THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 16, Issue of April 19, pp. 14211–14215, 2002
Printed in U.S.A.

Expression of L-Histidine Decarboxylase in Mouse Male Germ Cells*

Received for publication, January 23, 2002, and in revised form, February 1, 2002
Published, JBC Papers in Press, February 4, 2002, DOI 10.1074/jbc.M200702200

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Histamine synthesis in male reproductive tissues remains largely unknown. The interaction between stem cell factor and its receptor, c-Kit, has been found to be essential for the maturation of male germ cells and peripheral mast cells. Based on this analogy, we investigated the expression of histidine decarboxylase (HDC), the rate-limiting enzyme of histamine synthesis, in mouse male germ cells. Immunohistochemical analyses revealed that HDC is localized in the acrosomes of spermatids and spermatozoa. In the testis, epididymis, and spermatozoa, a significant amount of histamine and HDC activity were detected. W/W^V mice, known to lack most of their germ cells in the seminiferous tubules, were found to lack HDC protein expression as well as HDC activity in the testis. An *in vitro* acrosome reaction induced by a calcium ionophore, A23187, caused the release of histamine from epididymal spermatozoa. Our observations indicate that histamine is produced in and released from the acrosomes.

L-Histidine decarboxylase (HDC[1]; EC 4.1.1.22) catalyzes the decarboxylation of L-histidine to form histamine. It is the only enzyme that synthesizes histamine in mammals. Histamine is well known to act as an important physiological modulator. Histamine is produced by a variety of cell types such as mast cells, basophils, enterochromaffin-like cells, and neurons (1–3). Among these cells, histamine production in mast cells has been best characterized. We previously demonstrated that the 74-kDa precursor form of HDC is a short lived protein and is degraded via the ubiquitin-proteasome system in the cytosol of a rat basophilic/mast cell line (4). We also revealed that the 74-kDa form is processed post-translationally into its mature 53-kDa form and that histamine is produced in two distinct compartments, the cytosol and the granules of mast cells (5).

The maturation of mast cells in peripheral tissues such as the skin is known to be largely dependent on the presence of fibroblasts (6). The interaction between c-Kit (SCF receptor) of mast cells and the membrane-bound SCF of fibroblasts is required for the maturation of mast cell progenitors *in vitro* (6). The interaction between SCF and c-Kit has also been found to induce histamine synthesis in cultured mast cells (7). Such an interaction between SCF and c-Kit has also been found to occur during spermatogenesis (8, 9). W/W^V mice, which possess a point mutation in the kinase domain of c-Kit, are known to lack peripheral mast cells and a large proportion of their male germ cells (10–12). Spermatozoa are known to contain a granule-like organelle, the acrosome. Fertilizing spermatozoa are believed to undergo an acrosome reaction, the release of a variety of hydrolytic enzymes from the acrosome onto the surface of the zona pellucida before penetrating it (13). The acrosome reaction has been reported to require a massive influx of extracellular calcium (14), which is also known to be essential for the degranulation of mast cells.

We investigated the expression of HDC mRNA in various mouse tissues and found a large amount of mRNA in the testis. This observation led us to the hypothesis that the maturation of male germ cells may be accompanied by histamine synthesis. In this study, we demonstrate the expression of HDC in mouse male germ cells, which may be responsible for histamine production in the acrosomes.

EXPERIMENTAL PROCEDURES

Animals—Adult male ICR mice (9–12 weeks of age), WBB6F$_1$-W/W^V (W/W^V), and WBB6F$_1$-W/W$^+$ (W/W$^+$) mice (8 weeks of age) were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan).

RT-PCR—Various male reproductive tissues and stomach were collected from ICR mice, immediately frozen in liquid nitrogen and stored at −80 °C until use. Total RNAs were extracted with ISOGEN (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions. The reverse transcription reaction was performed using Moloney murine leukemia virus reverse transcriptase (New England Biolabs, Beverly, MA) in the presence of random hexamers. The PCR was performed with *Taq* DNA polymerase (TOYOBO, Tokyo, Japan) using the first strand as a template. The primer pair used for amplification of HDC transcripts were as follows: HDC (forward), 5′-CGC TCC ATT AAG CTG TGG TTT GTG ATT CGG-3′; HDC (reverse), 5′-AGA CTG GCT CCT GGC TGC TTG ATG ATC TTC-3′.

