ฤทธิ์ของสเตรอยด์แบบนอน-จีโนมิก และความเกี่ยวโยงกับระบบประสาทต่อมไร้ท่อ : ความเป็นไปได้ของไนโตรฟูราโซนต่อการเกิดมะเร็ง

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บทคัดย่อ

การทำงานของสเตรอยด์ฮอร์โมนโดยกลไก "นอน-จีโนมิก" (non-genomic) ไม่ได้ขึ้นอยู่กับการถอดรหัสทางพันธุกรรมหรือการสังเคราะห์โปรตีน แต่เกี่ยวข้องกับโปรตีนควบคุมในไซโตปลาสซึม และโปรตีนที่ผนังเซลล์ ได้แก่ การกระตุ้นไมโตเจน-แอ็คทิเวท-ไคเนส (แม็บไคเนส) ฟอสฟาติดิลอิโนซิตอล-ทรี-ไคเนส และ ไทโรซีน ไคเนส โดยโปรเจสเทอโรนและเอสโทรเจนผ่านกลไกนอน-จีโนมิก ความเข้าใจกลไกการทำงานของสเตรอยด์แบบนอน-จีโนมิก สามารถประยุกต์ใช้เพื่อศึกษาความเป็นพิษและการก่อมะเร็งของสารที่รบกวนกลไกควบคุมสเตรอยด์ เช่น สารไนโตรฟูแรน ซึ่งเป็นยาต้านจุลชีพที่พบตกค้างในอาหารทำจากเนื้อสัตว์ และในปัจจุบันนี้กลไกความเป็นพิษและการเกิดมะเร็งของสารนี้ยังไม่ชัดเจน แต่พบความเกี่ยวข้องระหว่างการได้รับไนโตรฟูแรนกับการเปลี่ยนแปลงสเตรอยด์ฮอร์โมนในหนูขาว

คำสำคัญ : สเตรอยด์, ฤทธิ์ของสเตรอยด์แบบนอน-จีโนมิก, ไนโตรฟูราโซน, การก่อมะเร็ง

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Non - Genomic Steroid Action and Neuroendocrine Interconnectedness : Possible Basis for Nitrofurazone Modifying Effect on Carcinogenesis

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Abstract

Novel non-transcriptional mechanisms of signal transduction through steroid hormone receptors have been identified. These so-called 'non-genomic' effects do not depend on gene transcription or protein synthesis but involve steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins. Ubiquitous regulatory cascades such as mitogen-activated protein kinases (MAPKs), the phosphatidylinositol 3-OH kinase (PI3K) and tyrosine kinases are modulated through non-transcriptional mechanisms by steroid hormones such as progesterone and estrogen. The understanding of non-genomic steroid action can be applied to study the mechanisms of toxicity and mammary carcinogenesis by steroid-modulating agents such as nitrofurazone, an antimicrobial drug residue found in food stuffs of animal origin. At present time, the mechanisms of toxicity and mammary carcinogenesis by nitrofurazone remain to be clarified. The relation between nitrofurazone-exposed rats and increase in steroid levels has been reported.

Key words : Steroid, Non-genomic steroid action, Nitrofurazone, Carcinogenesis

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Review Article

steroid hormone receptors have been identified. These so-called 'non-genomic' effects do not depend on gene transcription or protein synthesis and involve steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins .

Membrane receptors which are completely distinct from the traditional theory of steroid action. Classic steroid receptor actions are provided by evidences of steroid binding to intracellular receptors and modulate nuclear transcription after translocation of steroid-receptor complexes into the nucleus. Being sensitive to inhibitors of transcription and translation, e.g. actinomycin D and cycloheximide, long lasting physiological responses are classified as genomic actions of steroids.

As practical rules, non-transcriptional effects can be indicated as[4]:

1. Actions that are too rapid to be compatible with RNA and protein synthesis (i.e. that ensue within seconds to minutes from the challenge with the hormone).
2. Actions that can be reproduced in the presence of inhibitors of RNA or protein synthesis.
3. Actions that can be reproduced by using steroid hormones coupled to cell membrane-impermeable molecules.
4. Actions that steroid hormones induce in cells with highly compacted chromatin, in which RNA and protein synthesis are absent (such as spermatozoa).
5. Actions that are elicited by steroid hormones via binding to receptors containing mutations which make them incapable of activating transcriptional processes.

