamin D receptor	Tishkoff <i>et al.</i> (2008) (49)
	Alterations of cytosolic calcium concentration in mouse osteoblasts	Unknown	Lieberherr (1987) (50)
Thyroid hormones	Formation of new blood vessels in the chick chorioallantoic membrane model	Integrin $\alpha_v\beta_3$	Davis <i>et al.</i> (2004) (51)
	ERK/MAPK activation in CV-1 cells	Classical thyroid hormone receptor	Davis <i>et al.</i> (2000) (52)
Androgens	Alteration of calcium levels in activated T-cells, male rat osteoblasts	Unknown	Benten <i>et al.</i> (1997) (53) and Lieberherr & Grosse (1994) (54)
	Activation of MAPK kinases in prostate cancer cells	Classical androgen receptor	Peterziel <i>et al.</i> (1999) (55)
	Prolactin release from lactotrophs (type 2) in the male pituitary	Unknown	Christian <i>et al.</i> (2000) (56)
	Antiproliferative effect of testosterone on LNCaP human prostate cancer cells	Membrane androgen receptor	Hatzoglou <i>et al.</i> (2005) (57)
	Proliferative effect of dihydrotestosterone on human breast cancer cells	Integrin $\alpha_v\beta_3$	Lin <i>et al.</i> (2009) (58)

Physiological concentrations of the different steroids used as eligibility criterion: progesterone: pM– μ M (dependent on the site of action); estradiol: up to 10 nM; aldosterone 0.1 nM; 1 α ,25(OH)₂-vitamin D₃: 1–0.1 nM in plasma; thyroid hormones: 0.01 nM; androgen: 0.1–10 nM (dependent on the site of action); glucocorticoids: 10 nM (free cortisol).

Nonclassical receptors for rapid steroid action

Besides the rapid actions of steroids mediated through classical receptors, there are several examples of alternative molecules which mediate these rapid actions. Often, these are already known proteins, with distinct biological functions. For example, there is strong evidence that integrins mediate rapid effects of thyroid hormones (8). The digitalis receptor (digitalis being a steroid) is a membrane enzyme, namely sodium–potassium ATPase (9). The odorant receptors, through which we may smell steroidal pheromones, have not been identified yet, but they must exist (10). In plants, brassinosteroids do not stimulate transcription, but bind to transmembrane receptor kinases (11). Additionally, it was demonstrated that neurosteroids act through GABA receptors (12). Current data suggest that neurosteroids may alternatively act via an as yet unidentified G-protein-coupled receptor (13). The identification of completely unknown structures, which may mediate rapid steroid actions, has turned out to be difficult. All evidences for alternative membrane receptors transmitting rapid steroid action, which are not related to known structures, are still controversially discussed. This particularly applies to GPR30 as a potential receptor for estrogen and the membrane progesterone receptor (14, 15).

Physiological and pharmacological relevance of rapid steroid actions

A conceptual problem of many studies dealing with rapid steroid actions is the use of very high, supraphysiological steroid concentrations. This renders the identification of physiologically relevant rapid actions of steroids difficult. Table 1 summarizes some examples of steroid effects observed at physiological concentrations. It is important to consider the concentration of the steroid at the site of action, which may significantly differ from the circulating one. Progesterone may serve as an example: in the case of sperm stimulation, it is present at very high concentrations (exceeding plasma levels by a factor of 1000) at the site of action (reviewed in (16)). In general,

experiments using physiological concentrations of steroids at their site of action must be encouraged in future studies to better identify the physiological role of rapid steroid effects.

Nevertheless, also rapid actions of steroids mediated by supraphysiological concentrations may be relevant in pharmacological use, and therefore have clinical implications. Examples for this are the use of glucocorticoids to decrease the airway mucosal blood flow in asthma patients (17), the use of these steroids in acute phases of rheumatic diseases (18), and the use of neuroactive steroids as anesthetics or antidepressants (19).

Another problem that has received little attention until now is the fact that circulating hormone levels do not change rapidly, and therefore rapid effects should be persistent. Comparably little is known about the desensitization processes and secondary genomic impact of nongenomically initiated steroid actions which have been shown to exist (20).

Translational relevance

In the 1990s, there was great enthusiasm and hope to utilize the novel findings on rapid steroid hormone action for improved patient care. Overall, this hope has not been realized. In the past 15–20 years, no drug based on any kind of mechanism of rapid steroid action has been developed and marketed. However, some steroids that act rapidly, for example, glucocorticoids which are used in acute rheumatic diseases, have been used therapeutically for many years (18). In Table 2, the few successfully translated rapid actions of steroids are summarized. Most of them have been translated years ago without recognition of the rapid effect they are based on.

Furthermore, there are promising candidates which either are being developed or await funding to be taken into development. A prominent example is STX (2-(4-hydroxyphenyl)-3-phenylpent-2-enoic acid (4-(2-dimethylaminorthoxy)-phenyl)amide, E isomer), a selective estrogen receptor modulator (SERM), with potential impact on menopause symptoms and anti-obesity effects, which has been shown to act rapidly (21). The deaminated thyroid hormone analog tetrac may be useful in the treatment of cancer (22).

Table 2 Rapid steroid actions that have already been successfully translated (in part unwittingly).

Steroid action	Clinical use
Anesthetic effects of progestins	Althesin (mixture of alphaxolone and alphadolone) was formerly used in humans, but was stopped due to severe side effects, and is still in use in veterinary medicine (59)
Fluticasone and budesonide decrease the airway mucosal blood flow	Treatment of asthma by inhalation of these glucocorticoids (18)
Membrane effects of high-dose glucocorticoid application	Acute phases or particular severe forms of rheumatic diseases such as lupus erythematosus, vasculitis, polymyositis, and rheumatoid arthritis (17)

For vitamin D analogs, a role as anticancer and diabetes-preventing agents has been proposed based on their extranuclear effects (23).

Recommendations

The organizing committee of the RRSB series states that

- i) There is evidence for rapid steroid effects through both classic steroid receptors and unrelated structures presumably residing in biological membranes.

Future research should be focused on rapid physiological effects of steroid hormones to elucidate the involved biological pathways. Therefore, it is important that the concentrations used in the experiments are critically reflected. Studies analyzing rapid effects of steroids in cell lines and isolated tissues should be translated into intact animal models. Furthermore, the careful analysis of dose dependence of these effects in physiological concentrations is necessary. The pharmacodynamics of the involved receptors is another important issue, which should be analyzed in the future.

- ii) The identification of new 'receptor structures' that mediate rapid actions is difficult and, until now, most approaches have failed to do this or led to contradictory results. Future research should focus on the identification and validation of these structures. The difficulties of these approaches should also be recognized and appreciated by the funding agencies.
- iii) Increased emphasis must be placed on possible clinical application of experimentally demonstrated rapid actions of steroids. Until now, only limited translational success in the area of rapid responses to steroid hormones is evident. However, there are promising candidates, and others should urgently be identified and developed into potential drugs. Basic researchers are encouraged to seek advice from clinically oriented or translationally experienced researchers.

As a general concern, we anticipate that the area could face increasing funding problems if research were consumed by the very prominent area of classic steroid receptor research, or no clinical applications became evident in the near future.

Thus, this position paper stresses that the scientific community, funding agencies, and journal editors should structurally and financially acknowledge the opportunities of rapid and nonclassical steroid research. Scientists from neighboring areas (for example, those working on G-proteins or other rapid signaling pathways, or researchers from clinical areas of relevance, such as rheumatic diseases) should be

involved in the opportunities and challenges of this still novel research field.

Declaration of interest

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