Northern Blot Analysis—Total RNAs (15 μg) from testis, epididymis, and stomach were separated by electrophoresis on a 1.5% agarose gel and transferred onto a nylon membrane (Biodyne-A, Pall, Port Washington, NY). Hybridization was performed with a ^{32}P-labeled cDNA fragment specific for mouse HDC (*Pvu*II-digested fragment) at 65 °C in 6× SSC (1× SSC is composed of 0.15 M NaCl and 0.015 M sodium citrate), 0.5% SDS, and 5× Denhardt's solution. After hybridization, filters were washed at 68 °C in 2× SSC, 1% SDS, and the hybridized bands were detected by autoradiography. The filters were then rehybridized with a ^{32}P-labeled cDNA fragment specific for glyceraldehyde-3-phosphate dehydrogenase (CLONTECH, Palo Alto, CA).

In Situ Hybridization—*In situ* hybridization was performed as described previously (15). Testes collected from ICR mice were immediately frozen. Sections (10 μm in thickness) were cut on a Jung Frigocut 3000E cryostat and thaw-mounted onto poly-L-lysine-coated glass slides. Antisense riboprobes were synthesized by transcription with T3 RNA polymerase (Stratagene, La Jolla, CA) in the presence of [α-^{35}S]CTP. The sections were fixed with 4% formalin and acetylated with 0.25% acetic anhydride. Hybridization was carried out in a buffer containing 50% formamide, 2× SSC, 10 mM Tris-HCl, pH 7.5, 1× Denhardt's solution, 10% dextran sulfate, 0.2% SDS, 100 mM dithio-

* This work was supported by grants-in-aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: HDC, L-histidine decarboxylase; RT, reverse transcriptase; SCF, stem cell factor.

