110 nM. ONO-AE1-259, a recently developed EP_2 agonist, selectively binds to this subtype with higher affinity with a K_i value of 3 nM. No significant binding is observed with other EP agonists such as 17-phenyl-PGE_2, sulprostone, M&B-28767, GR-63799X, or 1-OH-PGE_1. PGE_2 and PGE analogs bind to the human EP_2 receptor and mouse EP_2 receptor with a similar rank order of potency (PGE_2 > PGE_1 > 16,16-dimethyl-PGE_2 > 11-deoxy-PGE_1 > butaprost > AH-13205, 19R(OH)-PGE_2>1-OH-PGE_1, M&B-28767>sulprostone=0). PGE_2, 1-OH-PGE_1, AH-13205, and butaprost work as full agonists of the human receptor with EC_{50} values of 43, 2000, 3100, and 5800 nM, respectively.

The EP_2 receptor, like the EP_4 receptor, is coupled to G_s, and the activation of the EP_2 receptor subtype leads to an elevation of cAMP concentration, consistent with its ability to relax smooth muscle in vivo [13,14]. However, these two receptors have been suggested to have differential sensitivities to phosphorylation and desensitization (see below).

2.1.3. EP_3 receptor

In many animal species including the mouse, rat, cow, rabbit, and human, alternative splicing of the EP_3 receptor gene results in several isoforms [15–24]. This alternative splicing generates receptor isoforms which have alternate sequences in the carboxy-terminal region after the seventh transmembrane domain, but have nearly identical ligand-binding properties.

The mouse EP_3 receptor binds most EP ligands. The rank order of affinity for the mouse EP_3 receptor is sulprostone, M&B-28767, PGE_2, PGE_1, 11-deoxy-PGE_1, GR-63799X, 16,16-dimethyl-PGE_2, 17-phenyl-PGE_2 > misoprostol, AH-13205 > 1-OH-PGE_1. Their K_i values are 0.60, 0.68, 0.85, 1.1, 1.5, 1.9, 1.9, 3.7, 67, 82, and 330 nM, respectively (Table 2). Notably, the mouse EP_3 receptor also binds three IP ligands, iloprost, carbacyclin, and isocarbacyclin, and one TP ligand, STA_2, with K_i values comparable to those for their respective receptors. In addition, the mouse EP_3 receptor binds two other IP ligands, beraprost and cicaprost, with K_i values of 110 and 170 nM, respectively. Finally, this receptor binds $PGF_{2\alpha}$, I-BOP, and PGD_2 with K_i values of 75, 100, and 280 nM, respectively. These findings agree well with the reported agonist order of potency of some of these compounds in rabbit cortical collecting tubule cells: PGE_2, PGE_1, 16,16-dimethyl-PGE_2 > carbacyclin, $PGF_{2\alpha}$ > PGD_2 [25]. Although sulprostone, M&B-28767, 16,16-dimethyl-PGE_2, and 11-deoxy-PGE_1 also bind to other receptors, they show the highest affinities for the EP_3 receptor (Table 2). Sulprostone shows affinities for both the EP_1 and FP receptors. M&B-28767, which is known as an EP_1 and EP_3 receptor agonist, also binds to the FP receptor with a K_i value of 124 nM. 16,16-Dimethyl-PGE_2 binds to the EP_2 and EP_4 receptors with relatively high affinities. 11-Deoxy-PGE_1 shows affinities for the EP_2, EP_4, and FP receptors. Misoprostol, known as an EP_2 and EP_3 receptor agonist, shows K_i values of 118, 254, 66.8, and 66.8 nM for the EP_1, EP_2, EP_3, and EP_4 receptors, respectively (Table 2). AH-13205, a known EP_2 agonist, also binds to the EP_3 receptor with a higher affinity. On the other hand, GR-63799X and the recently developed ONO-AE-248 show high affinities only for the EP_3 receptor, indicating the high selectivity of these agonists for this receptor. The human EP_3 receptor binds to PGE_2 and M&B-28767 with K_d and K_i values of 0.7 and 0.2 nM, respectively [15]. Curiously, this receptor also binds to AH-6809 with a calculated K_i of 1.3–4.7 nM. This is in contrast to the mouse EP_3 receptor that does not show binding affinity to AH-6809.

The ligand-binding specificities are almost identical among the EP_3 receptor isoforms. Number of studies have proposed functional differences among these multiple isoforms, although the major signaling pathway of the EP_3 receptor is the inhibition of adenylate cyclase via G_i. These differences among isoforms include the coupling to distinct G proteins [19], different efficacies of G protein coupling [22], different sensitivities to agonist-induced desensitization [20], and different extents of constitutive activity [26–28]. For example, the bovine EP_3A receptor is coupled to G_i and induces the inhibition of adenylate cyclase, whereas the EP_3B and EP_3C receptors are coupled to G_s and act to increase levels of cAMP. The EP_3D receptor is coupled to G_q, in addition to G_i and G_s, and evokes a pertussis toxin-insensitive phosphatidylinositol (PI) response [19]. Moreover, the bovine EP_3C receptor has been found to demonstrate a novel type of receptor–G protein interaction, in addition to the conventional stimulation of G_s. When an agonist is bound to this receptor, the activity of G_o is directly inhibited due to an apparent increase in its affinity for GDP but not for GTP [29]. The finding that the carboxy-terminal tail of a receptor has an important role in determining G protein coupling specificity explains the previously reported multiplicity of signal transduction pathways that operate via the EP_3 receptors [4,30,31]. Such mechanisms where G protein specificity is determined by the carboxy-terminal tail may also be important in the signal transduction with other rhodopsin-type receptors.

