Histamine \(H_1\) and \(H_2\) Receptor Gene and Protein Levels Are Differentially Expressed in the Hearts of Rodents and Humans


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ABSTRACT

Histamine is highly concentrated in the heart of animals and humans. Excessive release in pathophysiological conditions, such as immediate hypersensitivity and septic shock, causes cardiac dysfunction and arrhythmias. Previous pharmacological studies revealed that \(H_1\) and \(H_2\) receptors mediate these effects. Yet, an accurate estimate of the distribution and molecular characteristics of cardiac histamine receptors is missing. Recently, the genes encoding \(H_1\) and \(H_2\) receptors have been cloned, and the amino acid sequence and protein structure have been elucidated. Accordingly, we analyzed gene and protein expression levels of \(H_1\) and \(H_2\) receptors in atria and ventricles of guinea pig, rabbit, rat, and human hearts. With immunocytochemical techniques, we examined the regional expression of \(H_1\) and \(H_2\) receptor proteins in the sinoatrial and atrioventricular nodes and surrounding myocardium of the guinea pig heart. Northern and Western blot studies revealed that cardiac histamine \(H_1\) and \(H_2\) receptors are variably distributed among different mammalian species and different regions of the heart, whereas \(H_2\) receptors are abundantly expressed in human atrial and ventricular myocardium. These findings agree with those of previous pharmacological studies, clearly demonstrating that the responses of the heart to histamine depend on the expression level of \(H_1\) and \(H_2\) receptors. The highly abundant expression of \(H_2\) receptors in the human heart substantiates histamine arrhythmogenicity in various disease states. The new knowledge of a differential distribution of histamine receptor subtypes in the human heart will foster a better understanding of histamine roles in cardiovascular pathophysiology and may contribute to new therapeutic approaches to histamine-induced cardiac dysfunctions.

The actions of histamine as a cardiac stimulant have been appreciated for more than 90 years since the seminal work of Dale and Laidlaw (1910). The primary and direct actions of histamine in the heart are characterized by an increase in sinus rate and ventricular automaticity, a decrease in AV conduction velocity, and an increase in force of contraction (Levi et al., 1991; Hattori, 1999). Because histamine is present in high concentrations in cardiac tissues in most animal species, including humans (Bristow et al., 1982; Wolff and Levi, 1986), its release from cardiac stores and its subsequent actions on the heart may be of importance in pathological conditions associated with histamine release (Levi, 1988). In this context, we recently reported that superinduction of histamine receptor gene products and histamine overproduction contribute to the hemodynamic derangement characteristic of septic shock (Matsuda et al., 2002). The negative dromotropic and positive chronotropic effects of histamine invariably result from the activation of \(H_1\) and \(H_2\) receptors, respectively (Levi et al., 1991; Hattori, 1999). In contrast, there is a marked species difference in the subtype of histamine receptors mediating the positive inotropic effect. Moreover, different receptor subtypes mediate the inotropic effect in different parts of the heart within the same species. In the guinea pig heart, only \(H_1\) receptors are responsible for the positive inotropic effect of histamine in left atrium, whereas \(H_2\) receptors predominantly mediate its positive inotropic effect in ventricle (Steinberg and Holland, 1975; Verma and McNeill, 1977; Hattori et al., 1994). In contrast, in the rabbit heart, \(H_2\) receptors mediate the posi-
tive inotropic effect of histamine in left atrium, whereas H₁ receptors are predominantly involved in the ventricle (Hattori et al., 1988, 1990, 1994). On the other hand, the positive inotropic effect of histamine in the human heart, whether in the atria or in the ventricle, is invariably blocked by cimetidine, and not by pyrilamine, indicating an exclusive H₂ receptor involvement (Eckel et al., 1982; Du et al., 1993). In yet another mode, a positive inotropic response in the rat occurs only with very high concentrations of histamine and is blocked by propranolol or reserpine pretreatment, suggesting the involvement of adrenergic mechanisms (Laheer and McNeill, 1980).

Whether such species differences result from a different distribution of H₁ and H₂ receptors remains an open question. To date, the distribution of histamine receptors in different mammalian tissues has been studied with selective radioligands for each receptor subtype. Yet, these studies have been hampered by high nonspecific binding and/or significant binding to secondary nonhistamine-receptor sites (Chang et al., 1979; Hill and Young, 1980; Foreman et al., 1985; Rising and Norris, 1985; Liu et al., 1992). Thus, many of the radioligands used to label H₁ and H₂ receptors seem to be of limited value for an accurate estimate of the distribution and molecular characteristics of cardiac histamine receptors (Hattori et al., 1991, 1994).