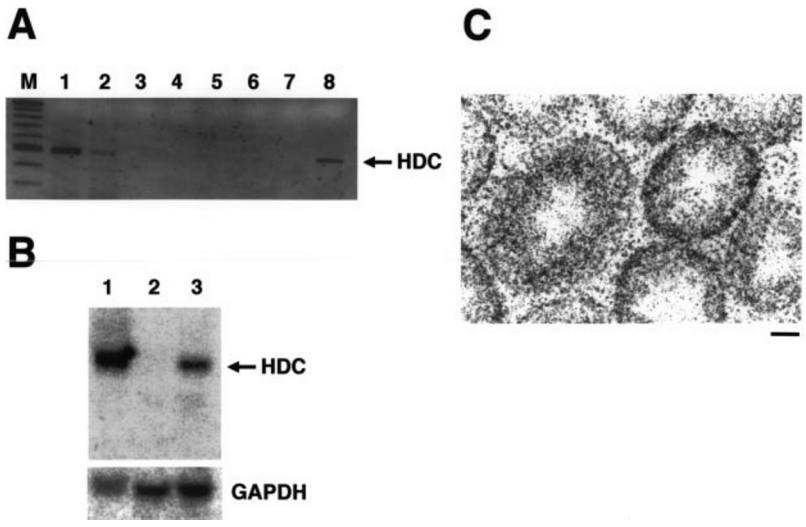

FIG. 1. **Expression of HDC in mouse male reproductive tissues.** *A*, various male reproductive tissues and stomach were collected from ICR mice (10 weeks of age). The RT-PCR was performed as described under "Experimental Procedures." *Lane M*, 100-bp DNA ladder marker; *lane 1*, testis; *lane 2*, epididymis; *lane 3*, vas deferens; *lane 4*, penis; *lane 5*, seminal vesicle; *lane 6*, prostate; *lane 7*, musculus bulbospongiosus; *lane 8*, stomach. The *arrow* indicates the amplified transcripts of HDC (505 bp). *B*, total RNAs (15 μg/lane) were loaded on a 1.5% agarose gel, and Northern blot analysis was performed using ^{32}P-labeled probes (HDC and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*)) as described under "Experimental Procedures." *Lane 1*, testis; *lane 2*, epididymis; *lane 3*, stomach. *C*, the testis of an ICR mouse was collected, and cryostat sections (10 μm in thickness) were prepared. *In situ* hybridization was performed using a ^{35}S-labeled antisense riboprobe as described under "Experimental Procedures." *Bar*, 100 μm.

threitol, 500 μg/ml sheared single-stranded salmon sperm DNA, and 250 μg/ml yeast tRNA. The ^{35}S-labeled riboprobes were added to the hybridization mixture at 1.5×10^5 cpm/μl. After incubation at 60 °C for 5 h, the slides were washed for 1 h in 2 × SSC. The sections were treated with 20 μg/ml ribonuclease A, followed by an additional wash in 0.1× SSC at 60 °C for 1 h. The slides were then dipped in nuclear track emulsion (NTB3; Eastman Kodak Co.). After exposure for 5 weeks at 4 °C, the dipped slides were developed, fixed, and counterstained with hematoxylin and eosin. The specificity of the signals for each probe was verified by its disappearance when an excess amount of unlabeled probe was added (data not shown).

Immunohistochemistry—Testes were collected and treated with Bouin's fixative (Muto Pure Chemicals, Tokyo, Japan) for 24 h at 4 °C. Sections (10 μm in thickness) were cut on a Jung Frigocut 3000E cryostat. The sections were incubated with a rabbit polyclonal antibody raised against glutathione *S*-transferase fusion HDC (16) (1:200) for 1 day at 4 °C. After incubation with biotinylated secondary antibody against rabbit IgG (1:2000, Vector, Burlingame, CA) for 2 h, the antibodies were detected with the avidin-biotin-peroxidase complex (diluted 1:2000; Vector, Burlingame, CA). Development was performed by incubation with 50 mM Tris-HCl, pH 7.6, containing 0.02% 3,3′-diaminobenzidine, 0.0045% H_2O_2, and 0.6% nickel ammonium sulfate for 3 min to obtain brown stained products. The sections were counterstained with methyl green for nuclear staining. For the immunofluorescence study, a rhodamine-conjugated anti-rabbit IgG antibody (1:200) was used as a secondary antibody. The nuclear staining was performed using a SYBR green dye (Molecular Probes, Inc., Eugene, OR). All of the immunoreactive signals obtained using the anti-HDC antibody were undetectable in the presence of an excess amount (10 μg/ml) of the antigen, glutathione *S*-transferase fusion HDC.

Histidine Decarboxylase Assay—Testes, epididymides, and spermatozoa were collected and homogenated in 50 mM HEPES-NaOH, pH 7.3, containing 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5′-phosphate, 0.2 mM phenylmethylsulfonyl fluoride, 0.1 mM benzamidine, 10 μg/ml E-64, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1% Triton X-100. The homogenates were centrifuged at 13,000 × *g* for 15 min at 4 °C, and the resulting supernatants were assayed for histidine decarboxylase activity as described previously (5). The assay mixture (1 ml) was composed of 0.8 μmol L-histidine, 0.2 μmol of dithiothreitol, 0.01 μmol of pyridoxal 5′-phosphate, 10 mg of polyethylene glycol 300, 100 μmol of potassium phosphate, pH 6.8, and the crude extracts. The reaction was performed at 37 °C for 4 h and was terminated by adding 0.04 ml of 60% perchloric acid. The histamine formed was extracted and separated on a cation exchange column, WCX-1 (Shimadzu, Kyoto, Japan) by high pressure liquid chromatography and then measured by the *o*-phtalaldehyde method (17).