2.1.4. EP_4 receptor

The rank order of affinity of the ligands for the mouse EP_4 receptor is PGE_2, PGE_1 > 11-deoxy-PGE_1, 16,16-dimethyl-PGE_2, misoprostol > 1-OH-PGE_1, GR-63799X, M&B-28767 > 17-phenyl-PGE_2. Their K_i values are 1.9, 2.1, 23, 43, 67, 190, 480, 500, and 1000 nM, respectively (Table 2). In addition to these EP ligands, STA_2 binds to this receptor with a K_i value of 350 nM. As for the human EP_4 receptor, only qualitative information based on a radioligand displacement experiment is available [32]. This study shows that the human EP_4 receptor has equal binding affinity for PGE_2 and PGE_1, and a relatively high affinity for M&B-28767; their respective IC_{50} values are ~1 and 9 nM, respectively. Butaprost and AH-6809 bind to this receptor with only weak affinity; their IC_{50} values are >10 μM. Recently, specific agonists and antagonists for the EP_4 receptor, ONO-AE-329 and EP_4A, respectively, were developed [33]. ONO-AE-329 binds to the mouse EP_4 receptor with a K_i value of 10 nM, and EP_4A binds to the human and rat EP_4 receptors with K_i values of 24 and 32 nM, respectively.

Similar to the EP_2 receptor, the EP_4 receptor is coupled to G_s and mediates increases in cAMP levels. However, the EP_4 receptor has a longer carboxyl tail that contains multiple possible phophorylation sites, whereas the EP_2 receptor has a shorter tail. This structural difference may account for the differential properties in agonist-dependent desensitization between these two receptors; the mouse EP_4 receptor showed short-term agonist-induced desensitization, while no such desensitization was found for the mouse EP_2 receptor [34]. Indeed, truncation of the carboxyl tail of the human EP_4 receptor abolished this desensitization, suggesting a role for the cytoplasmic tail of EP_4 in agonist-induced desensitization [35].

2.2. FP and TP receptors

There is no evidence for FP and TP receptors subtypes in any animal species, although two alternatively spliced variants have been described for the ovine FP and human TP receptors

[36,37]. Similar to the EP_3 receptors from a variety of animal species as described above, this alternative splicing occurs in the carboxy-terminal region after the seventh transmembrane domain. The alternative splicing results in the creation of receptor isoforms that differ only in their carboxyl tails, and have almost identical ligand-binding properties. However, the isoforms of the human TP receptor are coupled to different G proteins and differ with respect to internalization and phosphorylation; isoforms of the ovine FP receptor differ with respect to constitutive activity and phosphorylation (see below).

The mouse FP receptor only shows high binding affinity with $PGF_{2\alpha}$ and fluprostenol; their K_i values are 3.4 and 3.7 nM, respectively. Although some prostanoids can cross-react with this receptor, the binding affinities are at least 10-fold lower than the above two compounds, with a rank order of PGD_2, 17-phenyl-PGE_2 > STA2, I-BOP, PGE_2, M&B-28767 > 16,16-dimethyl-PGE_2, sulprostone > U-46619, 19R(OH)-PGE_2. Their K_i values are 47, 60, 97, 100, 100, 124, 350, 580, 1000, and 1000 nM, respectively. It is notable that fluprostenol was only able to bind to the FP receptor, indicating a high selectivity of this ligand. The fact that a variety of non-FP ligands show relatively high binding affinity for the FP receptor indicates that the ligand-binding specificity of the FP receptor is broader than was previously suspected. This is more marked in the human FP receptor than in the mouse, and the human FP receptor shows a similar rank of binding affinity of $PGF_{2\alpha}$, fluprostenol > PGD_2 > PGE_2 > U-46619 > iloprost, with respective calculated K_i values of 2.1, 2.7, 5.4, 65, 112, and 920 nM, respectively [38].

The rank order of affinity of ligands for the mouse TP receptor is I-BOP, S-145 > GR-32191, SQ-29548, STA2 > U-46619. Their K_i values are 0.56, 0.68, 13, 13, 14, and 67 nM, respectively. The rank order and K_i values are consistent with other reports including that of Morinelli et al. [39] describing a rank order of I-BOP > SQ-29548 > STA2 > U-46619, with respective IC_{50} values of 2.2, 4.7, 17, and 62 nM in ligand-binding competition experiments on human platelets. Other ligands known to act on other types of prostanoid receptors have no affinity for the TP receptor, except for M&B-28767, which shows weak binding with a K_i value of 1300 nM. PGD_2- or $PGF_{2\alpha}$-induced bronchoconstriction in humans has been reported to be mediated by the TP receptor [40], and $PGF_{2\alpha}$ and PGE_2 have been shown to play a role in the rat aortic ring contraction via the TP receptor [41]. However, PGD_2, $PGF_{2\alpha}$, and PGE_2 show no affinity for the TP receptor in the mouse, indicating that the TP receptor is quite specific for putative TP ligands. On the other hand, STA_2 binds to the EP_3, EP_2, and EP_4 receptors with K_i values of 23, 220, and 350 nM, respectively, and I-BOP binds to the FP, EP_3, and EP_2 receptors with K_i values of 100, 100, and 220 nM, respectively. Although there have been no reports demonstrating that TP ligands act on these receptors, these results should be taken into consideration when using these compounds.

