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EXPRESSION OF PROGESTERONE RECEPTORS IN HIPPOCAMPAL NEURONS IN RABBIT MALES

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Abstract

The aim of this study was to investigate the presence of progesterone receptors in hippocampal neurons of male rabbits and the distribution of these receptors in different areas of the hippocampus. The examinations were carried out on brains of five males, New Zealand breed, age of 2 years, weighing 2-3 kg. Immunohistochemical detection of receptors was performed using DAKO LSAB + Kit Peroxidase method. The sections were incubated with primary monoclonal antibody for progesterone receptor NCL-L-PGR-AB. The obtained results have shown the presence of progesterone receptors in neurons of CA1, CA3, and CA4 regions of the hippocampus. The reaction also occurred in the region of granular cells of the dental gyrus. The reaction was seen in the cytoplasm and nuclei of neurons simultaneously, whereas some neurons did not show any reaction in the nucleus and cytoplasm. Occurrence of progesterone receptors in most neurons of the hippocampus confirms a direct influence of progesterone on the hippocampus and its functions in rabbit males.

Key words: male rabbit, hippocampus, progesterone receptor.

In adult males progesterone is produced by adrenal glands. In blood of the male rats it reaches the level of about 1.96 ng/mL (22). Both females and males have progesterone receptors in different brain regions during development and in adulthood. They are among others in neurons of the hippocampus, amydgala, hypothalamus (1, 2, 3). Progesterone has an impact on typically masculine behaviour, including sexual. Already in the 60s it was claimed that progesterone may influence the behaviour of males and its role was emphasised as an anti-androgenic hormone. This theory was rejected in the later years, while in the 90s of the last century a renewed attention to the hypothesis has been gained. At present, the role of progesterone and its receptors in males during reproductive processes is examined. Brain areas responsible for behaviour connected with reproduction are examined (9, 13). Progesterone administration to uncastrated males weakens, and in large doses actually inhibits sexual behaviour. Progesterone administered simultaneously with androgens may eliminate aggressive behaviour in males (12, 15). Some brain areas in animals show sexual dimorphism. Already many years ago, differences in the structure of nuclei of the hypothalamus in rat males and females were observed. Especially, it concerned medial nucleus of preoptic area called sexually dimorphic (7). In rat males it is greater than in females, the difference has been observed already in the prenatal period. In females, the above mentioned nucleus affects the ovulatory cycle, in males it is responsible for copulatory behaviour. Sexual dimorphism was also observed in ventro-medial nucleus of the hypothalamus in rats and rhesus monkeys, which is responsible for the occurrence of lordosis in females. In males during copulation the neuronal activity of this nucleus was not observed. Sexual dimorphism concerns not only brain areas connected with reproduction (11). In animals, differences in the amygdala, orbitofrontal cortex, and hippocampus were observed. These regions are primarily associated with the processes of memorising and learning. Sexual dimorphism is here associated with differences in the way of learning, perception of certain phenomena, eases to remembering between females and males. All the above mentioned information encouraged us to conduct research on the structure of the hippocampus in rabbit males.

The presence of progesterone receptors in rabbit females was proved in the cerebral cortex, nuclei of the hypothalamus, nucleus of the infundibulum, preoptic area, and in the hippocampus (4, 6, 22). The examinations were carried out on preparations of the hippocampus of rabbit males. The hippocampus is a structure of the limbic system and is responsible for the processes associated with memory. The aim of this study was to investigate if hippocampal neurons in rabbit males contain progesterone receptors and what is the distribution of these receptors in different areas of the hippocampus. Intracellular localisation of IHC reaction for the presence of progesterone receptors was specified. CA1, CA2, CA3, and CA4 sectors of the hippocampus underwent examination. The analysis of granular cells' layer of the dental gyrus was also carried out.

Material and Methods

The examinations were carried out on brains of five New Zealand male rabbits, 2 years of age, weighing 2-3 kg. The material was obtained from a rabbit slaughterhouse. The brains were collected immediately after the slaughter. Histological slides were prepared cutting hemispheres in frontal plane into 4 µm-thick sections. In order to determine location of the hippocampus, atlases describing the structure of rabbit brain were used (8). Cross sections showing the hippocampus were at 5.5 mm in relation to the bregma. Immunohistochemical detection of receptors was performed using DAKO LSAB + Kit Peroxidase method. The sections were incubated with primary monoclonal antibody for progesterone receptor NCL-L-PGR-AB (Novocastra). Next, monoclonal, biotinylated secondary anti-mouse IgG antibody was used. The slices were incubated with streptavidine-peroxidase complex. Then, DAB was used. For the staining of cell nuclei, Mayer's haematoxylin was applied. The nuclei were stained blue, brown staining of the cytoplasm and nuclei indicated the presence of the reaction, proving the occurrence of progesterone receptors (colour reaction with DAB). Negative control of immunohistochemical reaction specificity was performed by omitting the incubation stage with primary antibody. The results of the reaction were observed under a light microscope and later morphometric examinations using a digital camera TIF and cell D programme were carried out.

