



Differential Distribution of GABA and Gat1 in Mouse Epididymis



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Abstract

The present study showed a detailed description of distribution of GABA and its transporter protein GAT1 in mouse epididymis and gave insight into the physiological function of GABA in mouse epididymis. The location of GABA and GAT1 in epididymis was detected by immunohistochemistry assay. Histological distribution of GABA and GAT1 was observed under microscope in different resolution. The staining of different parts of epididymis was quantified. The semi-quantification data showed that the GABA staining of epididymis caput is dramatically stronger than corpus and cauda, and GABA distribution in mouse epididymis paralleled with GAT1. These data suggest that GABA system performs its function mostly in the caput of mouse epididymis and epididymis caput might be crucial for sperm maturation.

Keywords: GABA; GAT1; Epididymis; Immuno histo chemistry

Abbreviations: GABA; γ -Aminobutyric Acid; GAT1: GABA Transport Protein I

Introduction

Γ -amino butyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian central nervous system [1]. Neuronal inhibition is induced by GABA binding with ionic GABAA/GABAC receptor and metabolic GABAB receptor of postsynaptic membrane. Synaptic transmission is mainly terminated by reuptake effect of GABA transport protein (GAT), and GAT1 is the primary neuron transport protein in rodent brain among the four GATs (GAT1-GAT4) [2,3]. Except for CNS, GABA system is also identified in many peripheral tissues [4], including the endocrine organs pituitary, pancreas and ovary. Previous study has shown that GABA exists in testis, sperm, deferent duct and epididymis in rat male reproductive system [5], but its function is still not clearly understood. Similar to progesterone, GABA can cause depolarization of cell membrane and stimulate acrosome reactions in precapacitated human spermatozoa, this response is concentration-dependent manner (with a narrow range of concentrations showing a considerable stimulatory effect and higher concentrations being less stimulatory) [6].

Previous studies demonstrated that GABAA and GABAB receptors were expressed in sperms of rats, which might be related with acrosome reaction mediated by GABA and progesterone [7-11]. GABA also modulates sperm kinematic parameters and increases hyper activation. These effects have the same magnitude of those produced by progesterone and mediated mainly by the GABAA receptor [12-14]. GABA may be a physiological regulator of sperm function. The GABA transporter protein GAT1 was also identified in testes, epididymis and sperms of rats and mice [15-17]. Testis and sperms of GAT1 over-expressed mice were significantly abnormal when compared with wild type mice. The reproductive capacity of the GAT1 transgenic mice was severely affected [15]. In the present study, GABA and GAT1 distribution was detected by immunohistochemistry and the signal intensity was quantified. Interestingly, GABA and GAT1 distributed in parallel and mostly in the caput of epididymis, suggesting that GABA system performs its function mainly in the caput of epididymis and the caput of epididymis plays an important role in sperm maturation and activation.

Materials and Methods

Animals

C57 mice were fed in plastic cages with enough food and water which met the standard of rodents. Temperature of $22 \pm 2^\circ \text{C}$ and diurnal cycle of 12 hours were maintained. All experimental protocols met rules of Shanghai Animal Administration Committee on feed and practice of SPF-grade experimental animals and were approved by the committee. Four groups of C57 mice (Every group contains 10 mice.) were used for each study.

Histology Assay

Epididymis samples from C57 one-month-old mice were sacrificed by cervical dislocation and then they were dissected and perfused with 4% paraformaldehyde after anesthesia, then followed by ethanol dehydration, xylene clearance and paraffin embedment. Subsequently, 7 μm paraffin-sections were cut on Leica microtome. Nonspecific binding sites were blocked with the rabbit serum-free protein. For detection, GABA and GAT1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Sections were incubated with the primary anti-GABA or anti-GAT1 (1:50) overnight at 4°C . After washing, the sections were incubated with the secondary antibodies (biotinylated rabbit anti-goat IgG) at room temperature. Then the sections were incubated with the horseradish peroxidase-labeled streptavidin (goat streptavidin/peroxidase Kit-SP 9003, Zhongshan Golden Bridge Biotechnology, Co LTD, China). Detection was done with the AEC substrate. The color development was stopped under the microscopic control by adding PBS. Finally, sections were counterstained with haematoxylin. The negative controls were performed by omitting the primary antibody. The slides were then examined and photographed under Leica microscope. Two sections per mouse were measured. Semi-quantification of the immunohistochemistry results was done by Axioplan 2 images microscopic image analysis system (Zeiss Company, Germany).