In recent years, the genes encoding each of the H₁ and H₂ receptor subtypes have been cloned (Gantz et al., 1991b; Yamashita et al., 1991a; Fujimoto et al., 1993; Horio et al., 1993; Traiffort et al., 1995), and the amino acid sequence and structure of their proteins have been elucidated. Thus, the application of molecular biology techniques to the study of histamine receptor subtypes has enabled us to assess by Northern and Western blotting the expression of their mRNAs and proteins in the heart. In the present study, we analyzed the gene and protein expression levels of H₁ and H₂ receptors in atria and ventricles from guinea pig, rabbit, rat, and human hearts. Furthermore, we used immunocytochemical techniques to examine the regional expression of H₁ and H₂ receptor proteins in the sinus node, AV conduction system, and surrounding myocardium of the guinea pig heart.

We report evidence from Northern and Western blot studies that cardiac histamine H₁ and H₂ receptors are variably distributed not only among different mammalian species but also in different regions of the heart and that H₂ receptors are abundantly expressed in human atrial and ventricular myocardium.

**Materials and Methods**

**Animal and Human Tissue.** Male Hartley guinea pigs (250–350 g), New Zealand White rabbits (2.2–2.5 kg), and Wistar rats (180–220 g) were used in these experiments. The animals were killed by exsanguination under anesthesia with an overdose of pentobarbital (guinea pigs and rabbits) or gaseous diethyl ether (rats), and the hearts and brains were harvested. All animal procedures were reviewed and approved by the Hokkaido University School of Medicine Animal Care and Use Committee.

Right atrial tissue was obtained from patients undergoing aortic-coronary bypass surgery; these patients had no heart failure, or only a moderate form of it, as measured by echocardiography. Also, portions of left ventricle were excised from patients with moderate heart failure during surgical resection of left ventricular aneurysms. The apex of the left temporal lobe of the brain was taken from patients undergoing internal defibrillation after subarachnoid hemorrhage with severe cerebral edema. Written informed consent was obtained for use of organs in research from patients undergoing cardiac surgery, or in the case of neurosurgery, from their relatives.

**Northern Blot Analysis.** Total RNA was extracted from tissues by the guanidinium thiocyanate-phenol-chloroform method with Iso- gen (Nippon Gene, Toyama, Japan) used routinely in our laboratory (Matsuda et al., 1999). RNA purity was determined by the ratio of optical density measured at 260 and 280 nm (OD₂₆₀/OD₂₈₀), and RNA quantity was estimated at OD₂₆₀.

RNA (30 μg/lane) was subjected to electrophoresis on agarose/formaldehyde gels and then transferred to a Hybond-N nylon membrane (Amersham Biosciences UK, Ltd., Little Chalfont, Buckinghamshire, UK). The membrane was prehydrized in prewarmed rate-enhanced hybridization buffer (Rapid-hyb buffer; Amersham Biosciences UK, Ltd.) at 65°C for 60 min. The amino acid sequence of the human histamine H₁ receptor displays an 81 and 75% homology with the rat and guinea pig receptors, respectively. The comparision of the amino acid sequence of the human histamine H₂ receptor with that of the rat and guinea pig H₁ receptors revealed an overall identity of 85 and 86%, respectively. The amino acid sequence of the rabbit H₁ and H₂ receptors has not been yet reported, but their amino sequences would be predicted to show high homology with those of other rodents. We thus used guinea pig H₁ receptor cDNA (Horio et al., 1993) and the rat H₁ receptor cDNA (Fujimoto et al., 1993), both of which were kindly provided by Prof. E. Senba (Department of Anatomy, Wakayama Medical College, Wakayama, Japan). The human H₂ receptor cDNA (accession no. NM_022304) was isolated from gel after electrophoretic separation of the products that were amplified by the PCR using two oligodeoxynucleotide primers (sense, 5'-CTCTACCGCATGCAAGATCA-3' and antisense, 5'-C CCCAGGTGGATAGACAGAAA-3') (Matsuda et al., 2002). The cDNA probes were radiolabeled using a random primer labeling system (Rediprime; Amersham Biosciences UK, Ltd.) in the presence of [³²P]dCTP (6000 Ci/mmol; Amersham Biosciences). The blot was washed in 2× standard saline citrate/0.1% SDS at room temperature for 10 min and in 1× standard saline citrate/0.1% SDS twice at 65°C for 5 min. The histamine H₁ or H₂ receptor mRNA was quantified by counting the radioactivity using a bioimaging analyzer (Fujix BAS 2000; Fuji Photo Film, Tokyo, Japan), as described previously (Mat- suda et al., 1999). Ethidium bromide staining was used as a control to verify gel loading, and the expression of H₁ or H₂ receptor mRNA was normalized as the ratio of the H₁ or H₂ receptor mRNA over 28S ribosomal RNA.

