

## Expression of Nucleoside Transporters in the Mouse Testis and Ovary

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### ABSTRACT

Nucleoside transporters play key roles in the physiology of nucleosides and nucleobases in mammals and also contribute to the pharmacological actions produced by their analogs. Several mammalian nucleoside transporters have been identified at the molecular level. The aim of this study was to examine the expression of the major nucleoside transporter isoforms in the mouse testis and ovary. Equilibrative Nucleoside Transporter 1 (ENT1) and ENT2 were expressed in the mouse testis and ovary, which was consistent with the general consideration that these transporters are ubiquitous in mouse tissues. The expression of Concentrative Nucleoside Transporter 2 (CNT2) and CNT3, but not CNT1 was detected in the mouse testis. These three CNT isoforms were also expressed in the ovary. Therefore, we concluded that the mouse testis and ovary simultaneously exhibit multiple transport processes for nucleosides and bases and also that these transporters are responsible for the homeostatic control of biological levels of nucleosides and bases in these tissues.

**Keywords:** Nucleoside Transporter, Nucleoside, Nucleobase, Testis, Ovary

### 1. INTRODUCTION

Nucleosides and nucleobases are important precursors for nucleotide synthesis (Kong *et al.*, 2004). Individual nucleosides, such as adenosine and guanosine, also exert a number of specialized functions (Osti *et al.*, 1997; Shryock and Belardinelli, 1997). The therapeutic uses of these compounds as antineoplastic agents in clinical cancer chemotherapy have greatly advanced, because the various structural analogs of nucleosides and bases are cytotoxic (Marce *et al.*, 2006; Nagai *et al.*, 2007a; Marechal *et al.*, 2009). Nucleosides, bases and their analogs require specific transporters for permeation through cell membranes because of their relative high hydrophilicities (Kong *et al.*, 2004).

Five major isoforms of the mammalian nucleoside transporter have been reported (Yao *et al.*, 1997;

Kong *et al.*, 2004; Nagai *et al.*, 2007b). Two genetically distinct Equilibrative Nucleoside Transporters (ENT) were identified, termed ENT1 and ENT2. ENT1 is a 460 residue protein that is predicted to have 11 transmembrane segments, the sequence of approximately 50% of these being identical to that of ENT2. ENT1 was shown to be completely inhibited by 100 nM Nitrobenzylmercaptapurine Riboside (NBMPR), whereas ENT2 was unaffected by exposure to NBMPR at a concentration lower than 1  $\mu$ M. A broad range of natural nucleosides and bases was reported to be transported *via* ENT1 in a Na<sup>+</sup>-independent manner, as well as ENT2. In addition to ENTs, three Na<sup>+</sup>-dependent Concentrative Nucleoside Transporters (CNT), CNT1, CNT2 and CNT3, were cloned. CNT1 and CNT2 preferentially transport pyrimidine nucleosides and purine nucleosides, respectively, whereas CNT3 transports both nucleosides.

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The testis and ovary are essential tissues that are responsible for reproduction. The expression profiles of the Nucleoside Transporter (NT) have so far been reported in various tissues and cells (Kong *et al.*, 2004). Whether various NT isoforms are expressed in testicular tissue remains unclear; however, the expression of CNT1 was shown at the cellular level (Kato *et al.*, 2005). Furthermore, the expression of NT has not yet been investigated in the ovary. In the present study, we examined the expression profiles of NT isoforms in the testis and ovary.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

The total RNAs of the spleen, testis and ovary were obtained from Ambion Inc., (Austin, TX, USA). All other reagents were of commercial or analytical grade and required no further purification.

### 2.2. RT-PCR

RT-PCR analysis was performed according to our previously reported method (Nagai *et al.*, 2003). The brain, small intestine, liver, kidney, lung and skeletal muscle were dissected from ddY male mice aged 5 weeks (Japan SLC Inc., Hamamatsu, Japan). The total RNAs of these tissues were extracted with Sepasol RNA-I super (Nacalai Tesque Inc., Kyoto, Japan) and purified with a GenElute™ Mammalian Total RNA kit (Sigma Chemical Co., St. Louis, MO, USA) according to the manufacturer's instructions. Each total RNA was reverse-transcribed into cDNA by means of Oligo-T priming and Moloney murine leukemia virus reverse transcriptase (Sigma Chemical Co.) and cDNA was then PCR-amplified at 94°C for 30 sec, at 55°C for 30 sec and at 72°C for 60 sec with exTaq DNA polymerase (Takara Bio Inc., Otsu,

