Original Article

$GABA_A$ 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: $GABA_A$Rs and $GABA_B$Rs. The $GABA_A$ receptor is derived from various subunits such as alpha1-alpha 6, beta1-beta 3, gamma1-gamma 4, delta, epsilon, pi, and rho1-3. 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 $GABA_A$ receptors in sperm prompted us to explore the existence of $GABA_A$ 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 $GABA_A$R subunits (forward and reverse primers) with other required materials. Reactions were carried out using a PCR machine to investigate the existence of $GABA_A$ 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 $GABA_A$Rs composed of α5, β1, β3, and γ1 subunits were expressed in both testis and sperm. These results indicate that, in sperm, $GABA_A$ receptors might have important functions.

Conclusion:
Sperm could be a novel site of $GABA_A$ expression. Further studies should be taken to explore the role of these receptors on sperm acrosome reaction.

Keywords: $GABA_A$R, RT-PCR, Testis, Sperm

Introduction

The amino acid gamma-aminobutyric-acid (GABA) is a major inhibitory neurotransmitter in the CNS (1) that mediates most of its effects through $GABA_A$ receptors. There are two major classes of GABA receptors: ionotropic GABA type-A receptors that form ion channels ($GABA_A$Rs) and metabotropic GABA type-B ($GABA_B$Rs) which are G protein-coupled receptors. The $GABA_A$R is a member of the cysteine-loop family of ligand-gated ion channels that also includes the nicotinic acetylcholine receptor cation channel (nAChR), the 5-HT3 receptor cation channel (5-HT3R) and the glycine receptor chloride channel ($GABA_A$R). Receptors of this class comprise 5 subunits arranged symmetrically around a central ion-conducting pore. Each subunit consists of 4 α-helical transmembrane (TM) domains and a large extracellular amino-terminal domain that harbors the ligand binding sites and the signature cysteine-loop.

Molecular biology revealed the complex subunit structural design of this receptor channel, in which a pentameric assembly resulting from five of at least 21 subunits, grouped in the eight classes alpha (α1-6), beta (β1-4), gamma (γ1-4), delta, pi, epsilon, theta, and rho (ρ1-3) (2, 3) permits an immense number of putative receptor isoforms. Their variety extended the existence of several splice forms, for example, the α6, β2 and γ2 subunits (4). The subunit combination of a $GABA_A$Rs determines the specific effects of allosterical modulators of benzodiazepines, barbiturates, steroids, general anaesthetics, some convulsants, polyvalent cations, and ethanol. These agents act in different binding sites, some of which are not yet known (5). Drugs and endogenous ligands bind either to the extracellular domain or channel domain of the $GABA_A$RS and act as positive or negative allosteric modulators (1). In heterologous expression systems, the presence of alpha and beta classes are needed to have functional channels. These gamma subunits are required in order to mimic the full repertoire of native receptor responses to drugs. Knowledge of the complex pharmacology of $GABA_A$Rs might eventually enable site-directed drug design to further our understanding of

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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. $GABA_A$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 $GABA_A$Rs 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 demonstratethe presence of $GABA_A$Rs 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 (2-3μg) 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 MgCl, 0.2mM dNTP and 10pM of specific $GABA_A$R 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 $GABA_A$Rs 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).

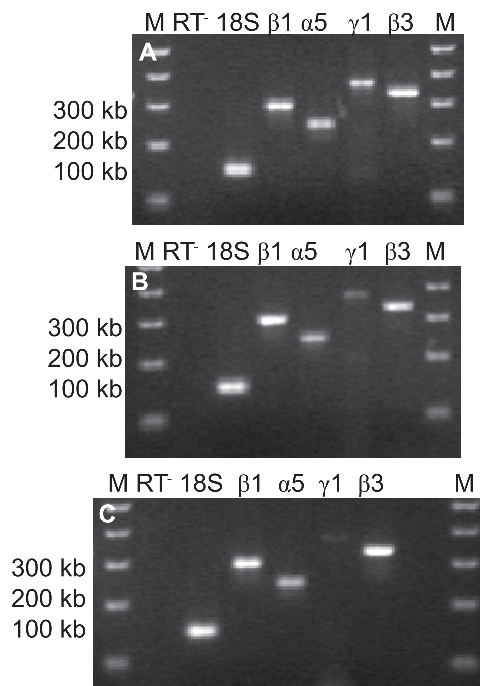

Fig 1: RT-PCR results of $GABA_A$Rs. (A-C) PCR amplification of $GABA_A$R subunits in rat brain, sperm and testis respectively. M: marker; $GABA_A$R subunits are indicated in the figure.

