Journal of Environmental Radioactivity xxx (2013) 1–9

Contents lists available at SciVerse ScienceDirect

Journal of Environmental Radioactivity

journal homepage: www.elsevier.com/locate/jenvrad

The low dose gamma ionising radiation impact upon cooperativity of androgen-specific proteins

Gennady N. Filchenkov, Eugene H. Popoff*, Alexander D. Naumov

Lab. of Biochemistry, Institute of Radiobiology, National Academy of Sciences of Belarus, Feduninskogo 4, Gomel 246007, Belarus

ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form
23 January 2013
Accepted 10 February 2013
Available online xxx

Keywords:
Androgens
Androgenic receptor
Hexokinase
Ionising radiation
Testosterone-binding globulin

ABSTRACT

The paper deals with effects of the ionising radiation (γ-IR, 0.5 Gy) upon serum testosterone (T), characteristics of testosterone-binding globulin (TeBG) and androgen receptor (AR) in parallel with observation of androgen (A) responsive enzyme activity – hexokinase (HK). The interdependence or relationships of T-levels with parameters of the proteins that provide androgenic regulation are consequently analyzed in post-IR dynamics. The IR-stress adjustment data reveal expediency of TeBG- and AR-cooperativity measurements for more precise assessments of endocrine A-control at appropriate emergencies.

1. Introduction

The modeling of Chernobyl radioecological effects on laboratory animals reveals how the ionizing radiation (IR) can cause derangements of life processes. They depress adaptive possibilities of an organism and its reproductive potential. According to medical data (Popoff and Kapich, 2010) the IR-induced malfunction of androgen-dependent regulation is an important radiobiological problem. For instance, at present over 90% of the post-Chernobyl IR effects in Belarus are caused by ^{137}Cs contamination. At the same time the number of IR-exposed schoolboys that suffer from mineral metabolism disorder and growth impairment (asthenic syndrome) has risen five-fold. Their body weight deficiencies are coupled with the lack of muscle strength and a shortage of lung capacity (Kapitonova, 2001) – all indicate a reduced level of anabolic regulation by androgens (A).

A-regulation is provided by specialized proteins – transporter (TeBG) and receptor (AR). However up to now coordination in their work (transfers of steroid signals) escaped from attention (Popoff and Filchenkov, 2011). That is why this research is aimed to estimate the γ-IR effects by monitoring specified parameters of T, TeBG and AR in a synchronized mode.

The main idea of investigation was to elucidate functional interrelations between extra- & intracellular A-specific proteins after IR-impact in dynamics. We realized our goal comparing characteristics of T and TeBG from blood samples with A-relevant parameters of typical A-target – prostate gland: viz. AR's concentrations and cooperativities, nuclear ARCs-binding capacities, activity of A-responsive enzyme – hexokinase (Sato et al., 2008).

2. Materials and methods

2.1. Animals

Experiments were performed during November–January 90-day-period using Wistar male rats (Rappolovo, St Petersburg, Russia) initially of 3-month age maintained under standard vivarium conditions (in plastic rat cages provided with food and water

Abbreviations: A, androgen(s); aa, amino acids; ABP, androgen-binding protein of rat i.e. $_r$TeBG; AC, adenylate cyclase; AR, androgen receptor; ARC, androgen receptor complex; B_t, total binding of [^{3}H]-A; *B*, quantity of specifically bound A ([^{3}H]-DHT by TeBG and [^{3}H]-MT by RA); B_{max}, maximal *B*′ value calculated by approximation in Scatchard plot; B_{nsp}, nonspecific binding of A; ChNPP, Chernobyl nuclear power plant; DCC, dextran-coated charcoal (1:9); *DF*, dimerization factor; DHT, 5α-dihydrotestosterone; Hb, hemoglobin; IR, ionising radiation; ISS, International Space Station; K_a, equilibrium association constant; K_d, equilibrium dissociation constant; *MF*, monomerization factor; *MT*, methyltrienolone; *OF*, oligomerization factor; PM, external plasma(tic) membrane of cell; SIS, standard incubation system; T, testosterone; T_{add}, final concentration of [^{3}H]-DHT added in incubation system; $_h$TeBG, human testosterone-binding globulin; *U*, unbound [^{3}H]-A that removed by DCC after incubation; η_{Hill}, the Hill coefficient indicating a degree of cooperativity for A-binding by TeBG or AR (calculated in Hill plot, see Figs. 2 and 3).