TP receptors are classically characterized by signaling via G_q. More recently, several other species of G proteins have also been reported to participate in signaling via the TP receptor. Specifically, TP can couple to G_q and an unidentified 85-kDa G protein [42], G_q and G_{i2} [43], and G_{12} and G_{13} [44]. Several pharmacological studies suggest that there is heterogeneity of TP receptors, not only among tissues [1,45] but also within a single cell type, such as in the blood platelet [46,47]. These observations, together with the existence of isoforms of the TP receptor, may account for the multiplicity of signal transduction pathways that are activated via this receptor. It is interesting in this respect that the TP receptor mutant

in human platelets with a point mutation at Arg-60 [48] cannot induce aggregation but can induce shape change in platelets and the activation of phospholipase (PL) A_2 [49]. Hirata et al. [50] examined the signal transduction pathways of the two splice isoforms of TP. They found that both isoforms couple to PLC activation equally well, but have opposite effects on adenylate cyclase; one isoform, TPα, activates adenylate cyclase, whereas the other, TPβ, inhibits adenylate cyclase. The Arg60Leu mutation impairs PLC activation by both isoforms; it impairs adenylate cyclase activation by TPα but the mutational TPβ retains the ability to inhibit the cyclase. On the basis of these findings, Hirata et al. [50] suggested that the pathway linked to adenylate cyclase inhibition, or some other pathway(s) not affected by the Arg60Leu mutation of TP, may be involved in some of TP-mediated platelet responses, such as shape change and PLA_2 activation. Recently, it has also been reported that TPβ is internalized to a much greater extent than TPα in response to agonist exposure [51]. Various dominant negative mutants were used to demonstrate that the internalization of TPβ is dependent on dynamin, G protein receptor coupled kinase, and arrestin in HEK293 cells, suggesting the involvement of receptor phosphorylation and clathrin-coated pits in this process. Thus, alternatively spliced forms of the human TP receptor may differ with respect to phosphorylation, arrestin binding, and receptor internalization. Walsh et al. [52] suggested a physiological role for the alternative splicing of the human TP receptor. They found that TPα, but not TPβ, is subject to cross-desensitization by the IP receptor mediated by direct protein kinase A phosphorylation, and proposed that TPα may be the isoform physiologically relevant to TP:IP-mediated vascular hemostasis.

The cloned FP receptor is also coupled to the activation of PLC via G_q. Functional coupling of the FP receptor with G_q has been shown by an experiment using anti-G_q antibodies [53]. In NIH 3T3 cells, $PGF_{2\alpha}$ induces DNA synthesis through this pathway [54]. Although coupling was not observed with G_i or G_s in FP receptor-transfected CHO cells, $PGF_{2\alpha}$ has been shown to inhibit gonadotropin-stimulated cAMP formation in luteal cells [55]. Pierce et al. [37] identified two alternatively spliced isoforms of the FP receptor, designated FPA and FPB, cloned from a sheep corpus luteum library. Functionally, both isoforms are able to stimulate inositol phosphate accumulation to the same extent, but the basal level of hydrolysis is higher with FPB than with FP_A. In addition, the longer FP_A isoform has four putative protein kinase C (PKC) phophorylation sites, and this isoform is selectively phosphorylated when expressed in cell culture [56]. This differential phosphorylation is suggested to lead to selective desensitization of the FP_A isoform but not the FP_B isoform. Another type of bovine FP receptor isoform, FP_a, has recently been cloned [57]. FP_a is generated by alternative splicing in the middle of the sixth transmembrane domain, resulting in a lack of a seventh transmembrane domain and the carboxyl tail. Cotransfection of an excess amount of FP_a markedly reduces the original FP-mediated PKC response, suggesting that FP_a could play a role as a negative regulator to attenuate normal FP function.

2.3. DP and IP receptors

The existence of subtypes and isoforms of the DP and IP receptors has not been reported. The rank order of affinity of the DP ligands for the mouse DP receptor is PGD_2 > BWA868C, BW245C > STA2. Their K_i values are 21, 220, 250, and 1600 nM, respectively. The cloned human DP receptor has a different order of affinity from the mouse receptor and shows

almost equal ligand binding affinities for PGD_2, BW245C, and BWA868C at 1.1, 0.9, and 1.7 nM, respectively [58]. In spite of this difference in binding affinities between the two species, agonist potencies of BW245C and PGD_2 are almost the same. These agonists both act as full agonists for the DP receptor, and their EC_{50} values for cAMP elevation are 0.54 and 6.8 nM, respectively, for the mouse receptor, and 0.7 and 6 nM, respectively, for the human receptor. The response of a presumed DP receptor antagonist, BWA868C, is limited, indicating that this compound is a partial agonist. BWA868C showed a pK_B of 8.7 for BW245C-induced relaxation in the rabbit jugular vein, which is consistent with its K_i value for the human receptor [59]. The DP receptor, therefore, only binds to its own putative ligands with high affinity. The binding affinities of other prostanoids and their analogs are more than two orders of magnitude lower than these compounds. On the contrary, PGD_2 binds to the mouse FP receptor with an affinity comparable to that for the mouse DP receptor with a K_i value of 47 nM, indicating that PGD_2 may evoke an FP receptor-mediated response. Indeed, PGD_2-induced bronchoconstriction in the anesthetized dog could be mediated via the FP receptor [60].