Protein Assay—Protein concentrations were determined by the method of Bradford (18) using bovine serum albumin for the standards.

Immunoblot Analysis—Testes, epididymides, and epididymal spermatozoa were homogenized in 99.2 mM NaCl containing 2.68 mM KCl, 0.36 mM NaH_2PO_4, 0.5% Triton X-100, and 0.2 mM phenylmethylsulfonyl fluoride, and crude extracts were prepared by centrifugation at 13,000 × *g* for 10 min at 4 °C. Aliquots were separated by SDS-PAGE (10% slab gel) and electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Tokyo, Japan). Immunoblot analysis was performed as previously described (5). An anti-HDC antibody (1:500) was used as the primary antibody, and a horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:3000; Dako, Glostrup, Denmark) was used as the secondary antibody. The membrane was stained using the ECL kit (Amersham Biosciences) according to the manufacturer's instructions. Partially purified HDC (53 kDa) was prepared from a mouse mastocytoma cell line, P-815, as described previously (19). Sf9 cells expressing recombinant 74-kDa HDC were prepared using a baculovirus-insect cell expression system as described previously (20).

Acrosome Reaction—Spermatozoa were collected from the cauda epididymis in modified fertilization medium reported by Whittingham (21) after 1 h of "swim up" at 37 °C in a fully humidified atmosphere with 5% CO_2 to allow capacitation. Motile spermatozoa (3.5×10^6 cells/ml) were carefully collected and incubated for 1 h at 37 °C in the medium in the presence or absence of 10 μM A23187. The acrosome reaction was confirmed by the loss of Coomassie Brilliant Blue R-250-positive spermatozoa. In our experimental conditions, only a small proportion of spermatozoa underwent acrosome reactions spontaneously ($4.80 \pm 0.374\%$, $n = 5$), whereas a large proportion of spermatozoa did so in the presence of A23187 ($86.4 \pm 1.81\%$, $n = 5$).

RESULTS

Expression of HDC mRNA in Mouse Male Reproductive Tissues—The expression of HDC mRNA in various male reproductive organs of ICR mice was investigated. HDC mRNA expression was detected in the testis and epididymis by RT-PCR analyses. The 2.7-kb transcripts of HDC could only be detected in the testis upon Northern blot analyses (Fig. 1*A*). The amount of HDC mRNA accumulation in the testis was comparable with that in the stomach, which has been reported to exhibit significant levels of HDC activity (22). HDC mRNAs were found to be expressed mainly in the cells inside the seminiferous tubules by *in situ* hybridization (Fig. 1*B*).

Immunohistochemical Analyses with an Anti-HDC Antibody—Immunohistochemical analyses with an anti-HDC anti-

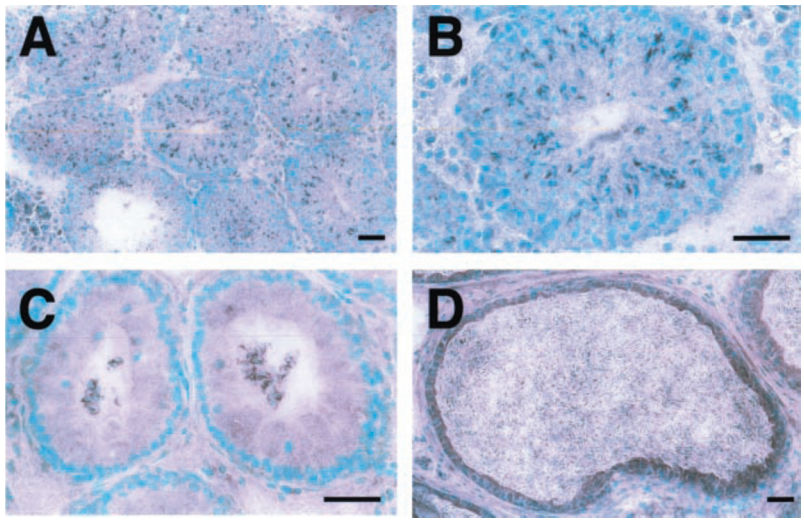

FIG. 2. **Immunohistochemical analysis with an anti-HDC antibody in the testis and epididymis of ICR mice.** Testis (*A* and *B*) and epididymis (caput (*C*) and cauda (*D*)) were collected from ICR mice (10 weeks of age), and cryostat sections (10 μm in thickness) were prepared. The sections were incubated with an anti-HDC antibody (1:200) and stained as described under "Experimental Procedures." The sections were counterstained with methyl green for nuclear staining. *Bar*, 100 μm.