Japan). PCR products were then separated by 1.0% agarose gel electrophoresis. The synthetic oligonucleotide primers (Takara Bio Inc.) used to investigate the expression of NT isoforms are listed in **Table 1**. RT-PCR analysis was performed three times in order to confirm the reproducibility of the expression profiles of NT isoforms. The detectable PCR products of the NT isoforms were subcloned into the pGEM-T vector (Promega Co., Madison, WI, USA) and then sequenced.

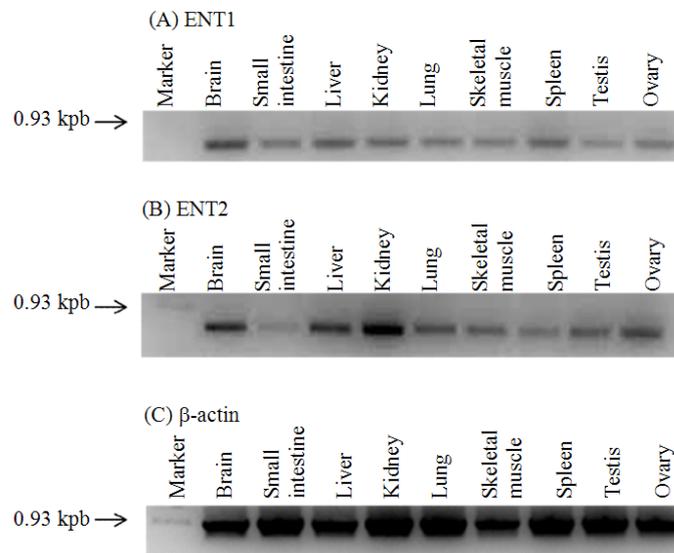
## 3. RESULTS AND DISCUSSION

NT is essential for nucleosides, bases and its analogs to exert their physiological and pharmacological functions in mammals and obtaining information on the detailed expression profiles of NT is of practical importance. Therefore, we examined the expression of the major NT isoforms in the mouse testis and ovary.

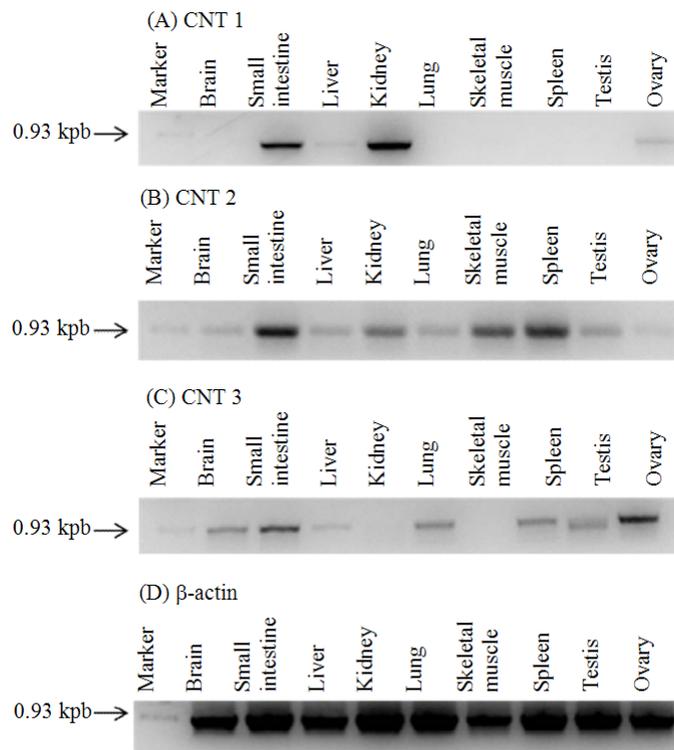
The results of the present study revealed that ENT1 and ENT2 mRNAs were expressed in the mouse testis and ovary, as well as in tissues that had previously been shown to express ENTs (**Fig. 1**). Except for the ovary and testis, the expression profiles of CNTs were consistent with those reported previously (Lu *et al.*, 2004). The expression of CNT1 was not observed in the testis; however, the mRNAs for other two CNT isoforms were detected (**Fig. 2**). Three CNT isoforms were expressed in the mouse ovary. A previous study demonstrated that CNT1 was expressed in rat primary-cultured Sertoli cells (Kato *et al.*, 2005), a finding that was not consistent with our results. Although we cannot clearly explain the reason for this contradiction, it may have been due to differences in the experimental animals used in the respective studies (rat *versus* mouse).

