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Immunohistochemical localization of HCA2 receptors in reproductive system of male rats

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Abstract

The study investigates presence and localization of hydroxy-carboxylic acid receptor 2 (HCA2 receptor) protein in different parts of reproductive system of male rats by using immunohistochemical approach. Right lung (as positive control), testes, epididymis (head, body and tail), vas deferens and penis as well as accessory glands including vesicular gland, coagulating gland, preputial gland, gland of vas deferens and ventral prostate of six adult male Sprague Dawley rats were immediately removed after euthanasia. Transverse sections were made for immunohistochemical evaluation by using rabbit polyclonal antibody against rat HCA2 receptor as the primary antibody and mouse polyclonal anti-rabbit IgG (HRP-conjugated) as secondary antibody. In testes, spermatogenic cells, except for spermatogonia which moderately stained, showed no reactivity as well as Sertoli cells and spermatozoa. In interstitial tissue Leydig cells demonstrated receptor expression with strong intensity. Epithelial cells of epididymis showed no reactivity. Epididymal spermatozoa were also unstained. No reactivity was observed in columnar pseudo stratified epithelium of vas deferens. In penile tissue, only ciliated pseudo stratified epithelium showed a very weak reactivity. In coagulating gland, epithelial cells were moderately stained. Ciliated pseudo stratified epithelium of vesicular gland was weakly positive. No or very weak reactivity was observed in epithelial cells of preputial gland as well as pseudo stratified epithelium of prostate and gland of vas deferens. Smooth muscle cells in different parts showed weak reactivity. HCA2 receptors are present only in some cells of reproductive system of male rats especially Leydig cells and their presence and relative density is cell-type specific.

Key words

HCA2 receptor, rat, male reproductive system, immunohistochemistry.

Introduction

Rats are frequently used as an animal model in physiological research as well as pharmacological studies on infertility disorders caused by different agents in both sexes. Moreover this laboratory animal has the potential to be used for drug safety assessment and evaluation of environmental toxicants on reproductive system. Therefore, exploration and identification of molecular and cellular characteristics of male reproductive system of this laboratory animal including detection and localization

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of different receptors may be useful from both physiological and pharmacological aspects. Testes as the male gonads in animals have the important functions of spermatogenesis and production of androgens, primarily testosterone. Two types of tissues are present in the testis, the interstitial tissue and the seminiferous tubules. The major component of the interstitial tissue is testosterone-secreting Leydig cells where seminiferous epithelium in seminiferous tubule is responsible for spermatogenesis. The epididymis is the connective tube between testis and vas deferens in the male reproductive system. The epididymis can be divided into three main regions: The head which receives spermatozoa from the testis and is histologically characterized by a thin myoepithelium, the body, and the tail which has a thicker myoepithelium than the head region and connects to vas deferens.

Anatomy, biology, function, and number of the male accessory sex glands are different among species. In mammals, the male accessory sex glands include the prostate, coagulating gland, seminal vesicle, ampullary gland, the bulbourethral (Cowper's) gland, the urethral gland, and the preputial gland (Creasy et al., 2012). The products of male accessory sex glands have different functions including nourishing and activating the spermatozoa. For instance, it has been shown that removal of the dorsolateral lobes of the prostate or vesicular glands in rats is associated with complete infertility (Queen et al., 1981).

Hydroxy-carboxylic acid (HCA) receptors including HCA1, HCA2 and HCA3, previously known as GPR81, GPR109A and GPR109B, are G protein-coupled receptors with intermediates of energy metabolism, all hydroxy-carboxylic acids, as their endogenous ligands. HCA2 receptors are predominantly expressed on adipocytes and mediate the inhibition of lipolysis by coupling to Gi–type proteins (Blad et al., 2011). Ketone body 3-hydroxy-butyrate is endogenous ligand for HCA_2 receptor and this receptor is most extensively studied since it is the target of the antidyslipidemic drug nicotinic acid (or niacin) (Soga et al., 2003; Tunaru et al., 2003; Wise et al., 2003).

HCA2 is highly expressed in human and murine white and brown adipose tissue, moreover the HCA2 receptor is expressed in various immune cells (Schaub et al., 2001; Kostylina et al., 2008), keratinocytes (Hanson et al., 2010), and retinal pigment epithelium as well as in the intestinal epithelium (Yu et al., 2014; Cresci et al., 2016). In 2011, Titgemeyer et al. identified HCA_2 receptors in bovine liver, muscle and brain. The tissue distribution of HCA2 receptor mRNA in hamster and guinea pig has also been determined by quantitative real-time PCR by Torhan et al. (2007). The researchers reported that the expression is fairly widespread, with highest expression in lung, spleen, testis, and adipose tissue of hamsters and spleen, lung, stomach, skeletal muscle, ovary, and adipose tissue of guinea pigs.