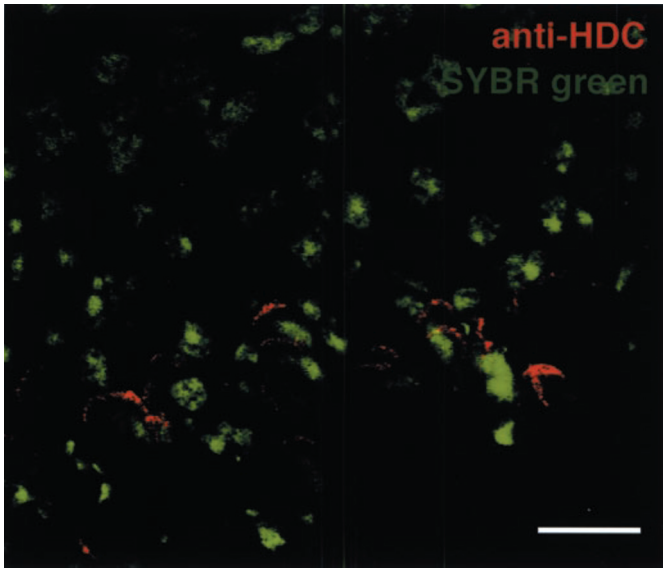

FIG. 3. **Immunofluorescent study using an anti-HDC antibody in the testis of ICR mice.** The preparations of the sections of the testis were performed as described in the legend to Fig. 2. The sections were incubated with an anti-HDC antibody (1:200) and stained with a rhodamine-conjugated anti-rabbit IgG antibody. The nuclear staining was performed with a SYBR green dye. The fluorescent images were obtained using confocal microscopy (MRC-1024; Bio-Rad). *Bar*, 10 μm.

TABLE I
Enzymatic activity of HDC and histamine content in the testis, epididymis, epididymal spermatozoa, and stomach

Testis, epididymis, epididymal spermatozoa, and stomach of ICR mice were homogenized and subjected to the assay for HDC activity and histamine as described under "Experimental Procedures." Data are represented as the means ± S.E. (n = 5).

	HDC activity	Histamine
	fmol/min/mg protein	*pmol/mg protein*
Testis	53.8 ± 3.31	8.00 ± 2.34
Epididymis	9.96 ± 1.82	39.0 ± 4.93
Spermatozoa	46.0 ± 2.44	93.7 ± 6.33
Stomach	5880 ± 487	8030 ± 763

body also demonstrated that the immunoreactive cells were localized in the seminiferous tubules (Fig. 2, *A* and *B*). A majority of immunoreactive signals demonstrated a characteristic shape of the acrosomal cap of elongating spermatids. Small signals were also detected in the round spermatids. Confocal microscopic analysis demonstrated that the immunoreactive signals were localized mainly in the cells that possessed an