The data suggested that α5, β1α5, β1, β3 and γ1 subunits were always expressed, which provided evidence that those genes existed in rat testis.

Table 1. Primer sequences for real-time PCR of GABA$_A$R subunits

Submit	Direction	Primer sequence 5´-3´	Product size(bp)
α1	Forward	AGCTATACCCCTAACTTAGCCAGG	304
	Reverse	AGAAAGCGATTCTCAGTGCAGAGG	
α2	Forward	ACAAGAAGCCAGAGAACAACAAGCCAG	333
	Reverse	GAGGTCTACTGGTAAGCTCTACCAG	
α3	Forward	CAACATAGTGGAACCACCTATCC	351
	Reverse	GGGGTTTGGGGATTTTGGATGCTTC	
α4	Forward	TGCCAGATCCAGAAGGTGGT	437
	Reverse	AAGATATCAAAACCTCCTCC	
α5	Forward	CAAGAAGGCCTTGGAAGCAGCTAA	338
	Reverse	GGTTTCCTGTCTTACTTTGGAGAG	
α6	Forward	TTCCTGGCTGCAAACTACTCGACA	348
	Reverse	AAGCCCCCGGTAGCAAAGTCAAAA	
β1	Forward	CCTGGAAATCAGGAATGAGACCAG	341
	Reverse	GGAGTCTAAACCGAACCATGAGAC	
β2	Forward	TGAGATGGCCACATCAGAAGC	317
	Reverse	TCATGGGAGGCTGGAGTTTAGTTC	
β3	Forward	GAAATGAATGAGGTTGCAGGCAGC	355
	Reverse	GCAGGGTAATATTTCACTCAG	
γ1	Forward	CAGAGACAGGAAGCTGAAAAGCAA	360
	Reverse	CGAAGTGATTATATTGGACTAAGC	
γ2	Forward	TGTGAGCAACCGGAAACCAAGCAA	374
	Reverse	CGTGTGATTCAGCGAAGACCC	
γ3	Forward	CATCCAGATTCAACAAGATG	255
	Reverse	AGCTCAGACGTCAATG	
δ	Forward	TGAGGAACGCCATTGTCCTCTTCT	333
	Reverse	ACCACCGCACGTGGTACATGTAAA	
ρ1	Forward	GAGAAAATTGACCAGTATA	348
	Reverse	GAGAAGATCTCCTGCACCTGT	
ρ2	Forward	GACAAATTTCCATGCACGTGT	210
	Reverse	GGAAAACACTGACCAATAAAT	
ρ3	Forward	CACATATATACCCCAGTAAA	350
	Reverse	GGAAAGATATCTGGAATGTA	
ε	Forward	CAAGTTAAGGCAAACAAGCCAGT	390
	Reverse	GTGACTTCATGGTCATGCGATTTC	
18S	Forward	GTAACCCGTTGAACCCCATT	150
	Reverse	CCATCCAATCGGTAGTAGCG	

RT-PCR in rat sperm

Because the GABA$_A$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 GABA$_A$Rs 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$_A$Rs have been detected in pig, ram and human sperm membrane preparations by radiolig-

and-binding experiments (12, 13). It is suggested that $GABA_AR$ 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 α5 or β (β1 or β3) 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 $GABA_ARs$ may be involved in important fertilization events, such as depolarization as seen during progesterone-initiated AR capacitating and hyper-activated motility.

We propose that $GABA_ARs$. can be therapeutically targeted for contraception or dysgenesis treatment. Additionally we are awaiting a deeper understanding of the mechanisms and functions of $GABA_AR_S$ in male reproductive tissues. We propose to study the expression of $GABA_ARs$ 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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There is no conflict of interest in this article.

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