* Corresponding author. Tel.: +375 0232 682640; fax: +375 0232 570706.
E-mail address: ehpopoff@mail.ru (E.H. Popoff).

0265-931X/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jenvrad.2013.02.002

ad libitum; lights from 07:00 to 19:00 h; temperature, 20 ÷ 24 °C). The guidelines of the Institutional Animal Ethics Committee regarding experimental research in small animals (Kasatkina and Kaplansky, 2000) were strictly followed. In particular (before blood sampling procedures) rats were anesthetized by Na-pentobarbital (60 mg/kg *ip*).

2.2. Chemicals

Isotope chemicals used were labeled androgens: 5α-dihydro-/1,2,6,7-[$^3H_4$]/-testosterone ([^{3}H]-DHT) with specific activity of 3630 GBq/mmol (Izotop, St Petersburg, Russia) and [^{3}H]-R1881 i.e. methyltrienolone ([^{3}H]-MT) with specific activity of 3219 GBq/mmol (NEN, Boston, MA, USA); scintillation liquid *ZhS-8* (Monocrystalreactiv, Kharkov, Ukraine); other chemicals were as follows: R1881 i.e. unlabeled MT (NEN, Boston, MA, USA), aprotinin, the activated *Norit A* charcoal, dextran *T70*, α-monothioglycerol, dithiothreitol, mersalyl acid (Sigma, St Louis, MO, USA); glucose-6-phosphate dehydrogenase (Sigma–Aldrich, Tokyo, Japan), 5α-dihydrotestosterone (DHT), Tris–HCl, Triton X-100, triamcinolone acetonide, glycerol et al. of analytical grade (Serva, Heidelberg, Germany).

Radiochemical purity of [^{3}H]-DHT was checked by thin layer chromatography on Siluphol UV254 plates (Kavalier, Sázava, Czech Republic) using the 3-solvents system of "chloroform–methanol–ethyl acetate" in the ratio of 70:10:30 (by volume).

2.3. External irradiation of animals in experimental conditions

Acute external γ-irradiation of rats (0.5 Gy from ^{137}Cs source, 10.33×10^{-4} Gy/s) was executed in apparatus Igur-01 (Igur, Brest, Belarus).

2.4. Sampling of rat sera

Samplings of rat blood (venous – from the tail vein and arterial – by means of cardio puncture) were performed into chilled glass tubes (4 °C). Serum samples were obtained 1, 3, 10, 30 and 90 days after completion of external irradiation by centrifugation of blood (2000*g*, 20 min at 4 °C). Serum was either analyzed immediately or placed into the Eppendorf type tubes, frozen in liquid nitrogen and kept at −50 °C until use.

2.5. TeBG-analysis

It was earlier found that an estrogen-binding glycoprotein (EBG) is the main blood protein, which specifically binds (with high affinity) E_2 molecules. Its characteristics significantly differ from the characteristics of the other blood sex steroid-binding glycoprotein (SSG), TeBG:EBG poorly interacts with [^{3}H]-DHT and the affinity of TeBG for E_2 binding is two orders of magnitude lower than the former one (Popoff and Kapich, 2008). Taking into consideration these features we have used [^{3}H]-DHT for characterization of TeBG.

The tests on time dependence of TeBG interactions with the A-ligand [^{3}H]-DHT at various incubation temperatures as well as determinations of qualitative and quantitative characteristic in the investigated samples of blood plasma and serum have shown identical results during immediate use of blood samples and also after a single freeze-thawing procedure; these tests also optimized routine procedures employed for analysis of characteristics of this glycoprotein.