The rank order of affinity of the ligands for the mouse IP receptor is cicaprost, iloprost, isocarbacyclin > beraprost, PGE_1 > ONO-1301 > carbacyclin > 11-deoxy-PGE_1. Their K_i values are 10, 11, 15, 16, 33, 47, 110, and 1000 nM, respectively. A similar rank order of binding affinity has been reported for the human IP receptor with iloprost >> carbacyclin > PGE_2 >> PGD_2, $PGF_{2\alpha}$, and U-46619 [61,62]. These results are consistent with the rank order of potency of ligands, cicaprost, iloprost > carbacyclin, in platelets from several species [63]. Isocarbacyclin, beraprost, and ONO-1301 also show high affinities for the IP receptor, as previously reported [64–66]. Interestingly, all of the IP ligands used in this study bind to the EP_3 receptor with K_i values ranging from 22 to 740 nM. Among these ligands, iloprost, carbacyclin, and isocarbacyclin show affinities comparable to those for the IP receptor. Thus, it is possible that IP ligands act on the EP_3 receptor. Indeed IP ligands have recently been suggested to cross-react with presynaptic EP_3 receptors in the guinea pig vas deferens [67]. Moreover, carbacyclin has been reported to act on the EP_3 receptor [25]. It is notable that only iloprost is able to bind to the EP_1 receptor; the actions of this compound on the EP_1 receptor have already been reported [68].

Taking advantage of the high homology between the DP and IP receptors, Kobayashi et al. [69,70]employed various chimeric mouse DP/IP receptors and site-directed mutagenesis to examine the regions involved in ligand binding to these receptors. As described above, IP shows high binding affinity to prostacyclin analogs such as iloprost and carbacyclin as well as PGE_1, but not to PGE_2 or other types of prostanoids, whereas DP shows selective binding to the type D PG. Kobayashi et al. indicated that the binding specificity of IP is determined by the recognition of both the ring structure and the side chain configuration of PGs, and suggested that the latter may be primarily recognized by the sixth to seventh transmembrane domain to discriminate a structural difference in the α-side chain between PGE_1 and PGE_2. They further found that the binding domain of IP that recognizes the ring structure of PGs was located in a region from the first transmembrane domain to the first extracellular loop and can accommodate the rings not only of PGI and PGE, but also of PGD. Moreover, subsequent detailed analyses revealed that the region from the second transmembrane domain to the first extracellular loop contained amino acid residue(s) conferring iloprost binding activity and that Ser50 in the first transmembrane domain of the mouse IP contributed to its broad

binding properties. On the other hand, the high affinity for PGD_2 and the strict specificity of ligand binding observed with the mouse DP appeared to be determined by Lys75 and Leu83, respectively, in the second transmembrane domain. Kedzie et al. [71] have also reported a similar line of study, where they also made use of the high degree of homology between the relaxant group of the prostanoid receptors. They introduced point mutations in amino acid residues conserved in the EP_2 and EP_4 receptors but not in the IP receptor and examined the residues conferring responsiveness to IP ligands. A Leu304Tyr mutation in the seventh transmembrane region of the EP_2 receptor was found to enhance the potency of iloprost ~100-fold, to a value almost equal to that of PGE_1. This may be in good agreement with the above proposal by Kobayashi et al. that the sixth and seventh transmembrane regions are responsible for accommodating the α-chain of IP ligands. However, the question still remains as to the mechanism of the selectivity of the IP receptor, since IP can bind to both iloprost and PGE_1 but not PGE_2, while their mutant receptor binds to PGE_2 preferentially over iloprost and PGE_1.

The DP and IP receptors have been known to be coupled to G_s and stimulate adenylate cyclase. However, it has been further clarified that the IP receptor mediates not only cAMP elevation but also a PI response [72]. In several lines of cultured cells, PGI2 has been reported to induce an elevation of free intracellular Ca^{2+} concentration [73,74]. Because the IP receptor-induced PI response in CHO cells is not inhibited by either pertussis toxin or cholera toxin, it is likely that the G_q family of G proteins is participating in this response [72]. In contrast, no PI response was observed during DP receptor signaling [58,75], although stimulation of the human DP receptor expressed in HEK 293 cells induced a transient increase in intracellular free Ca^{2+} concentration possibly via a cAMP system [58].

3. Expression and regulation of the prostanoid receptors

Prostanoid receptors have been shown to be present in many tissues throughout the body, however, information as to the exact expression site and regulation remains mostly unknown due to the relatively low expression levels of these receptors and the expression of multiple receptors in a single cell. Recently, Northern and in situ hybridization techniques have provided valuable insights as to where each receptor is distributed and when the expression levels are altered. Moreover, some researchers have begun to use antibodies raised against prostanoid receptors to analyze receptor at the protein level.