**Antibody Specificity to Histamine Receptors.** The coding regions of the human H₁ receptor gene and of the human H₂ receptor gene were subcloned into a plBluescript SK+ vector (Stratagene, La Jolla, CA). Then, nucleotide sequences that encoded 10-amino acid peptides (EQKLISEEDL) of the human c-myc epitope were inserted between the amino-terminal initiator methionine and the second amino acid of the human H₁ or H₂ receptor by PCR. PCR was carried out with the following primers using plBluescript vectors containing the human H₁ or H₂ receptor gene as template. Primary pairs (forward, 5'-GCCGCCGGATCCATGAAGAGACCATGCTA AGCCTTCCCAATTTCTGCGCC-3'; and reverse, 5'-GCGGGCGCTTA ACCTCGCTTATCAGCTTCTAAGA-3') were used for the H₁ receptor. Primary pairs (forward, 5'-GCCGCCGGATCCATGAAGAGACCATGCTA AGCCTTCCCAATTTCTGCGCC-3'; and reverse, 5'-GCGGGCGCTTA ACCTCGCTTATCAGCTTCTAAGA-3') were used for the H₂ receptor. The resulting PCR products were digested and ligated into the plBluescript vectors. The entire coding region, including the epitope tag, was sequenced and transferred into a mammalian expression vector, pDKC-R-dhfr. CHO cells deficient in dihydrofolate reductase were transfected with the plasmid constructs using the calcium phosphate precipitation method (Fujimoto et al., 1993). Cells were cultured in a-minimum essential medium without ribonucleosides and deoxyribonucleosides (Invitrogen, Carlsbad, CA, USA) with.
10% fetal calf serum. After 2 weeks of incubation, individual colonies were transferred to new plates.

Viewed by immunostaining, CHO cells were confirmed as being successfully transfected with the plasmid-containing the H1 or H2 receptor by the expression of c-myc. Transfected CHO cells expressing the H1 and H2 receptor proteins were stained with anti-human H1 and H2 receptor rabbit polyclonal antibody (Chemicon International, Temecula, CA) used in this study. However, control cells that were subjected to the transfection procedure with no plasmid present showed no reactivity with these antibodies. The specificity of the histamine receptor antibodies was also certified in transfected cells by Western blot (Fig. 1). Therefore, these data confirmed that anti-histamine receptor antibodies was also certified in transfected cells showed no reactivity with these antibodies. Western Blot Analysis. Samples of tissue homogenate (20 μg) were run on SDS-polyacrylamide gel electrophoresis (14% polyacrylamide gel), and electrotransferred to a polyvinylidene difluoride filter membrane. To reduce nonspecific binding, the membrane was preincubated for 60 min at room temperature in PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, and 1.5 mM KH2PO4) containing 1% bovine serum albumin. The membranes were then incubated overnight 4°C with anti-histamine receptor antibody at 1:500 dilution in PBS (10 μg/ml). After extensive washing with PBS 0.05% Tween 20, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit antibody (1:6000 dilution; Bio-Rad Laboratories, Hercules, CA) for 60 min at room temperature. The bands were washed twice in PBS-Tween buffer and subsequently visualized with an enhanced chemiluminescence detection system (Amersham Biosciences), exposed to X-ray film, and analyzed by NIH Image Software produced by Wayne Rasband (National Institutes of Health, Bethesda, MD). To check for protein loading/transfer variations, all blots were stained with Ponceau red (washable, before incubation with antibodies) and with Coomassie Brilliant Blue (permanent, after the enhanced chemiluminescence detection system). Intensity of total protein bands per lane was evaluated by densitometry. Loading/transfer variation between samples was negligible.