Statistical Analysis

SPSS13.0 was used for the statistical analyses. Data are presented as mean $\pm$ SD. Comparisons were made using t test. $P < 0.05$ was considered statistically significant.

Results

Immunohistochemistry Assay of Gaba and Gat1 of Mouse Epididymis

Immunohistochemistry was used to detect the distribution of GABA and GAT1 in mouse epididymis. The results showed that GABA existed over the whole epididymis, including epididymis caput, corpus, and cauda (Figure 1a). The staining of the caput was stronger than the corpus and cauda (Figure 1b & c). GAT1 immunohistochemistry staining pattern was similar to the results of GABA (Figure 2). There was little staining of the control as shown in Figure 3.

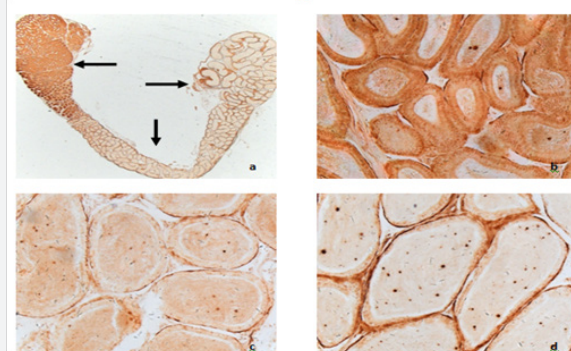


Figure 1: Immunohisto chemistry assay of GABA in epididymis of C57 mice (a). GABA staining over the epididymis ($\times 40$). The arrows showing the epididymis caput, epididymis corpus, and epididymis cauda from left to right (b). Epididymis caput ($\times 400$) (c). Epididymis corpus ($\times 400$) (d). Epididymis cauda ($\times 400$) Bar_____.

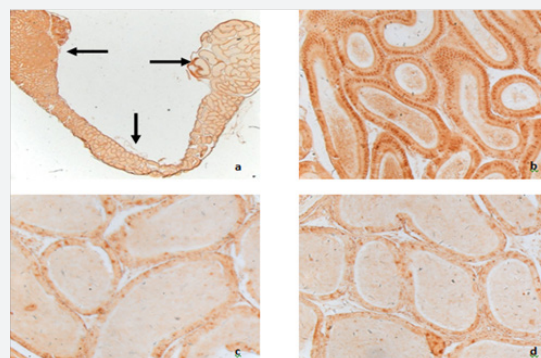


Figure 2: Immunohisto chemistry assay of GAT1 in epididymis of C57 mice (a). GAT1 staining over the epididymis ($\times 40$) (b). Epididymis caput ($\times 400$) (c). Epididymis corpus ($\times 400$) (d). Epididymis cauda ($\times 400$). Bar_____.

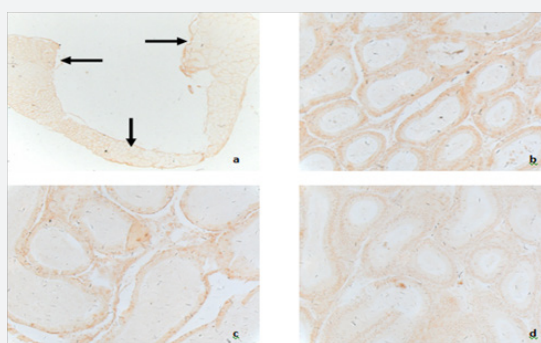


Figure 3: Negative control of immunohisto chemistry assay in epididymis of C57 mice (a), (b), (c), (d) are the whole epididymis ($\times 40$), epididymis caput ($\times 400$), epididymis corpus ($\times 400$), and epididymis cauda ($\times 400$) respectively Bar_____.