Several relevant biological actions of steroids have been associated with this kind of signaling. Ubiquitous regulatory cascades such as mitogen-activated protein kinases (MAPKs), the phosphatidylinositol 3-OH kinase (PI3K) and tyrosine kinases are modulated through non-transcriptional mechanisms by steroid hormones. Furthermore, steroid hormone receptor modulation of cell membrane-associated molecules such as ion channels and G-protein-coupled receptors (GPCRs) has been shown. Tissue traditionally considered as "non-targets" for classical steroid actions are instead found to vividly regulated by non-genomic mechanisms. To this aim, the cardiovascular and the central nervous system provide excellent examples, where steroid hormones induce rapid vasodilation and neuronal survival via non-genomic mechanisms, leading to relevant pathological consequences[4].

Progesterone administration is also known to regulate ion fluxes. Sperm cells are characterized by a highly compacted chromatin, and do not accomplish significant RNA or protein synthesis, thus representing an excellent model to study non-genomic actions of steroids. Progesterone induces rapid Ca^{2+} influx in spermatozoa by targeting a membrane Ca^{2+} channel[5] and therefore activating the acrosomal reaction. Control of Ca^{2+} influx at the cell membrane level may be linked to the local presence of progesterone receptors (PRs)[6]. Similar effects can be found in osteoblasts, where a membrane-impermeable form of progesterone increases intracellular Ca^{2+} by opening L-type Ca^{2+} channels and activating Ca^{2+} mobilization from the endoplasmic reticulum[7].

Introduction

Interconnectedness that ties together organ and hormone system is important in both human health and in animal toxicology studies. The mammalian reproductive system undergoes basic activation during development and fine-tuning as adults, both rely on the hormones and growth factors which can have effects both subtle and profound[1]. Mechanisms of toxicity in the reproductive system are complex, and risk evaluation depends on our understanding of how the reproductive system interacts with other hormonal and organ systems in the body. Possible involvement in the lesion and extent of damage are an important consideration in toxicological evaluation. Adverse health effects occur when the chemical or its metabolites bind reversibly or irreversibly to target molecules. When the amount bound has reached a certain level and the repair mechanisms have been overwhelmed.

In view of biological monitoring[2] the experts from a symposium on biological monitoring sponsored by the US Environmental Protection Agency (EPA), the World Health Organization (WHO), and the Commission of the European Communities (CEC), proposed that "a biological effect should be considered adverse if there was an impairment of functional capacity, a decreased ability to compensate the additional stress, a decreased ability to maintain homeostasis, and an enhanced susceptibility to other environmental influences or if such impairments were likely to become manifest in the near future".

Nitrofurazone (NF), a 5-nitrofuran derivative also known as nitrofural, is an anti-microbial drug with broad spectrum bactericidal effects, is used both therapeutically and prophylactically for domestic animals such as pigs, sheep, goats, cattle, chickens, and turkeys[3]. It also possesses some antiprotozoal activity. Dopamine antagonists, such as haloperidol inhibits dopamine release from the hypothalamus and cause increase of prolactin release. Takahashi *et al.* (2000)[3] proposed that nitrofurazone which influenced endocrine actions through unknown mechanism might be important for its enhancement of mammary carcinogenesis in female rats.

The committee (JECFA, Joint Expert Committee on Food Additives) concluded that it could not establish an acceptable daily intake for nitrofurazone because the effect levels had not been established. Before reviewing toxicological data again, the committee would wish to see data to support the view that tumour formation in rodents following nitrofural administration had an endocrine origin. The committee noted that nitrofuran had been reviewed by the International Agency for Research on Cancer, which concluded that there was limited evidence for the carcinogenicity of nitrofural in animals but inadequate evidence for humans.

Steroid Hormone Actions

Steroid hormone receptors have been traditionally considered to act via the regulation of transcriptional processes, involving nuclear translocation and binding to specific response elements, and ultimately leading to regulation of gene expression. However, novel non-transcriptional mechanisms of signal transduction through

Nitrofurazone and Risk Analysis

Nitrofurazone and furazolidone are antimicrobial drugs residues found in food stuffs of animal origin. Critical effects such as mammary carcinogenesis, testicular degeneration (atrophy of germinal epithelium and aspermatogenesis) and degeneration of vertebral and knee articular cartilage in both sexes are issues of concern.