Regarding the wide spread use of rats as an animal model in experimental studies and the lack of knowledge about the tissue distribution pattern of HCA2 receptors in this laboratory animal, we previously screened different organs of rats for HCA2 receptor mRNA by RT-PCR, that showed the presence of its mRNA in different organs including testicular and epididymal tissues (Shomali et al., 2014). In the present study we report localization of these receptors in reproductive tissues of male rats by immunohistochemical detection of different cell types that express the receptor protein.

Materials and methods

Six adult male Sprague Dawley rats with a body weight of 200-250 g were used. Rats were acclimatized for one week to the ambient conditions (temperature about 23°C and a 12h/12h, light/dark cycle) and had free access to commercial chew pellets and tap water. After adaptation period, all animals were sacrificed under deep ether anesthesia and right lung, testes and epididymis (head, body and tail), vas deferens and penis as well as all accessory glands (except for Cowper's gland), including vesicular gland, coagulating gland, preputial gland, gland of vas deferens and ventral prostate, were immediately removed. Anatomical localization of different organs and accessory glands was according to Rowett (1968).

Five μm-thick paraffin transverse sections were made from above mentioned tissues. Endogenous peroxidases were inactivated by 3 minute incubation in 3% hydrogen peroxide in methanol. The primary antibody for immunohistochemical staining was rabbit polyclonal GPR109A antibody (1:300, overnight at 4°C) which reacts with rat HCA2 receptor. Secondary antibody was mouse polyclonal anti-rabbit IgG (HRP) (1:200, 30 min at room temperature). Both antibodies were prepared by Biorbyt Ltd. (Cambridge, UK).

Visualization was made by DAB substrate kit (Abcam®, Cambridge, MA, USA), (2-5 min at room temperature). Slides were counterstained with hematoxylin. Negative controls were treated with secondary antibody only. Lung tissue was used as a positive control for presence of the receptor as suggested by the antibody manufacturer.

Color images of the slides under light microscope were prepared and evaluated for detection of cells that express the receptor.

All procedures used in the present study are in accordance with the institutional ethical guidelines for care and use of animals in experiments.

Results

Figures 1-3 show photomicrographs of different parts of reproductive tract of male rats used for immunohistochemical evaluation of cells that express HCA2 receptor.

In testes, spermatogenic cells, except for spermatogonia that were moderately stained, showed no reactivity as well as Sertoli cells and spermatozoa. In interstitial tissue Leydig cells demonstrated receptor expression with strong intensity (Fig. 1).

Epithelial cells in head, tail and body of epididymis showed no reactivity. Epididymal spermatozoa were also unstained. Fig. 2 shows a photomicrograph of epididymal tail without reactivity.

No reactivity was observed in columnar pseudo stratified epithelium of vas deferens. In penile tissue, ciliated pseudo stratified epithelium showed a very weak reactivity (Fig. 3). Other cells were negative for receptor expression.

In coagulating gland, epithelial cells were moderately stained. Ciliated pseudo stratified epithelium of vesicular gland were weakly positive. No or very weak reactivity was observed in epithelial cells of preputial gland as well as pseudo stratified epithelium of prostate and gland of vas deferens.

Smooth muscle cells of coagulating gland, vesicular gland, preputial gland, prostate, gland of vas deferens as well as vas deferens and epididymis showed weak reactivity.