Results

The obtained results have shown the presence of progesterone receptors in neurons of CA1, CA3, and CA4 regions of the hippocampus (Fig 2). The reaction also occurred in the region of granular cells of dental gyrus (Fig. 1). The reaction occurred in the cytoplasm and nuclei of neurons simultaneously, whereas some neurons did not show any reaction in the nuclei and cytoplasm (Fig 2). Seventy-nine percent of cells of the dental gyrus granular layer showed no reaction for the progesterone receptor. A small percentage of granular neurons showed the reaction in the nucleus (only 4%), in the cytoplasm the reaction concerned 10% of neurons, whereas in both the nucleus and cytoplasm the reaction occurred in 8% of nerve cells. The neurons of CA4 area showed the reaction in the mere cytoplasm in the highest percentage, reaching as much as 22%. In the cell nucleus of this area, the reaction occurred only in 4% of the nuclei, in the nucleus and cytoplasm in 18% of CA4 area's cells. In 42% of neurons, there were no progesterone receptors. Pyramidal cells of hippocampal CA3 area had the highest percentage of progesterone receptors in the cytoplasm-31%, in 13% of this area neurons PR occurred only in the cell nuclei. Twenty-four percent of CA3 area neurons showed positive reaction in both the nucleus and cytoplasm. In 32% of the cells of the examined area there was no immunohistochemical reaction for PR.

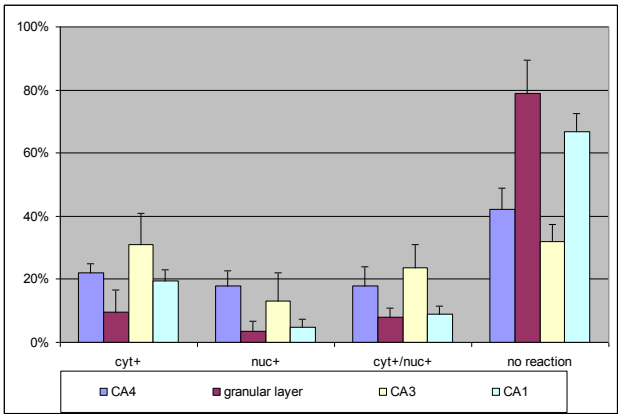


Fig 2. Immunohistochemical expression of progesterone receptors in the hippocampus of male rabbits. Error bars show the standard deviation (SD).

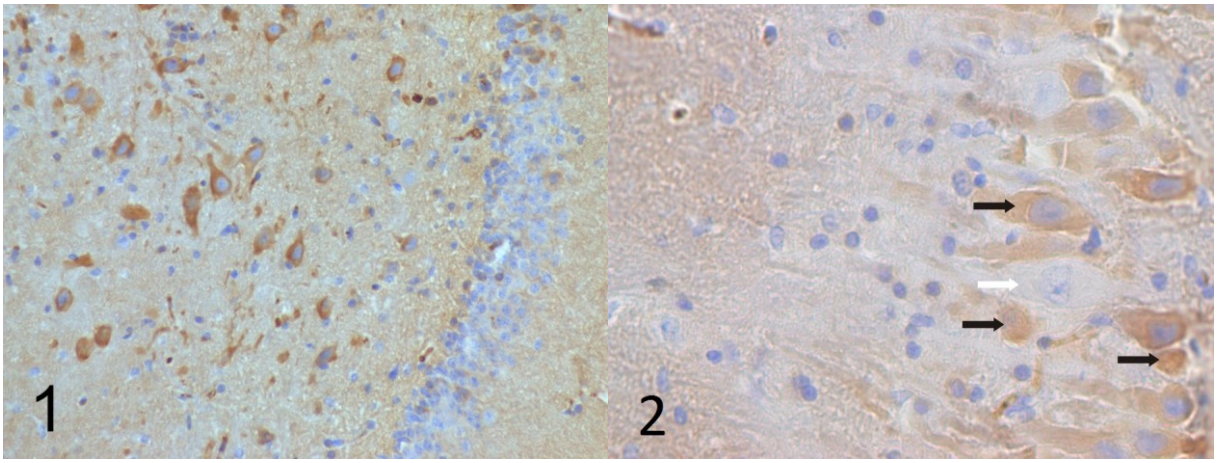


Fig. 1. Expression of progesterone receptors in the hippocampus of male rabbit: 1. PR in CA4 field and cells of granular layer About 100x, 2. Expression of progesterone receptors in neurons of CA3 field. The black arrows indicate PR-IR neurons but white arrow indicates no PR-IR neuron. The top (black) arrow indicates neuron with reaction in cytoplasm, while the two black arrows below indicate the neurons with the reaction product in nucleus. About 600x.