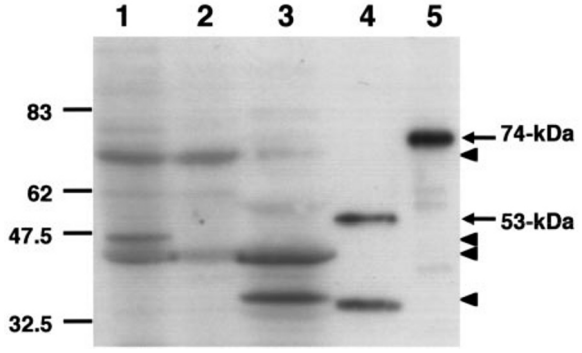

FIG. 4. **Immunoblot analysis using an anti-HDC antibody in the testis, epididymis, and spermatozoa.** Crude protein extracts were prepared from the testis (*lane 1*, 50 μg of protein), epididymis (*lane 2*, 50 μg), and spermatozoa (*lane 3*, 100 μg) of ICR mice and subjected to SDS-PAGE (10% slab gel). Immunoblot analysis was performed using an anti-HDC antibody (1:500) as described under "Experimental Procedures." Partially purified 53-kDa HDC (*lane 4*, 30 ng of protein) from a mouse mastocytoma cell line, P-815, and the crude extracts of SF9 cells expressing mouse full-length HDC cDNA (*lane 5*, 1 μg of protein) were loaded for comparison. The *arrows* indicate 53- and 74-kDa HDC, and the *arrowheads* indicate the multiple forms of HDC.

elongating nucleus (Fig. 3). No immunofluorescence was detected in the cells distributed near the basement membranes of the seminiferous tubules. In the epididymis, the luminal spermatozoa and epithelial cells were also immunoreactive to the anti-HDC antibody (Fig. 2, *C* and *D*).

Enzymatic Activity of HDC and Tissue Histamine Content in the Testis, Epididymis, and Spermatozoa—The enzymatic activity of HDC and tissue histamine content in the testis, epididymis, and spermatozoa obtained form the cauda epididymis were determined. Significant amounts of enzymatic activity and tissue histamine were detected in these tissues and cells, although the levels were much lower than that in the stomach (Table I). Similar levels of specific activity of HDC were detected in the testis and epididymal spermatozoa, but a higher histamine content (more than 10-fold) was observed in the spermatozoa.

Immunoblot Analyses in the Testis, Epididymis, and Spermatozoa—The molecular species of HDC detected in the mouse male reproductive system was different from those observed in a rat mast cell line. Three immunoreactive bands (69, 45, and 39 kDa) were detected in the testis by immunoblot analyses using an anti-HDC antibody (Fig. 4). In the epididymis, 69- and 39-kDa forms were detected, whereas 39- and 35-kDa forms were detected in the epididymal spermatozoa. None of these

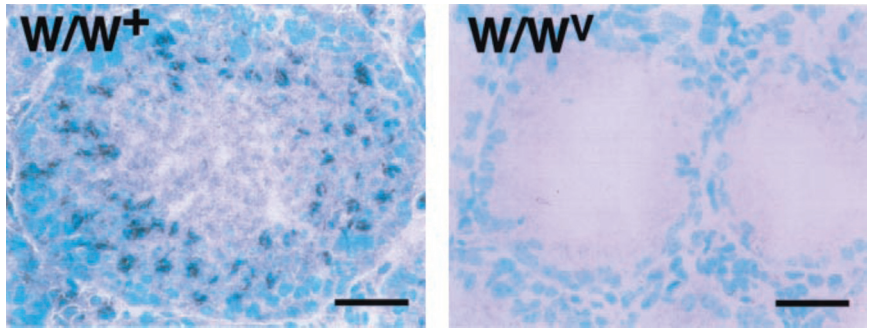

FIG. 5. **Immunohistochemical analysis with an anti-HDC antibody in the testis of W/W$^+$ and W/W^V mice.** The testes of W/W$^+$ and W/W^V mice (9–10 weeks of age) were collected, and immunohistochemical analysis was performed with an anti-HDC antibody (1:200) as described in the legend to Fig. 2. *Bar*, 100 μm.

bands were detected in the presence of an excess amount of the antigen, glutathione *S*-transferase fusion HDC (data not shown).