Takahashi *et al* (2000)[3] examined female wistar rats exposed to 1000 ppm of nitrofurazone containing diet ad libitum between 8 and 27 weeks of age. After week of 9,10 – Dimethyl , 1,2 - benzanthracene (DMBA) in 2% of sesame oil injection, the modifying effect of nitrofuran on mammary carcinogenesis was investigated.

Animals that exhibited at least three consecutive 4-day vaginal cycles were employed for hormonal assays, and five animals in each groups were killed by decapitation on diestrus day at 10.00 hr. and on proestrus day at 17.00 hr. respectively. Serum prolactin and progesterone were examined on diestrus day at 10.00 hr and on proestrus day at 17.00 hr and found to be significantly increased while other hormone (LH, FSH, TSH and E2) concentrations were similar in all groups.

Non-genomic steroid action and carcinogenesis induced by nitrofurazone may be mediated by progesterone receptor, c- Src tyrosine kinase and MAPK signaling cascades. The possible non-genomic steroid action of neuroactive steroids, in particular, progesterone is currently being investigated.

At present, the mechanisms of toxicity and mammary carcinogenesis by nitrofurazone remain to be clarified.

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Between the signaling machineries that are regulated by sex steroids are the MAPK cascades, several tyrosine kinases and lipid kinases.

Rapid activation of tyrosine kinases is accomplished by progesterone. In sperm cells, cell membrane PR interaction with protein tyrosine kinases accounts for the channel regulation that is required for the acrosomal reaction[6]. Although the mechanism of interaction of PR with tyrosine kinases is unclear, recent evidence indicates the existence of a specific polyproline motif in the NH_2-terminal domain of PR that mediates direct progestin-dependent interaction of PR with SH_3 domains of various cytoplasmic signaling molecules, including c-Src tyrosine kinases. PR interaction with SH_3-containing tyrosine kinases may also be the mechanism of progesterone-dependent MAPK activation[8].

Activation of PI3K by estrogens is important in breast cancer cells, where E_2 rapidly triggers association of ERα with Src and p85[9]. This complex probably favors hormone activation of Src- and PI3K-dependent pathways, which converge on cell cycle progression[9].

Nongenomic Effects of Steroids in Vivo

Steroids can rapidly activate signaling cascades within endothelial and smooth muscle cells. They seem to by pass the classical genomic receptors.

In addition to rapid effects of steroids on vasoregulation, nongenomic steroid actions on the central nervous system have been demonstrated. Administration of corticosteroid or progesterone modulates reproductive behavior, which application of progesterone or neurosteroids exerts sedative or anesthetic effects[10].

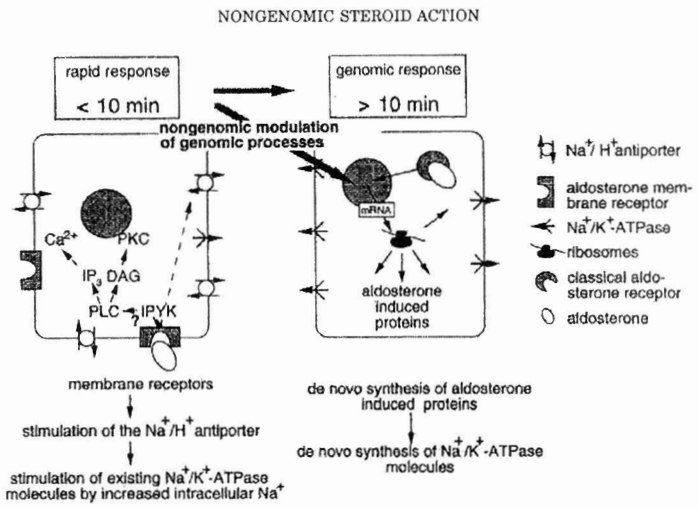

Figure 1 Scheme of the two-step model for steroid action comprising both genomic (right) and non-genomic (left) actions of steroids as exemplified for aldosterone[10].

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