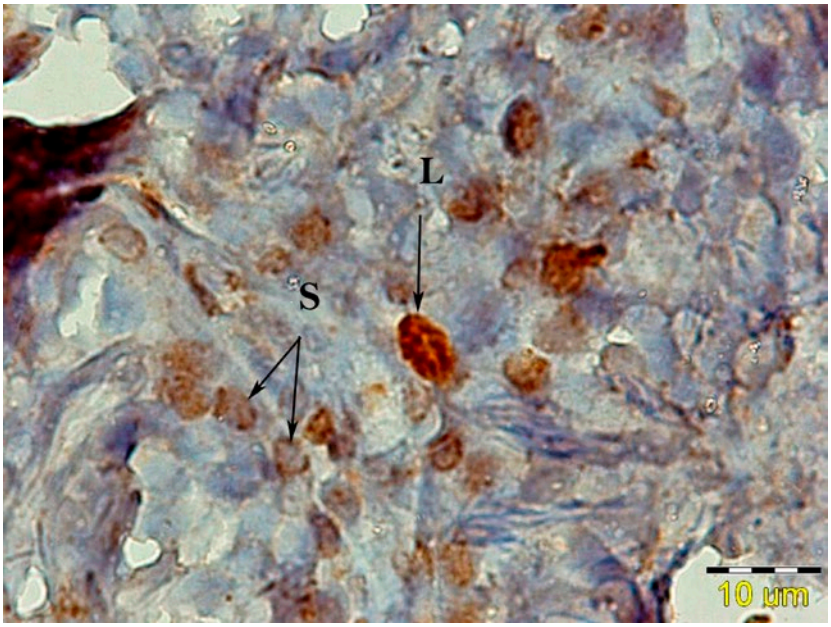

Figure 1. Photomicrograph of rat testicular seminiferous tubules upon immunohistochemical staining for HCA2 receptor: Leydig cell were strongly stained (L); spermatogonia (S) showed moderate reaction.

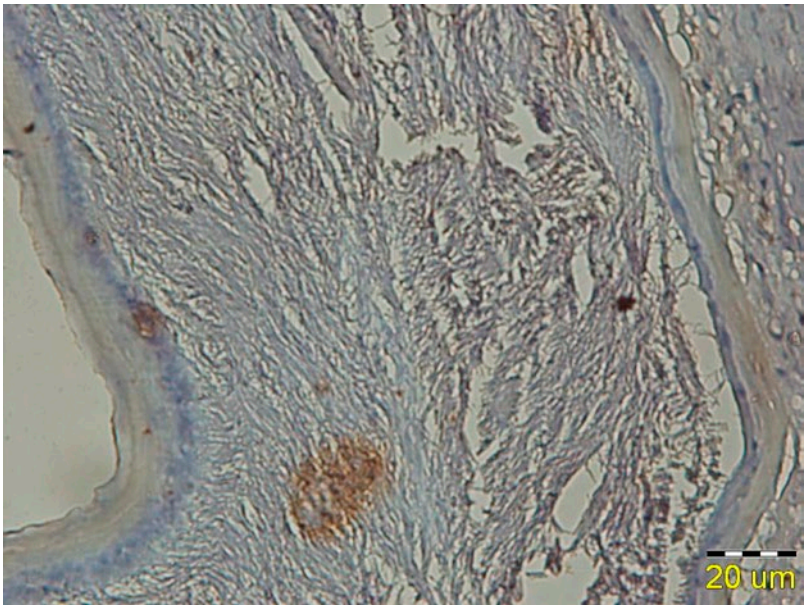

Figure 2. Photomicrograph of tail of rat epididymis upon immunohistochemical staining for HCA2 receptor: No reactivity was observed in epithelial cells as well as spermatozoa.

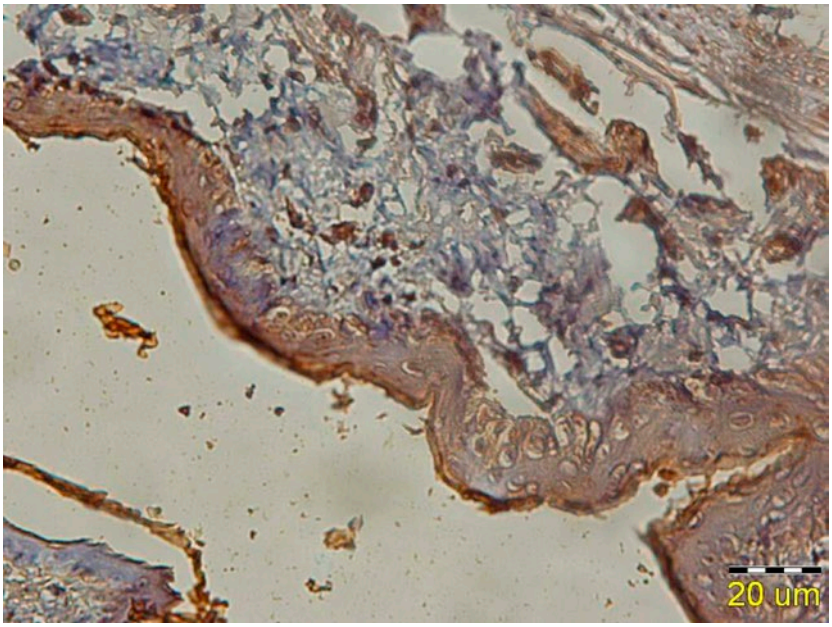

Figure 3. Photomicrograph of rat penile tissue showing signs of weak reactivity in pseudo stratified columnar epithelium. The immunoreactive cells in lamina propria are most probably fibroblasts. Immunohistochemistry for HCA2 receptor.