3.1. Kidney

In situ hybridization analyses have revealed the distribution of EP subtype mRNA in the mouse kidney [76,77]. The EP_3 receptor is expressed in the tubular epithelium in the outer medulla, and probably also in the thick ascending limb and cortical collecting ducts, the EP_1 receptor is expressed in the papillary collecting ducts, and the EP_4 receptor is found in the glomerulus. These expression patterns appear to correspond with the PGE_2-mediated regulation of ion transport, water reabsorption, and glomerular filtration, respectively [78]. A similar expression pattern has been reported in human kidney [79]. Interestingly, the expression of EP_3 and EP_4 transcripts could be altered in animals by treatment with either a

high-NaCl diet or salt deprivation [80]. Thus, region- and subtype-specific changes in PGE receptor expression are involved in renal adaptation to changes in dietary salt intake. The FP receptor is found in the distal convoluted tubule in the cortex, suggesting its effects on renal salt transport [81]. The IP and TP receptors are expressed in the afferent arterioles of the glomerulus and the glomerulus, respectively. The activation of these receptors may counteract and have roles in the regulation of glomerular filtration.

3.2. Gastrointestinal tract

A distinct localization of PGE receptor subtypes has also been found in the gastrointestinal tract [82]. EP_1 mRNA is expressed in the smooth muscle of the muscularis mucosa throughout the tract, but not in the proper smooth muscle layer, suggesting that this receptor is involved in the local movement and folding of the mucosa [83]. In contrast, EP_3 mRNA is found in the proper smooth muscle cells of the longitudinal muscle. Moreover, EP_3 mRNA is highly expressed in the neurons of the myenteric ganglia. EP_3 may thus regulate gastrointestinal motility both directly and indirectly through the modulation of the enteric nervous system [84]. The expression of EP_3 mRNA has also been detected in fundic gland epithelial cells, possibly in both the parietal and chief cells. This expression of EP_3 in parietal cells may be responsible for the role of PGE_2 in the inhibition of acid secretion [85]. On the other hand, high expression of EP_4 mRNA is found in the gland of the gastric antrum, indicating that this subtype is involved in PGE_2-mediated mucus secretion [86]. It is interesting that rebamipide, an anti-gastric ulcer agent, has been reported to increase EP_4 transcripts in rat gastric mucosal cells [87]. In addition, EP_4 mRNA is present in the epithelial cells, especially in the upper part of the villi, throughout the intestine. Since EP_4 is coupled to the elevation of cAMP, and PGE_2 stimulates chloride secretion and inhibits NaCl absorption in the intestine through the production of cAMP, EP_4 may be responsible for these two PGE_2-induced processes and also for PGE_2-induced diarrhea [88].

3.3. Nervous system

3.3.1. Brain

Different spatial expression of the prostanoid receptors appears to exist in the nervous system. In situ hybridization [89] and immunohistochemical [90] analyses have revealed that the EP_3 receptor is widely distributed in the neurons of the cortex, hippocampus, thalamus, hypothalamus, midbrain, and lower brain stem. In the hypothalamus, the EP_3 receptor is located in the neurons surrounding the organum vasculosa lamina terminalis (OVLT). The OVLT has been regarded as a key structure with a poor blood–brain barrier. Indeed, COX-2, an inducible form of cyclooxygenase, is induced in this region in response to the peripheral administration of lipopolysaccharide (LPS) [91]. It is likely that the EP_3 receptor in this region is involved in fever generation. The EP_3 receptor is expressed in monoaminergic neurons in the brain, such as in the locus ceruleus (adrenergic) and raphe nuclei (serotonergic), suggesting that this receptor modulates the autoregulation of monoaminergic neurons [76,92]. EP_3 agonists have been reported to depolarize the membrane by cationic conductance, leading to the excitation of dorsal raphe neurons [93]. In contrast, Zhang et al. [94] have reported that EP_4 and EP_2 mRNAs are expressed in small groups of neuronal and

non-neuronal cells. Interestingly, they further reported that some of these expression levels were increased with the peripheral administration of LPS.

In spite of the central actions of PGD_2 in the brain such as in the induction of sleep, little expression was found in the brain of mice as determined by Northern blot analysis [75]. Thus, DP, if present, must be expressed in limited areas or in specific cells in the brain. Oida et al. [95] found by an in situ hybridization study in the mouse brain that DP mRNA is expressed in the leptomeninges, but not in neurons or glia in the brain parenchyma. Functional activity of the DP receptor in the leptomeninges has recently been confirmed by Scammell et al. [96]. They detected intense Fos immunoreactivity in the leptomeninges below the hypothalamus, upon the infusion of PGD_2 into the subarachnoid space below the basal forebrain, which induced sleep. Thus, the DP receptor may indeed mediate a signal in the leptomeninges. Interestingly, Urade et al. [97] indicated that brain-specific PGD synthase is located in the leptomeninges and the choroid plexus. Thus, the enzyme responsible for PGD_2 synthesis and the receptor for this PG are both expressed in the leptomeninges. These results suggest that PGD_2 produced by cells in the leptomeninges acts in an autocrine or paracrine fashion on the cells in the leptomeninges. Interestingly, Matsumura et al. [98] examined the site responsible for the sleep-inducing action of PGD_2 by microinjecting PGD_2 into various parts of the brain and found that sleep is induced more effectively upon injection into the subarachnoid space than that into the brain parenchyma. From these results, they concluded that PGD_2 acts on the ventral surface of the rostal basal forebrain. However, no expression of the DP receptor was found in the parenchyma of the brain in this area. On the other hand, the leptomeninges is markedly thick in this area, with high levels of DP mRNA. Therefore, it is possible that PGD_2 injected into the subarachnoid space of this region works on the DP receptors in the leptomeninges to induce sleep. If this is the case, these findings suggest a new mode of regulation of brain function in which a humoral factor released from the leptomeninges is delivered to the brain and has an influence on its function. The leptomeninges is located in a strategic position between the peripheral circulation and the brain and may mediate the transmission of blood-borne signals such as cytokines to the brain by this mode of regulation.

