Evidence for a histaminergic system in the human testis

The complete lack of information about mast cells as a source of histamine and potential target cells for histamine in human testes prompted us to investigate these issues in testes of fertile and infertile patients using a combination of laser microdissection, reverse transcription-polymerase chain reaction (RT-PCR), and immunohistochemistry. We show for the first time the expression of the rate-limiting enzyme in histamine synthesis—histidine decarboxylase—by human testicular mast cells and the expression of the histamine (H) receptors 1 (H1) and 2 (H2) by germinal, interstitial, and peritubular cells in the testes of fertile and infertile patients. (Fertil Steril® 2005;83:1060–3. ©2005 by American Society for Reproductive Medicine.)

Knowledge about the precise repertoire of the secretory products of testicular mast cells (MCs) and, consequently, their role in testicular physiology and pathology in the human male is very limited. Recent studies have reignited interest in this subject and have linked MCs with male infertility. Thus, fibrotic thickening of the wall of the seminiferous tubules, which is regarded as the hallmark of male infertility (1), coincides with increased numbers of activated MCs (2–4). A causal link may exist via the major MC product tryptase, which is an established factor in fibrosis, and key steps of tryptase action have been identified in the testes of infertile patients (5).

However, human MCs are generally known to secrete a plethora of products. Presently, only tryptase and chymase have been identified in human testicular MCs (2, 4). Thus, it is not known whether human testicular MCs may synthesize and secrete another prototype MC product, namely histamine (H), and whether H receptor-bearing target cells are present in the human testis. It can be deduced from studies with histidine decarboxylase (HDC) knockout mice and from other studies in golden hamsters that H acting via H receptors may be involved in testicular functions in animal species (6, 7). To examine these points in humans, we investigated the expression of the rate-limiting enzyme in H synthesis (HDC) by human testicular MCs and searched for the H receptors 1 (H1) and 2 (H2) in germinal, interstitial, and peritubular regions of the testes of fertile and infertile patients.

Tissue samples were obtained from fertile (n = 3; age: 30, 32, 33; mean: 31.7 years) and infertile patients (n = 8; age: 26, 28, 32, 33, 34, 37, 42, 42; mean: 34.3 years). Patients underwent testicular biopsies; data about the general health status of each individual patient were not recorded. All participants granted written informed consent. The local ethical committee approved the study.

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Tissue sections (5 μm) of human testicular biopsies were transferred to membrane slides (P.A.L.M. Microlaser Technologie, Bernried, Germany) and counterstained with hemalaun. Laser microdissection (LMD) from human testicular biopsies was performed using the focused nitrogen laser of the Robot-MicroBeam (P.A.L.M. Microlaser Technologie) to circumscribe the target from surrounding material [see Frungieri et al. (5) for details]. Microdissected samples were ejected from the object slide directly into the cap of a microcentrifuge tube. In the case of MCs, cells were identified by immunohistochemical staining for tryptase before dissection. Dissected samples from one tissue section were pooled. A ribonucleic acid (RNA) stabilization reagent (50 μL, RNEasy protect minikit; Qiagen, Hilden, Germany) was added into the cap. Finally, the samples were frozen at −70°C until reverse transcription-polymerase chain reaction (RT-PCR) assays were performed.

Total laser-dissected RNA was prepared by using the Purescript kit (Biozym; Hessisch, Oldendorf, Germany). Reverse transcription was then performed by employing random hexamer as well as oligo deoxythymidine (dT) primers, followed by PCR amplification (5). For analysis of the LMD samples, a second PCR amplification step, with nested primers, was used. We designed oligonucleotide primers for the H1 receptor (first primers: 5′-CTACAAGGCCGTACGACA-3′ and 5′-CCTGCTCATCTGTCTTGA-3′; nested primers: 5′-GTGGTGGATCTGTCTTGA-3′ and 5′-GCCTGCATGTGCACAATA-3′), H2 receptor (first primers: 5′-TCTACCGCATGCAAGATC-3′ and 5′-CGAGGCTGATCATGAAGA-3′; nested primers: 5′-TCATCCTCATCACCGTTG-3′ and 5′-TGGTAGATGGCAGAGAAG-3′) and HDC (first primers: 5′-AAGTACAAGCTGCAGCAG-3′ and 5′-CTCTTGGCAGGAATTTCA-3′; nested primers: 5′-GTGTGAATCCCATCTACC-3′ and 5′-GGTCGTTTCTGACCA-