We have applied the following protocol of TeBG-assays:

(1) The serum samples were treated (15 min at 37 °C) with a suspension of dextran-coated charcoal (DCC) in buffer *Z* (20 mM Tris–HCl, 10% glycerol, 10^5 IU/L aprotinin, pH 7.4) with magnetic stirring. Final concentrations of dextran *T70* and charcoal *Norit A* were 0.25% and 2.5% (by weight). The serum/DCC ratio was 9:1 (by volume). Then the serum was diluted by buffer *Z* (various amounts).

(2) Diluted serum samples in a volume of 1.8 mL of buffer *Z* were incubated with [^{3}H]-DHT (total added final concentrations ranged from T_{add} = 0.1–10.0 nM) for 12 min at 24 °C and a further 90 min at 4 °C.

Aliquots of [^{3}H]-DHT hexane solution (primarily designed from a vial of proprietary product) were transferred into glass tubes. The hexane was evaporated by a gentle air stream and the [^{3}H]-DHT was redissolved in 0.1 mL of ethanol. A row of different [^{3}H]-DHT concentrations in buffer *Z* was prepared. In parallel we prepared a row of different DHT concentrations in buffer *Z* (in 200-fold molar excess as compared to [^{3}H]-ligand). Further manipulations for each [^{3}H]-label concentration were in polystyrene tubes. They included the making of SIS.

Buffer *Z* (1.0 mL); [^{3}H]-DHT solution (0.1 mL); another 0.1 mL of buffer *Z* (to the samples without inhibition of specific [^{3}H]-DHT–TeBG binding) or 0.1 mL of DHT solution (in 250-fold molar excess of DHT as compared to [^{3}H]-ligand) to the samples with inhibition of specific [^{3}H]-DHT binding. The reaction was started by the adding 0.6 mL/tube of diluted serum samples (executed in duplicates). After incubation the protein-unbound [^{3}H]-DHT and DHT were removed by incubation for 3 min at 4 °C with DCC (final concentrations of dextran *T70* and charcoal *Norit A* were 0.05% and 0.5% respectively) – 0.2 mL of the DCC suspension (magnetically stirred) was added to 1.8 mL of the SIS (final volume 2.0 mL). Then sedimentation of charcoal was run (5000*g*, 3 min) and the radioactivity in supernatants of samples was counted.

2.6. Measurement of [^{3}H]-radioactivities

The aliquots of samples (0.5 mL) were transferred into potassium-free glass vials. Then after the addition of the scintillation liquid (*ZhS-8*, 10 mL) vials were screwed up with lids and placed into β-spectrometer Mark III (Tracor Analytic, Elk Grove, IL, USA) where the β-radioactivity of the samples was counted.

2.7. Determination of testosterone in blood serum

Testosterone (T) was determined by radioimmunoassay (RIA) kit Steron-T-^{125}I (Institute of Bio-Organic Chemistry, Minsk, Belarus) using the standard protocol (with ethanol extraction of T) and γ-counting in the LKB-Wallac RiaGamma 1274 (Wallac OY, Turku, Finland).

HK-activities were measured according to Sato et al. (2008). Briefly: to determine enzyme activity of HK, 10 μL of each sample were incubated for 5 min at 30 °C in a 150-μL incubation mixture containing 125 μM Tris–HCl (pH 7.6) and 0.6 units of glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of 40 μL of 100 mM D(+)-glucose and was then determined spectrophotometrically at 340 nm for 5 min.

2.8. Experiments

Animals were sacrificed under diethyl ether anesthesia. Animal tissues were taken for experiments at 08:30 h within 1–90-day-period after completion of external γ-irradiation.

2.9. Prostate fractioning

Ventral prostate lobes were excised, separated from connective tissue, bathed in physiological solution, blotted and used immediately

Please cite this article in press as: Filchenkov, G.N., et al., The low dose gamma ionising radiation impact upon cooperativity of androgen-specific proteins, Journal of Environmental Radioactivity (2013), http://dx.doi.org/10.1016/j.jenvrad.2013.02.002