3.3.2. Sensory neuron

It has been reported that EP_1, EP_3, and EP_4 mRNAs are expressed in the sensory neurons of the dorsal root ganglion (DRG) [89,99]. EP_3 mRNA is found in one-half of the DRG neurons, which are mostly small in size, suggesting the involvement of this receptor in PGE_2-mediated hyperalgesia. Indeed, EP_3 and EP_4 receptors have been implicated in the PGE_2-induced augmentation of the release of substance P and calcitonin gene-related peptide in rat primary neurons of the DRG [100]. Moreover, IP mRNA is also abundantly co-expressed with the mRNA of EP receptor subtypes in some neurons. This may imply that EP and IP play overlapping or different roles in the transmission of pain.

3.4. Immune system

Macrophages produce a large amount of PGE_2 in response to inflammatory stimuli such as LPS. This PGE_2, at least in part, acts on macrophages themselves, causing inhibition of cytokine release. This inhibitory effect of PGE_2 is exerted through the elevation of cAMP,

suggesting the involvement of EP_2 and EP_4 receptors. Arakawa et al. [101] reported that EP_2 and EP_4 receptors were expressed in the murine macrophage-like cell line, RAW 264.7, and that the mRNA levels of these receptors were variable in response to LPS stimulation. In another macrophage-like cell line, J774.1, Katsuyama et al. [102] reported that the EP_2 receptor was effectively induced upon LPS treatment with a time course similar to that of COX-2, and that this induction of EP_2 was completely inhibited by the concomitant administration of interferon-γ. Ikegami et al. [103] also reported that mouse peritoneal macrophages also showed a similar induction of EP_2 mRNA upon LPS treatment. On the other hand, the abundant expression of EP_4 in pre-stimulated cells showed a decrease upon LPS stimulation, which may be mediated by LPS-induced PG-synthesis and EP_4 activation itself. Indeed, C3H/HeJ (LPS-hyporesponsive) macrophages exhibited a reduction in EP_4 expression upon PGE_2 treatment alone, but not upon LPS treatment. In contrast, the up-regulation of EP_2 expression was again observed in LPS-treated C3H/HeJ macrophages. Thus, the expression of EP_2 and EP_4 genes is differentially regulated during macrophage activation and, as proposed for COX-2, EP_2 may serve as an inducible type of PGE receptor in macrophages. As for immune cells other than macrophages, it has recently been observed that murine peritoneal neutrophils also express the transcripts for EP_2 and EP_4, which may differentially modulate cytokine production [104]. However, it remains unknown whether the expression levels of these receptors are variable in neutrophils upon stimulation.

TP mRNA is abundantly expressed in immune-related organs such as the thymus and spleen, as well as in highly vascularized organs, such as the lung, kidney, and heart [105]. Ushikubi et al. [106] examined cells expressing TP in the thymus using ligand binding. TP was found to be expressed in immature thymocytes such as CD^{4-8-} and CD^{4+8+} at a density as high as that in platelets. Although T-cell maturation caused a decrease in the expression of TP, a significant amount of TP expression was still observed in the peripheral T cells. Moreover, a TP receptor agonist was shown to evoke apoptotic cell death of immature thymocytes in a receptor-dependent manner. Thus, the TP receptor could be playing a role in thymocyte differentiation and development, in addition to its well-known roles in the cardiovascular and respiratory systems. In the thymus and spleen, IP mRNA is expressed in mature thymocytes and splenic lymphocytes [99]. However, the function of the IP receptor in these tissues remains unknown.

3.5. Female reproductive system

3.5.1. Ovary

The corpus luteum in the ovary is the tissue in which the expression of FP mRNA is the most abundant, as suggested by earlier ligand binding studies [107,108]. The expression pattern of FP mRNA in luteal cells was investigated in gonadotropin-primed pseudopregnant mice [109]. No expression of FP mRNA was detected in the ovaries of immature female mice treated with pregnant mare serum gonadotropin (PMSG) for 48 h. However, once ovulation was triggered by the treatment of human chorionic gonadotropin (hCG), the granulosa cells in the ruptured follicles initiated the expression of FP mRNA in vivo. The stimulatory effects of hCG and interleukin (IL)-1β, proposed mediators of ovulation, on FP mRNA in human granulosa-luteal cells in vitro have also been reported, providing further evidence that the expression of FP mRNA begins in response to the preovulatory

gonadotropin surge [110,111]. Interestingly, the expression level of FP mRNA increased until the luteal cells underwent apoptosis. Thus, the expression of FP mRNA in the corpora lutea shows variability during the estrous cycle, indicating a close relationship between FP gene expression and luteolysis, one of the most recognized actions of $PGF_{2\alpha}$. The mouse FP mRNA is also expressed in the kidney, heart, lung, and stomach, but expression levels in these tissues show no variability during the estrous cycle. A transgenic mouse study showed that the potential promoter region of the FP gene (up to 7.3 kb upstream from ATG) worked as a promoter for the expression in the kidney and the stomach but not in the ovary [109]. Thus, the control mechanism for luteal FP expression may be distinct from those of other tissues. It should be noted that EP_2 mRNA expression as well as COX-2 expression was also induced by a preovulatory gonadotropin surge in the cumulus cells around the ovum in vivo [112] and by IL-1β in human granulosa-luteal cells in vitro [113]. Such co-regulation of COX-2 and the EP_2 receptor during the ovulatory period suggests that both the synthesis of and sensitivity to the PGs may be under autocrine and/or paracrine control in the ovary.