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GAG-3′). Finally, we verified the identity of PCR products by sequencing with a fluorescence-based dideoxy sequencing reaction [see Frungieri et al. (5) for details].

For immunohistochemistry, deparaffinized tissue sections of human testes were treated with 3% H_2O_2 in methanol for 20 minutes to block endogenous peroxidase activity. The sections were then incubated with 5% normal goat serum for 30 minutes to prevent nonspecific antibody binding [see Frungieri et al. (5) for details]. The sections were incubated overnight at 4°C with a monoclonal antihuman MC tryptase antibody (M 7052, dilution 1:50; DAKO, Hamburg, Germany) or a polyclonal antihuman H1 receptor antibody (dilution 1:200; LifeSpan BioSciences, Suffolk, United Kingdom), and were probed with biotin-coupled goat antimouse antibody (1:500) or biotin-coupled goat antirabbit antibody (1:500). The sites of immunoreaction were visualized by the ABC-method (Vectastain Elite Kit; Vector Laboratories, Burlingame, CA) and the addition of 3′3′-diaminobenzidine tetrahydrochloride solution containing H_2O_2. Controls consisted of nonimmune rabbit or mouse serum (1:5,000) or omission of the antibody.

Results obtained in the human testicular specimen allowed us to identify MCs by immunohistochemical staining for tryptase, which is known to be contained in all human MC populations. As pictured in Figure 1A, MCs in infertile patients are located mainly in the interstitial and peritubular regions of the testis, while in fertile men, MCs are mainly located in the interstitial regions and are rarely found in the wall of seminiferous tubules (data not shown; see previous reports, e.g., Meineke et al. [3]). By performing PCR experiments with complementary deoxyribonucleic acid (cDNA) isolated from laser-dissected tryptase positive MCs from the testes of fertile and infertile patients, we found that these cells contained HDC (Fig. 1B). Because HDC is the rate-limiting enzyme in H synthesis, these cells are thus a potential source of H in the human testis.

To investigate whether H receptor-bearing cells are also in close proximity to the peritubular and interstitial testicular MCs, we performed RT-PCR experiments for the H receptors H1 and H2 (i.e., those receptors implicated in testicular functions in rodents), using laser microdissected material from germinal, interstitial, and peritubular regions (Fig. 1C–E) of human testicular specimens derived from fertile and infertile patients (Sertoli cell only [SCO] syndrome, germ cell arrest). As depicted in Figure 1F through Figure 1H, cells derived from germinal, interstitial, and peritubular regions express H1 and H2 receptors, irrespective of the pathological status, and are therefore potential targets for testicular MC-derived H.

Immunohistochemical studies performed with a specific human H1 receptor antibody revealed a consistent expression of the receptor in the germinal epithelium of all sections from a total of 11 patients, irrespective of state of fertility. Moreover, in 45% (5 out of 11) of the specimens derived from fertile and infertile patients, interstitial Leydig cells displayed H1 receptor reactivity. Weak signals for the H1 receptor were also associated with elongated cells in the peritubular region (data not shown).

Our results demonstrate for the first time that human testicular MCs are potential producers of H, and that H1 and H2 receptors are expressed in all compartments of the human testis, regardless of the state of fertility. Thus, in normal circumstances, particularly in cases of MC activation, which includes states of infertility (3), H is likely to interact with multiple testicular target cells.