Discussion

The presence of mRNA for HCA2 receptor has been previously described in testis and epididymal tissue of rats (Shomali et al., 2014). In this study, presence of HCA2 receptor protein was observed in Leydig cells and spermatogonia in testes however we did not find appreciable reactivity for this receptor in epithelium as well as smooth muscle cells of epididymis. The reason behind this discrepancy is possibly the fact that HCA2 receptors are also present on neutrophils and tissue macrophages (Schaub et al., 2001; Kostylina et al., 2008; Lukasova et al., 2011) the cells that may be present in samples used for PCR.

The immunoreactive cells in lamina propria of the tissue in Fig. 3 are most probably fibroblasts, since the immunoreactivity of this cell type for HCA2 receptors has been previously reported in rats by our team Shomali et al., 2015).

As stated elsewhere in the text, 3-hydroxybutyrate, a ketone body which is produced when fatty acid β-oxidation rate is high, is the endogenous ligand for HCA2 receptors. It is well established that, in situations of increased β-oxidation rates (e. g. during starvation) 3-hydroxybutyrate plasma level raises appreciably and by a negative feedback loop via HCA2 receptors results in inhibitory regulation of lipolysis (Blad et al., 2011).

The consequences following activation of HCA2 receptors expressed by cells outside the adipose tissue during starvation has not been completely clarified, yet. Although it is possible that activation of HCA2 receptors expressed on immune cells

by elevated 3-hydroxybutyrate levels during starvation may induce anti-inflammatory effects which could be advantageous under conditions of starvation. Moreover, the antidyslipidemic drug niacin also activates adipocytes' HCA2 receptors and results in inhibition of the release of free fatty acids from these cells (Blad et al., 2011).

Although there has been no previous report on the presence of HCA2 receptor in Leydig cells of rat, the presence of β-hydroxybutyrate dehydrogenase - enzyme which has a pivotal role in generation of 3-hydroxybutyrate - has been previously demonstrated in this tissue. In a study performed by Bara (1979), β-hydroxybutyrate dehydrogenase was present in both the Leydig and Sertoli cells of the guinea-pig testis. This enzyme has also been detected in isolated rat testicular germ cells (Bajpai et al., 1998).

Leydig cells utilize both glucose and a ketone body to maintain their steroidogenesis (Amrolia et al., 1988). The presence of peroxisomes containing peroxisomal fatty acid β-oxidation enzymes (Nemali et al., 1988) has been shown in Leydig cells in the interstitial tissue of the testis. The energy needed for proliferation and differentiation of spermatogenic cells is thought to be provided mainly by glucose and the products of glycolysis, pyruvate and lactate (Boussouar et al., 2004).

In a study by Fukasawa et al. (2010), Quantitative analysis by immunoblot and DNA microarray revealed that mitochondrial fatty acid β-oxidation enzymes occur abundantly in Leydig cells in the interstitial tissue but much less so in the seminiferous tubules. By immunohistochemical method these authors also showed that Leydig cells as well as Sertoli cells are equipped with a full set of mitochondrial fatty acid β-oxidation enzymes with relatively different expression among the cells.

Taken together, it seems that with regard to the presence of HCA2 receptor in Leydig cells and spermatogonia, which was demonstrated in this study, locally produced and/or plasma 3-hydroxybutyrate may be important in regulation of cellular functions of these cells. On the other hand, administration of niacin as a well-known drug for dyslipidemic conditions may affect lipid metabolism or possibly other functions of the above mentioned cells, which needs to be clarified in the future studies.

As stated in result section, in other parts of the reproductive tract that were examined in the present study (except for coagulating gland epithelium with moderate receptor expression), HCA2 receptors were weakly present or even absent, therefore it is quite hard to expect a considerable physiological or pharmacological role for these receptors in these parts of reproductive tract.

In conclusion, HCA2 receptors are present only in some cells of reproductive system of male rats especially Leydig cells and their presence and relative density is cell-type specific.

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Declaration

Authors have no conflict of interest to declare.

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