3.5.2. Uterus

The expression of the EP subtypes has been examined in the uterus of pseudopregnant mice [114]. There appeared to be considerable changes in the abundance and localization of mRNA for EP_2, EP_3, and EP_4 when mice were treated with PMSG and hCG to induce pseudopregnancy. The expression of EP_2 mRNA is hardly detectable before stimulation but is induced in luminal epithelial cells upon stimulation. This expression peaks on day 5 of pseudopregnancy and disappears quickly thereafter. Because EP_2 mRNA induction is in parallel with that of COX-2 in this tissue, and coincides with the period of blastocyst implantation, a process sensitive to indomethacin treatment, it is suggested that EP_2 might be involved in the implantation process. EP_4 mRNA expression shows a sharp increase on day 3 of pseudopregnancy and is maintained at high levels after day 5. EP_4 mRNA expression is limited to luminal epithelial cells at day 0 but is also expressed in endometrial stromal cells and the glandular epithelium after induction. PGE_2 has been shown to cause a phenotypic change in decidualization via elevation of cAMP levels, suggesting that EP_4 mRNA may be involved in the decidualization process. Indeed, stromal EP_4 mRNA is expressed at an abnormally low level in mice deficient in Hoxa-10, which show a phenotype of defective decidualization [115]. In contrast to the EP_2 and EP_4 receptors expressed in the endometrium, EP_3 mRNA is found in the smooth muscle layers. EP_3 mRNA expression is low on day 1, increases up to day 5, and then declines. The cellular localization of EP_3 mRNA changes during pseudopregnancy; it is expressed in the longitudinal muscle layer before stimulation, but in the circular smooth muscle layer on day 5. EP_3-mediated longitudinal and circular smooth muscle contraction might contribute to sperm transport and the retention and spacing of embryos, respectively.

The expression patterns for uterine EP_3 and EP_4 mRNAs in the implantation period are reported to be similar to those in ovariectomized mice with combined treatment of progesterone and estradiol [116]. Thus, EP_3 and EP_4 genes may be primarily under the control of ovarian steroids.

Recent studies suggest that the expression levels of prostanoid receptor mRNA in the myometrium change around the time of parturition. A large amount of PGs, especially PGE_2 and $PGF_{2\alpha}$, are produced and released in uterine tissues in late pregnancy and these PGs

have potent uterotonic activities, suggesting that PGs are implicated in uterine contraction during parturition [117]. EP_3, EP_4, and FP mRNAs in sheep myometrium [118] and FP mRNA in rat myometrium [119,120] have been shown to increase upon spontaneous term labor. Indeed, ligand-binding studies have suggested that the number of PGE_2 and $PGF_{2\alpha}$ receptors in the rat myometrium increase around the time of parturition [121]. It is likely that such up-regulation of PG receptors potentiates the uterotonic action of the PGs and thus play a role in the initiation and progression of parturition. Overall, the analyses of the uterine expression of the prostanoid receptors provide an interesting example of prostanoid receptor induction under physiological conditions.

4. Perspectives

The complex biological actions of the prostanoids may be at least partly due to the existence of multiple subtypes and isoforms of prostanoid receptors. Molecular cloning of the individual receptor subtypes and subsequent elucidation of their biochemical properties and expression patterns have been a pivotal step in our understanding of prostanoid physiology. We are now able to adequately evaluate our experimental results related to prostanoid actions, based on our knowledge of the cellular distribution and the biochemical properties of each receptor subtype. The importance of the activity of each prostanoid has also been assessed by the analyses of phenotypes of knockout mice for each receptor subtype, under various physiological and pathophysiological settings. The insights obtained by this powerful approach is reviewed in another article in this issue. The cloned receptors are now also promoting the development of selective agonists and antagonists by enabling the use of advanced mass screening systems. Specific information on the structure–function relationship and on the signal transduction pathway of each receptor subtype will contribute to the development of potential therapeutic compounds. Such analyses of the receptor molecules should lead to further insight into the use of prostanoid receptors as new targets for drug therapy in the near future.

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

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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-