**Table 1.** Forward and reverse oligonucleotide primer sequences used for RT-PCR screening of NT isoforms

Isoform	Direction	Sequence	Product
ENT1	Forward	5'-TGTC C CAG AATGTGTCCTCTCGG-3'	687 bp
	Reverse	5'-TGGGTGGAGAGTTGGGGC-3'	
ENT2	Forward	5'-TCTTTGCCCTGACAGCAGCG-3'	700 bp
	Reverse	5'-CAATCCATGACGTTGAAGAGC-3'	
CNT1	Forward	5'-GCTGAAGAGAGGTCTAGCCC-3'	821 bp
	Reverse	5'-CGACCTTCACTGAGATGGCA-3'	
CNT2	Forward	5'-TTTGGTGATACTGGTCC-3'	957 bp
	Reverse	5'-CCTGACCACAATCTTGAC-3'	
CNT3	Forward	5'-TCTTTGGGGAAAAGTATACAG-3'	959 bp
	Reverse	5'-TCTCATGGCTCCAGAGGCG-3'	



**Fig. 1.** Expression of ENTs in mouse tissues cDNA was PCR-amplified at 94°C for 30 sec, at 55°C for 30 sec and at 72°C for 1 min. PCR products were separated by electrophoresis with a 1.0% agarose gel. The sizes of the PCR products for ENT1 and ENT2 were 687 kb and 700 kb, respectively



**Fig. 2.** Expression of CNTs in mouse tissues cDNA was PCR-amplified at 94°C for 30 sec, at 55°C for 30 sec and at 72°C for 1 min. PCR products were separated by electrophoresis with a 1.0% agarose gel. The sizes of the PCR products for CNT1, CNT2 and CNT3 were 821 kb, 957 kb and 959 kb, respectively

Both the testis and ovary are important endocrine glands necessary for reproduction. The proliferation, differentiation and survival of spermatogenic cells in the mammalian spermatogenic pathway were observed by interacting with Sertoli cells (Russel *et al.*, 1990; Lui *et al.*, 2003). Ovarian folliculogenesis is a complex process, during which follicles undergo tremendous growth and maturation. Beginning as a single layer of pregranulosa cells surrounding the immature oocyte, granulosa cells actively proliferate and differentiate until the time of ovulation (Maruo, 1995). The testis and ovary possess rate-limiting enzymes involved in the purine and pyrimidine salvage pathways, such as hypoxanthine-guanine phosphoribosyltransferase, adenine phosphoribosyltransferase and uridine kinase, in order to biosynthesize DNA and RNA for proliferation and differentiation (Adair *et al.*, 1983; Haugen *et al.*, 1988; Steams *et al.*, 2002). Therefore, these transporters may be responsible for the uptake of nucleosides and bases by spermatogenic cells and ovarian granulosa cells. Furthermore, CNT3 appeared to have been more abundantly expressed in the ovary than in the other tissues. This result suggests that CNT3 makes a significant contribution to the homeostatic maintenance of the biological levels of nucleosides in ovarian cells.

The transporting capacity of NT was previously reported to be a factor determining the cytotoxic behavior of anti-tumor nucleoside and base analogs (Marce *et al.*, 2006; Nagai *et al.*, 2007a; Marechal *et al.*, 2009). Various NT isoforms were found to be expressed in the testis and ovary in the present study. This result strongly suggests that the onset of cytotoxicity also need to be considered in reproductive organs during chemotherapy.

#### 4. CONCLUSION

In the present study, we demonstrated that two ENT isoforms, CNT2 and CNT3, but not CNT1 were expressed in the mouse testis and the expression of all NT isoforms examined was confirmed in the mouse ovary. Such information will enhance understanding of the more detailed physiological and pharmacological roles of NT.

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