Absence of HDC in the Seminiferous Tubules of W/W^V Mice—W/W^V mice, which are genetically defective in c-Kit (stem cell factor receptor) function, have been reported to lack a significant proportion of their male germ cells. No immunoreactive cells were observed in the seminiferous tubules of W/W^V mice when immunostained with an anti-HDC antibody. On the other hand, a similar staining pattern to that of ICR mice was observed in the testis of W/W$^+$ mice (Fig. 5). Neither HDC activity nor histamine was detectable in the testis of W/W^V mice. In the epididymis, W/W^V mice showed a much lower HDC activity and histamine content than the W/W$^+$ mice (Table II).

Histamine Release Induced by an in Vitro Acrosome Reaction—In vitro acrosome reactions were performed using a calcium ionophore, A23187. Spermatozoa obtained from the cauda epididymis of ICR mice underwent acrosome reactions upon the addition of A23187. Intracellular histamine was completely lost during the reaction for 1 h and about 25% of the histamine was recovered in the medium (Table III). The addition of a diamine oxidase inhibitor, aminoguanidine, had no effect on the recovery of histamine (data not shown).

TABLE II
HDC activity and histamine content in the testis and epididymis of W/W$^+$ and W/W^v mice

The testes and epididymides of each mouse were homogenized and subjected to the assay for HDC activity and histamine as described under "Experimental Procedures." Data are represented as the means ± S.E. (n = 7).

	HDC activity		Histamine	
	Testis	Epididymis	Testis	Epididymis
	fmol/min/mg protein		*pmol/mg protein*	
W/W$^+$	98.9 ± 10.0	22.9 ± 2.50	9.58 ± 0.880	46.7 ± 3.84
W/W^v	<1.00	2.00 ± 2.00[a]	<0.25	3.33 ± 1.76[a]

[a] $p < 0.01$ by Student's t test.

TABLE III
Histamine release by the acrosome reaction induced by A23187

Spermatozoa were collected from the cauda epididymis in the fertilization medium. The acrosome reaction was induced by the addition of a calcium ionophore, A23187 (10 μM) as described under "Experimental Procedures." Histamine content in the A23187-treated spermatozoa and in the medium was measured. Data are represented as the means ± S.E. (n = 3).

	Histamine	
	None	+A23187
	pmol/10^7 cells	
Spermatozoa	14.3 ± 3.31	<0.500
Medium	<0.500	3.40 ± 0.270

DISCUSSION

The abundant expression of HDC mRNA was demonstrated in the mouse testis by Northern blot analysis. Since the function of histamine in the male reproductive system remains to be clarified, our current studies were performed to analyze the possible functions. HDC mRNA and protein were detected in the cells inside the seminiferous tubules. The most intense immunoreactive signals were found in the acrosomes of elongating spermatids. Since immature acrosomes of the round spermatids were also immunoreactive to the anti-HDC antibody, HDC protein expression in the seminiferous tubules may be synchronized with acrosome development. HDC mRNA was also detected by *in situ* hybridization in the cells located near the basement membranes of the seminiferous tubules, which were not immunoreactive to the anti-HDC antibody. Although we were unable to identify which types of cells near the basement membrane were positive upon *in situ* hybridization, these observations suggest that the expression of HDC in these cells may be regulated at the post-transcriptional level.

W/W^V mice, which possess a point mutation in the kinase domain of c-Kit (SCF receptor), are known to be sterile and to lack a significant portion of their germ cells in the seminiferous tubules (10). Studies with a monoclonal antibody, ACK-2, which blocks binding of SCF to c-Kit, have indicated that the SCF/c-Kit interaction is essential for the proliferation of type A spermatogonia in a normal genetic background (23, 24). W/W^V mice are known to lack spermatocytes, spermatids, and spermatozoa. The absence of the HDC protein in the seminiferous tubules and the lack of enzymatic activity in the testis of W/W^V mice is consistent with the observation that HDC is selectively expressed in the spermatids and spermatozoa. The relationship between membrane-bound SCF expression in Sertoli cells and c-Kit expression in male germ cells (spermatogonia and spermatids) is analogous to that between SCF in fibroblasts and c-Kit in immature mast cells. W/W^V mice are also known to lack peripheral mature mast cells, indicating that the SCF/c-Kit signaling is also essential for the development of peripheral mast cells (10). The treatment of interleukin-3-dependent bone marrow-derived mast cells with SCF has been reported to enhance the synthesis of histamine in these cells (7). The acrosome is believed to be analogous to the lysosome (25) and contains a large array of hydrolyzing enzymes such as acrosin and hyaluronidase (26). We previously demonstrated that HDC is localized in the granular fraction of a rat mast cell line, RBL-2H3 (5). Male germ cells and mast cells may share a common mechanism, a SCF/c-Kit signaling pathway, that may induce the expression and determine the intracellular localization of HDC.