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

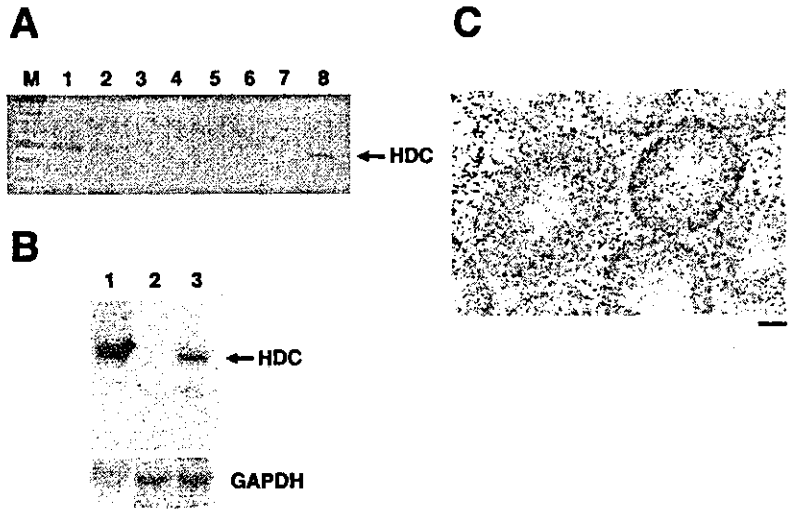

FIG. 1. **Expression of HDC in mouse male reproductive tissues.** *A*, various male reproductive tissues and stomach were collected from ICR mice (10 weeks of age). The RT-PCR was performed as described under "Experimental Procedures." *Lane M*, 100-bp DNA ladder marker; *lane 1*, testis; *lane 2*, epididymis; *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 ± 0.374%, $n = 5$), whereas a large proportion of spermatozoa did so in the presence of A23187 (86.4 ± 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-

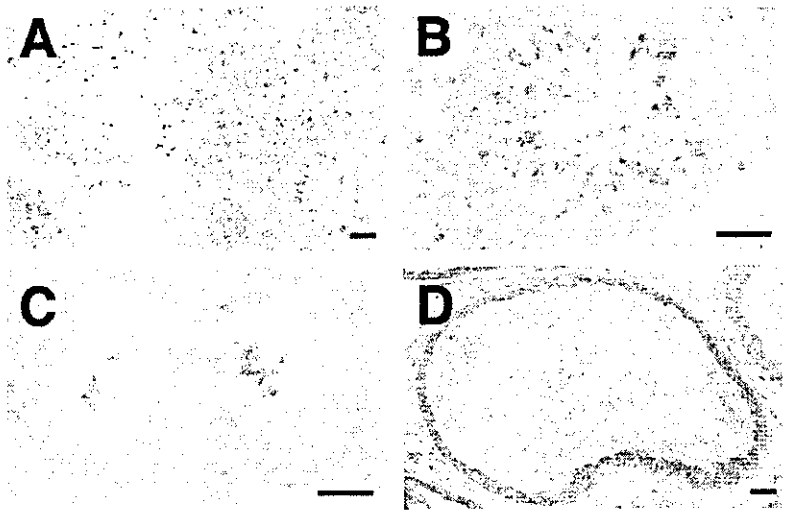

FIG. 2. **Immunohistochemical analysis with an anti-HDC antibody in the testis and epididymis of ICR mice.** Testis (*A* and *B*) and epididymis (caput (*C*) and cauda (*D*)) were collected from ICR mice (10 weeks of age), and cryostat sections (10 μm in thickness) were prepared. The sections were incubated 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.

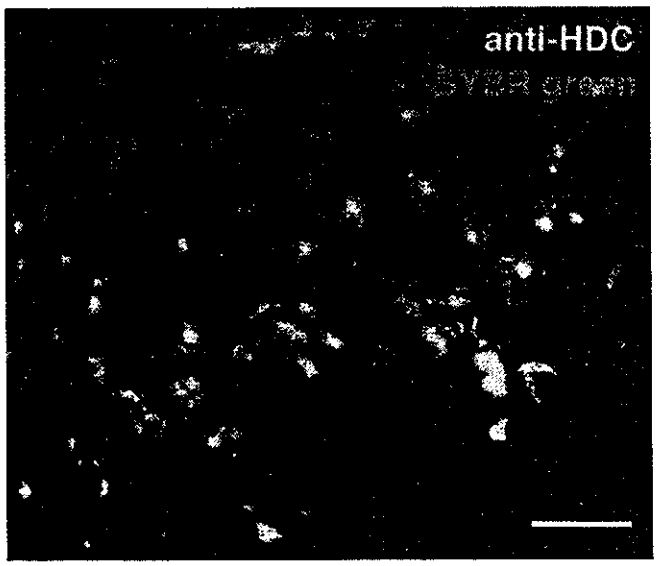

FIG. 3. **Immunofluorescent study using an anti-HDC antibody in the testis of ICR mice.** The preparations of the sections of the testis were performed as described in the legend to Fig. 2. The sections were incubated with an anti-HDC antibody (1:200) and stained with a rhodamine-conjugated anti-rabbit 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 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*).

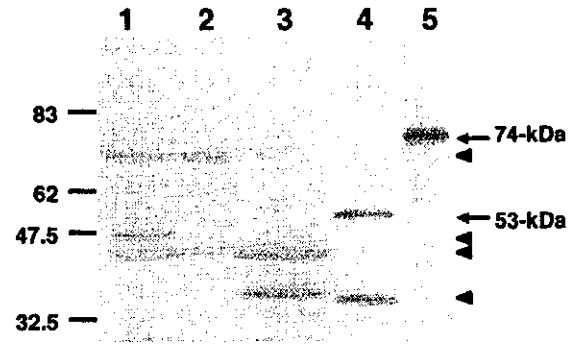

FIG. 4. **Immunoblot analysis using an anti-HDC antibody in the testis, epididymis, and spermatozoa.** Crude protein extracts were prepared from the testis (*lane 1*, 50 μg of protein), epididymis (*lane 2*, 50 μg), and spermatozoa (*lane 3*, 100 μg) of ICR mice and subjected to SDS-PAGE (10% slab gel). 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.

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