Semi-Quantification of the Immunohisto chemistry Staining Intensity of Gaba and Gat1

The intensity of GABA staining varied from the caput 95.5 ± 6.5 to the corpus 55.9 ± 3.5 and the cauda 59.2 ± 4.2 . The

intensity of GAT1 staining varied from the caput 75.3±2.0 to the corpus 52.0±2.9 and the cauda 54.5±1.7. The results identified that GABA distributed significantly ($p<0.01$) in the caput of epididymis, instead of the corpus and cauda. GAT1 has the similar distribution pattern as GABA (Table 1, Figures 3 & 4).

Table 1: immunohisto chemistry assay semi-quantitative data (a). GABA (b). GAT1.

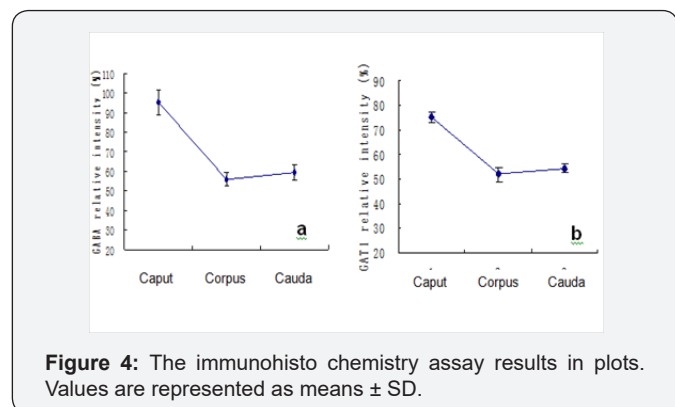
Table 1a: The semi-quantitative results of GABA immunohisto chemistry assay.

Group	1	2	3	4	Mean	SD
Caput	103.2	96.7	94.7	87.4	95.5*	6.5
Corpus	51.7	57.7	59.6	54.6	55.9	3.5
Cauda	55.6	62.5	63.2	55.6	59.2	4.2

* $p<0.01$ compared with corpus and cauda.

Table 1b: The semi-quantitative results of GAT1 immunohisto chemistry assay.

Group	1	2	3	4	Mean	SD
Caput	77.4	75.0	76.1	72.7	75.3*	2.0
Corpus	53.5	52.2	54.3	47.8	52.0	2.9
Cauda	56.6	53.8	54.9	52.6	54.5	1.7



Discussion

GABA protein was expressed in sperms, testes and epididymis, which plays very important role in spermiotocosis [13-15]. Our previous studies reported that testis and sperm morphology was abnormal in adult male GAT1 transgenic mice, but the epididymis was unremarkable [15]. The reproduction of male mice was affected significantly by GAT1 over expression [15,16]. The present study confirmed that GABA and GAT1 distributed differentially in mouse epididymis caput, corpus, and cauda. GAT1 is responsible for transfer of GABA from the epididymis lumen into the epithelium cells and maintain homeostasis of GABA in epididymis. The similar distribution pattern of GABA and GAT1 in epididymis can be explained by GAT1 is a membrane-transport protein regulating GABA concentration of the epididymis lumen.

The physiological function of epididymis is for sperm maturation. Spermatozoa enter the epididymal tubule from the

testis and, during transit, undergo a process of maturation that confers motility and the ability to fertilize ova [18]. GABA has already been proved that it can induce sperm acrosome reaction and increase human sperm motility in vitro [7,10,12]. Acrosome reaction is a critical step for fertilization. The motility is also very important for sperms to reach ova and start fertilization. Our data suggests that GABA perform those two functions on sperm in epididymis, especially in the caput. In conclusion, our observation offers the proof that GABA and GAT1 exists mostly in the caput of mouse epididymis, which is the major organ for sperm maturation and GABA plays an important role in epididymal function. This guided us that we should protect the intactness of epididymis caput during andrological surgery operation.

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