GABA<sub>A</sub> Receptor Subunits in Rat Testis and Sperm
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Abstract

Background: γ-Aminobutyric acid (GABA) is considered to be the predominant inhibitory neurotransmitter in mammalian central nervous systems (CNS). There are two major classes of GABA receptors: GABA<sub>A</sub>Rs and GABA<sub>B</sub>Rs. The GABA<sub>A</sub> receptor is derived from various subunits such as alpha<sup>1</sup>-alpha<sup>6</sup>, beta<sup>1</sup>-beta<sup>3</sup>, gamma<sup>1</sup>-gamma<sup>4</sup>, delta, epsilon, pi, and rho<sup>1</sup>-3. Intensive research has been performed to understand and establish the distribution and functions of these receptors in the CNS and peripheral tissues. The presence of some GABA<sub>A</sub> receptors in sperm prompted us to explore the existence of GABA<sub>A</sub> receptors in rat testis and sperm.

Materials and Methods: Total cellular RNA was isolated from Wistar rat sperm and testis and reverse transcriptased to cDNA. PCR reactions were performed in a 20μl (microliter) volume containing specific GABA<sub>A</sub>R subunits (forward and reverse primers) with other required materials. Reactions were carried out using a PCR machine to investigate the existence of GABA<sub>A</sub> receptor subunits in rat testis and sperm. The amplification products were analyzed on 2% agarose gels stained with ethidium bromide.

Results: Our results showed that GABA<sub>A</sub>Rs composed of α<sub>5</sub>, β<sub>1</sub>, β<sub>3</sub>, and γ<sub>1</sub> subunits were expressed in both testis and sperm. These results indicate that, in sperm, GABA<sub>A</sub> receptors might have important functions.

Conclusion: Sperm could be a novel site of GABA<sub>A</sub> expression. Further studies should be taken to explore the role of these receptors on sperm acrosome reaction.

Keywords: GABA<sub>A</sub>R, RT-PCR, Testis, Sperm
GABA-related disorders and of the complex interaction of excitatory and inhibitory mechanisms in neuronal processing.

GABA is a major inhibitory neurotransmitter in the mammalian brain. GABA Rs are constructed from a family of around 21 different subunits including six alpha (α1-6), four beta (β1-4), four gamma (γ1-4), one delta (δ), one epsilon (ε), one pi (π), one theta (θ), and three rho (ρ1-3) subunits, all of which are products of separate genes.

GABA has been discovered in the epididymis, seminal vesicle, and testicle of the adult rat (6). Recently, it has been shown that GABA AR subunits are expressed outside of the CNS in the adrenal glands, ovaries, testis, placenta, and uterus in a tissue-specific manner (7). It is suggested that GABA might regulate sperm functions such as capacitation and acrosome reaction via its interaction with the receptors that were originally found in the CNS (8). In the present study, we demonstrate the presence of GABA AR subunits in rat testis as well as in sperm.

**Materials and Methods**

All experiments were performed on adult Wistar rats in accordance with the Biology Department for the Care and Use of Animals for Scientific Purposes and approved by the Animal Ethics Committee in the University of Isfahan. Animals were housed in cages with free access to standard rodent chow and water. The colony room was maintained at 22±2°C with a 12 hour: 12 hour light: dark cycle. Animals were anesthetized with halothane and decapitated. Testis were quickly isolated and kept ice-cold. Tissues from testis were excised. Sperm contained in the caudal epidermis of male rats were released into PBS and washed twice with PBS.

Total cellular RNA was extracted using the RNeasy kit (Qiagen, Valencia, CA) following the manufacturer’s specifications. Total RNA was assessed by optical density measurements. DNase treated total RNA (2-3 μg) was reverse transcribed using random hexamer primers and Superscript III RT invitrogen according to the manufacturer’s instructions.

PCR reactions were performed in a 20μl volume containing 1U Taq (Fisher-Biotech), 1.5mM MgCl₂, 0.2mM dNTP and 10pM of specific GABA AR subunits (Table 1) forward and reverse primers with 5 ng of template. Samples were made up to 20ml with RNase-, DNase-free water. Reactions were carried out using a BioRad (Bio-Rad Laboratories, Inc., California, USA) PCR machine as follows: an initial 95°C denaturation step for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds, repeated 40 times. The amplification products were analyzed on 2% agarose gels stained with ethidium bromide. Products sizes were as expected for all subunits and specificity of the PCR products were confirmed by sequencing.

**Results**

**RT-PCR in rat testis**

The expression of 17 GABA AR subunits (Table 1) in rat testis assessed by RT-PCR analysis. Specific fragments from rat testis RT samples were obtained using selective primers. Products of corresponding size from rat brain RT samples via PCR with the same primers were also obtained. Subsequently, the amplified products were confirmed by sequencing analysis. It was found that the products from rat testis were identical to those from the rat brain. 18S cDNA served as a control of RT samples (Fig 1). In addition, no specific PCR product from the RNA samples that were omitted in reverse transcription could be observed over background, which verified the absence of genomic DNA contamination (Fig 1).

The data suggested that α5, β1α5, β1, β3 and γ1 subunits were always expressed, which provided evidence that those genes existed in rat testis.
Because the GABA Rs were present in rat testis, we wondered if they also existed in rat sperm. Results revealed that α5, β1α5, β1, β3 and γ1 were detected in rat sperm (Fig 1). Testis-specific 18S proved the origin of RNA which was absent of genomic DNA contamination (Fig 1). The data suggested that GABAARs were always expressed providing evidence of the existence of those genes in rat sperm.

### Discussion

Several laboratories reported the presence of GABA receptors and transporters in testis and sperm (7, 9-11) An important function of GABA in the process of sperm maturation was demonstrated. GABA_Rs have been detected in pig, ram and human sperm membrane preparations by radiolig-
and-binding experiments (12, 13). It is suggested that GABA$_A$R subunits are localized primarily in the plasma membrane overlying the equatorial segment of motile or fixed, permeabilized capacitated and incapacitated ejaculated human sperm (14). The fact that immunoreactivity was also detected on motile sperm supports the expected plasma membrane location for the receptor. Western blot studies of epididymal rat sperm demonstrated the presence of the $\alpha_5$ or $\beta$ ($\beta_1$ or $\beta_3$) subunits, and immunofluorescence experiments detected those subunits in the plasma membrane overlying the ‘ventral surface’ of the acrosome in fixed epididymal sperm (15).

**Conclusion**

In conclusion our results are in agreement with previous studies. Collectively these results show that sperm GABA$_A$Rs may be involved in important fertilization events, such as depolarization as seen during progesterone-initiated AR capacitating and hyper-activated motility. We propose that GABA$_A$Rs can be therapeutically targeted for contraception or dysgenesis treatment. Additionally we are awaiting a deeper understanding of the mechanisms and functions of GABA$_A$Rs in male reproductive tissues. We propose to study the expression of GABA$_A$Rs in fertile and infertile male sperm. This may lead to a better understanding of infertility and may result in novel treatments.

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**References**