Besides tryptase, which is present in all human MCs, H is regarded as one of the most important MC products. As described previously (3), tryptase-positive MCs are located in the peritubular as well as in the interstitial regions of the human testes. Laser microdissection in combination with RT-PCR clearly revealed the expression of the rate-limiting enzyme in H synthesis (HDC) by these testicular MCs derived from fertile and infertile patients. Thus, testicular MCs are a very likely source of H in the human testis. At least in animal species, H-producing cells also reside within the germinal epithelium, as Safina et al. (8) found expression of HDC in mouse male germ cells, but whether this is also the case in the human testis remains to be elucidated.

Although virtually nothing is presently known about the function of testicular MCs in the human testis, several contradictory reports have been published about the functions of testicular H and its target cells in animal species. Pap et al. (6) demonstrated that the tissue concentrations of testosterone and several androgenic steroids were elevated in the testes of HDC knockout mice, whereas studies performed with golden hamsters indicated acute stimulation of steroidogenic activity of Leydig cells by H, which can be blocked by the H1 receptor antagonist pyrilamine (7). The H2 receptor antagonist cimetidine, used in the treatment of gastric and duodenal ulcers, was demonstrated to be a testicular toxicant in rats when applied chronically, and Franca et al. (9) proposed testicular smooth muscle cells as H targets. Assuming a histaminergic system in all these rodent species similar to the one proposed by our study in humans, stimulatory acute effects of H on steroidogenesis do not correspond well with the described consequences of chronic lack of H in HDC knockouts. One must bear in mind, however, that in nonorgan-specific HDC knockout mice, systemic or neuronal changes may have an impact on the reproductive system.

Whether the previously mentioned findings implying testicular functions and targets of H are also relevant for the human testis is currently unknown, but nevertheless our study has clearly identified H1 and H2 receptors in the germinal, interstitial, and peritubular region of fertile and infertile patients. Additional studies are required to exam-