We previously reported that HDC is translated as a 74-kDa precursor and processed into a mature 53-kDa form in a mast cell line (5). The reported molecular mass of purified enzymes from various mammalian tissues has all been 53–55-kDa (19, 20, 27) while the mouse HDC cDNA codes a protein with a predicted molecular mass of 74 kDa (28). Immunoblot analyses demonstrated that different forms of HDC (69, 45, 39, and 35

kDa) are expressed in the male reproductive tissues. We recently generated a mouse strain, genetically lacking the pyridoxal-binding site of HDC, and exhibiting no HDC activity (29, 30). In this strain, no immunoreactive bands were observed in the testis, epididymis, and epididymal spermatozoa (data not shown). Since no splice variants of HDC were detected in the testis by RT-PCR (data not shown), these multiple forms of HDC may be generated by post-translational processing. It is possible that sperm-specific forms of HDC may be produced by a different processing pathway from that in mast cells, since acrosomes are known to contain various specific proteases. A previous study has suggested the existence of a testis-specific form of the c-Kit protein (31). In rat stomach, the existence of multiple forms of HDC have also been reported, where small forms of HDC (40 and 36 kDa) were found to be increased upon the refeeding of fasted rats (32, 33). Since the specific activity of these forms remains to be determined both in the rat stomach and in our study, it is unclear whether these small forms of HDC possess the enzymatic activity. However, since Engel *et al.* (34) have reported that the deletion of the amino-terminal 68 residues and the carboxyl-terminal 20-kDa region has no effect on the K_m value and enzymatic activity of rat HDC, it is possible that the small forms of HDC (39 and 35 kDa) in spermatozoa exhibit enzymatic activity.

In this study, we demonstrated the release of histamine from spermatozoa by an *in vitro* acrosome reaction induced by a calcium ionophore, A23187. Accumulating evidence suggests that fertilizing spermatozoa do not initiate the acrosome reaction *in vivo* until they come into contact with the zona pellucida (14). Although the acrosome reaction induced by a calcium ionophore may be quite different from the zona-induced reaction, it is generally believed that the differences most probably occur during the initial steps of the reaction and not in the downstream cascade of events that occur.

Although a function for histamine in fertilization has not been reported, our results suggest that histamine release may have some function in the fertilization process. We have observed normal fertility in the HDC-deficient mice (29), indicating that histamine production may not be essential in fertility. We are now investigating of male germ cell function and morphology using this mutant mouse strain. To clarify the mechanism as to how histamine modulates the process of fertilization, further experiments, such as *in vitro* fertilization, are surely required.

The recovery of released histamine was quite low in our *in vitro* acrosome reactions. Since the addition of a diamine oxidase inhibitor, aminoguanidine, did not increase the recovery of histamine, the other histamine-metabolizing enzyme, histamine *N*-methyltransferase, may be involved in the degradation of histamine. We have not been able to perform further studies on this point, since we could not obtain a specific inhibitor of histamine *N*-methyltransferase. The expression of diamine oxidase and histamine *N*-methyltransferase in spermatozoa remains unknown.

In summary, we have demonstrated for the first time that there is significant expression of HDC in the spermatids and spermatozoa of male mice and that the release of histamine from spermatozoa can be induced by an *in vitro* acrosome reaction. Our results raise the possibility that histamine may play a role in the mouse reproductive system.

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