The neurons of CA1 area also showed the presence of progesterone receptors. Progesterone receptors occur in the cytoplasm in 20% of the cells of the examined area, in the mere cell nuclei in only 5%, and in the nucleus and cytoplasm in 9%. No reaction indicating the occurrence of receptors was found in 67% of neurons.

Discussion

The research carried out so far mainly concerned the occurrence of progesterone receptors in females of various animal species. It should be noted that in females progesterone level in blood depends on gonadal activity and menstrual cycle, during which the level of sex hormones, including progesterone, varies considerably. In males, on the other hand, the level of progesterone in blood is subject to slight fluctuations (15). As the studies have shown, progesterone is not only a hormone responsible for functions of reproductive system but also for functions of the central nervous system (22). It is also important that it affects functions of the mentioned systems in both females and males, hence it is important to conduct the research on male brains. Our studies proved that in the hippocampus of rabbit males, the most neurons showing progesterone receptors occur in CA3 area. The next area to the number of neurons with PR reaction is CA4, followed by CA1, whereas granular neurons of dental gyrus are characterised by the smallest percentage of neurons showing immunohistochemical reaction indicating the presence of PR. These results support the hypothesis that progesterone through specific receptors exerts an influence on the structures such as the hippocampus and thus has an impact on the processes associated with memory. It should be stressed that differences in the distribution of receptors in neurons of different hippocampal areas are important, since neurons of CA1 area send axons to form synaptic junctions with neurons of the intraolfactory cortex. Through the intraolfactory cortex, information connected with processes of memorising from the whole area of cerebral cortex is transformed. Axons from pyramidal cells of CA3 area of the hippocampus form synaptic junctions with pyramidal cells of CA1 area and as reversible collaterals they form synapses with the cells of the same CA3 area. Efferent fibres, making up presynaptic endings with nerve cells of the thalamus and hypothalamus, come out of hippocampal neurons. Neurons containing PR in areas CA3 and CA1 have therefore a significant impact on functioning of the brain in a wide range. Our results also prove that the highest percentage of neurons show the presence of progesterone in the cytoplasm and in the mere nucleus they are present in much smaller numbers. Diverse localisation of receptors within the nerve cells proves different sensitivity of neurons to endogenous hormone level. Progesterone receptors were observed in the brain of rat males and females during developmental period and in adult individuals (9, 20, 21). The receptors occurred in the hypothalamus, hippocampus, cerebellum, preoptic area, frontal cortex, and olfactory bulbs. In rat females, the presence of PR in the hypothalamus, frontal cortex, and preoptic area was observed (14).

The presence of progesterone receptors was analysed in rabbit females (4, 6, 10). Authors described distribution of PR-immunoreactive cells in the forebrain of ovariectomised rabbits. They used mouse monoclonal PR antibody. The highest concentration of PR-immunoreactive cells was found in the infundibular nucleus, paraventricular, medial, and principal preoptic nuclei. Immunoreactive cells were observed in the nucleus X, paraventricular nucleus, and supraoptic and suprachiasmatic nuclei. Immunoreactivity was found only in the nucleus of cells. PR-immunoreactive cells were absent in the cerebral cortex, hippocampus, subiculum, and amygdala (4). The investigations of female rabbit brain by *in situ* hybrydisation were also carried out (6). The results showed the highest expression in preoptic area and hypothalamic anterior nuclei (paraventricular, periventricular, and arcuate nuclei). A high expression was detected in the hippocampus and cerebral cortex. In the hippocampus and hypothalamic and preoptic area regions oestradiol treatment induced an increase in PR mRNA expression. PR mRNA expression was observed in CA1 and CA3 hippocampal fields, and in the dentate gyrus. Our results were similar, the progesterone receptors were found in neurons of CA1, CA3, and CA4 fields and in the dentate gyrus. Other investigations showed co-expression of Erβ with Erα and progestin receptor proteins in the female rat forebrain (10). Erα immunoreactive neurons were present in preoptic area, the principal and medial nuclei of the BNST, the anterior, lateral, and ventromedial hypothalamus, the central, medial, and cortical amygdala, arcuate nucleus, piriform cortex, and hippocampus. Erβ immunoreactive cells were found in the periventricular preoptic area (PvPO), BNST, the paraventricular nucleus, supraoptic nucleus, and medial amygdala. PR immunoreactive cells were detected in the preoptic area, BNST, the lateral and ventromedial hypothalamus, the arcuate nucleus, the hippocampus, the piriform cortex, various amygdala nuclei, and in the III – IV layers of the cerebral cortex. Co-expression of Erβ immunoreactivity with Erα-ir or PgR-ir was observed in the PvPO, BNST, and medial amygdala. According to other authors (5), oestrogen receptors α in the female rabbit forebrain are present in the nucleus X and PVN, but the ventromedial hypothalamic nucleus and hippocampus lacked Erα-ir neurons. The differences in progesterone receptor expression depending on the gender of the tested animals confirm the examinations carried out on rats (16, 19). Specific differences were found in PR expression during prenatal period in neurons in rat females and males (20, 21).

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