FIGURE 1

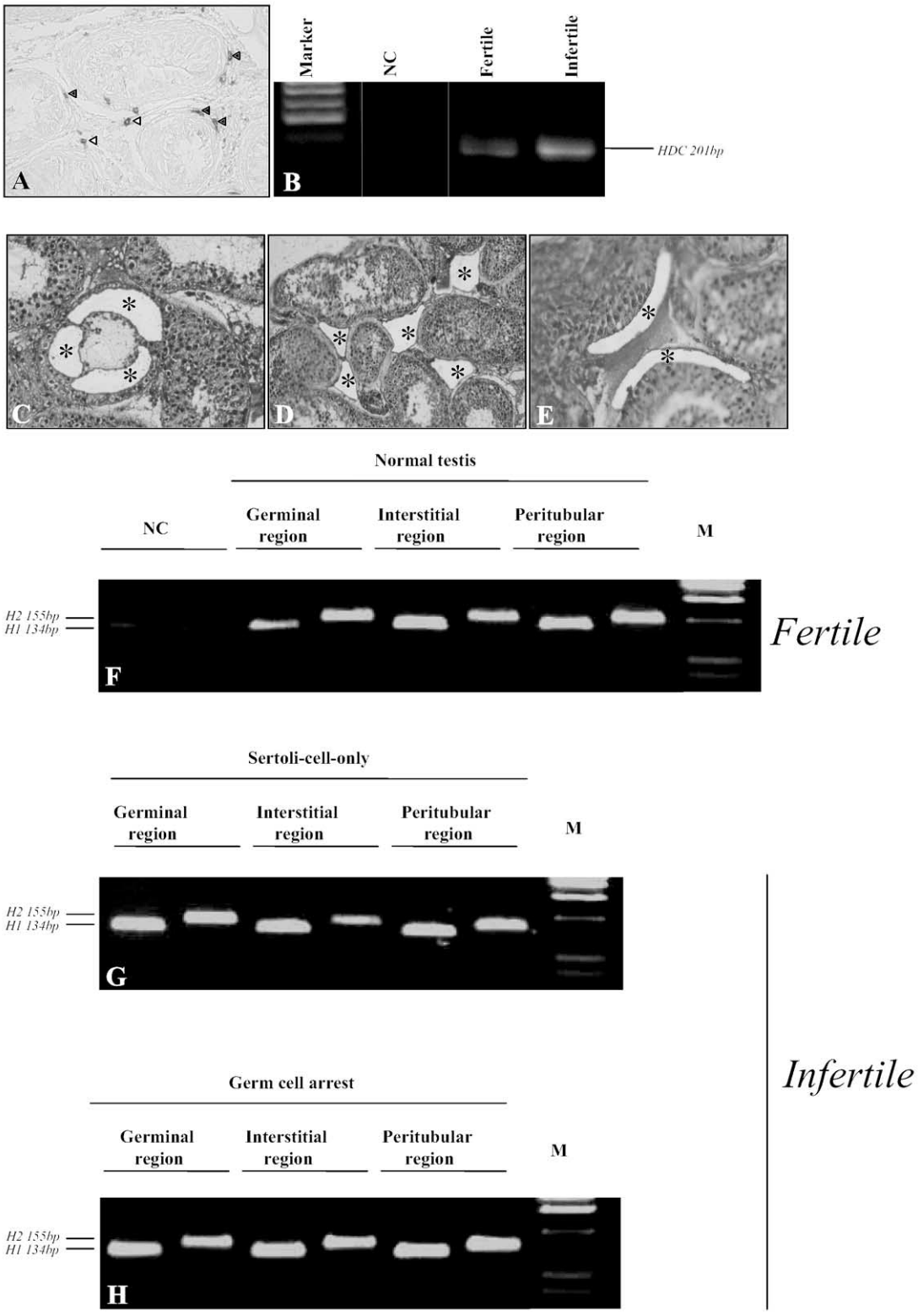

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FIGURE 1 Continued

H-producing and H-receptor-bearing cells are located within the human testis. Immunohistochemistry using an antitryptase antibody was performed to demonstrate the different localizations of tryptase positive MCs in the testis of an infertile patient with SCO (**A**). As previously described (3), tryptase positive MCs are located in the peritubular regions (black arrowheads) as well as in the interstitial regions (open arrowheads). Magnification X 200. Nested RT-PCR performed with HDC specific primers demonstrated the expression of the rate-limiting enzyme in H synthesis by laser microdissected tryptase-positive testicular MCs derived from fertile and infertile patients (**B**). Using laser microdissection, germinal (**C**), interstitial (**D**), and peritubular (**E**) testicular regions of fertile and infertile patients were isolated and subjected to RT-PCR studies using H1 and H2 receptor specific primers. The expression of H1 and H2 receptors is evident in all testicular regions investigated, in the normal (**F**) as well as in the pathologically altered human testis (**G, H**). Regions isolated and subjected to RT-PCR experiments are denoted with an asterisk.

ine the precise nature of the H1/H2 receptor-bearing cells in the human testis. This is important because, aside from the germ cells and endocrine cells of the testis (Leydig cells), connective tissue cells, vascular cells, and immune cells are also known to respond to H. Indeed, H via interaction with connective tissue cells, vascular, and immune cells is a major player in inflammation, allergy, and fibrotic processes. Because activated MCs not only coincide with fibrotic events but also with elevated numbers of several types of immune cells in testes of infertile men (10–13), it is tempting to speculate that MC-derived H may be involved in the pathogenesis of testicular inflammatory processes as well.

In conclusion, H-synthesizing MCs and H receptors are present in the various testicular compartments of the human fertile and infertile male gonad, implying a histaminergic system in the human testis. Additional studies are required to elucidate the physiological significance of this system and to study the possibility that treatment of men with drugs affecting MCs and H receptors may influence normal testicular function.

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