Immunohistochemistry. For immunohistochemical determination of histamine receptors, tissue specimens were fixed in 10% buffered-formalin solution, dehydrated, and then embedded in paraffin. The preparations were cut in 5-μm sections, deparaffinized, and treated for 10 min with citrate buffer (10 mM citric acid; pH 6.0) in a microwave oven (750 W) before immunostaining. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide for 7 min. After incubation with primary antibodies overnight at 4°C, the sections were washed with PBS and exposed to DAKO EnVision, Peroxidase, Rabbit (DAKO, Carpinteria, CA) for 30 min at room temperature. Slides were rinsed in PBS, incubated with DAKO Liquid Liquid DAB Large Volume Substrate-Chromogen System (DAKO), rinsed gently with distilled water, and counterstained. Coverslips were mounted with malinol (Muto Pure Chemicals, Tokyo, Japan). The specificity of immunoreactivity was confirmed by negative controls in which nonimmune IgG was used instead of the primary antibodies.

Results

Histamine H1 and H2 Receptor Gene Expression.

Typical Northern blots for detection of the H1 receptor mRNA in cerebral and myocardial tissues from guinea pigs, rabbits, rats, and human are shown in Fig. 2. The hybridization with the H1 receptor probes revealed two mRNAs for the H1 receptor at 3.3 and 3.9 kb. In the guinea pig, the H1 receptor transcripts were abundantly expressed in atrial tissue (Figs. 2A and 2D). Thus, the H1 receptor mRNA levels in atrium were 86 to 94% of the cerebral level. Guinea pig ventricular H1 receptor mRNA expression was analyzed in the atrium and ventricle of the guinea pig (A), rabbit (B), rat (C), and human (D) (representative Northern blots). H1 receptor mRNA was detected as two major bands of 3.3 and 3.9 kb using a 32P-labeled cDNA fragment of the guinea pig (0.50 kb) or rat (0.35 kb) H1 receptor as a probe. The locations of ribosomal RNA (28 S and 18 S) are indicated. Ethidium bromide staining was used as a control for gel loading (lower part of each panel).
tissue also exhibited a significant presence of the H1 receptor transcripts, but the transcript level (48–68% of the cerebral level) was evidently less than that in atrial tissue. In the rabbit, the H1 receptor transcripts were barely detectable in atrial tissue (Figs. 2B and 3B). However, the presence of the H1 receptor transcripts was evident in ventricle. Its H1 receptor mRNA level was 31 to 43% of the cerebral level. In the rat, abundant expression of the H1 receptor mRNA was detected in both atrial and ventricular tissues (75–80% and 72–73% of the cerebral level, respectively) (Figs. 2C and 3C). In the human atrium there was very little expression of H1 receptor mRNA (Figs. 2D and 3D). Furthermore, the expres-

Fig. 3. Quantification of steady-state levels of H1 receptor mRNA in cerebral and myocardial (atrium and ventricle) tissues from the guinea pig (A), rabbit (B), rat (C), and human (D). H1 receptor mRNA was normalized as the ratio of its mRNA level over the 18 S level and was expressed as percentage of the value obtained in each cerebral tissue. Values are means ± S.E.M. (n = 5). For the human, the average of two separate experiments is given. P < 0.05 indicates a significant difference between atrial and ventricular tissues.
sion level of H1 receptor mRNA was markedly lower in the ventricle compared with the cerebral level (12–26%).

Northern blot analysis using the H2 receptor probe revealed a major mRNA species of 3.2 kb in guinea pig and rabbit tissues (Fig. 4, A and B). In rat and human tissues, however, 7 kb H2 receptor mRNA was also detected (Fig. 4, C and D). In the guinea pig, H2 receptor mRNA expression was much higher in ventricle than in atrium (Figs. 4A and 5A). Its expression level was 26% in atrium and 64% of the cerebral level in ventricle. In contrast, in the rabbit, H2 receptor mRNA expression was higher in the atrium than in the ventricle (Figs. 4B and 5B). The levels of the H2 receptor mRNA were 66 and 30% of the cerebral level in atrium and ventricle, respectively. In the rat, the H2 receptor transcripts were less abundantly expressed in both atrial and ventricular tissues (Figs. 4C and 5C). Compared with the H2 receptor transcript level in cerebral tissue, the levels in atrial and ventricular tissues were 17 and 23%, respectively. In the human, a considerable expression level of the H2 receptor transcripts was detected in myocardial tissues (Figs. 4D and 5D). Thus, atrial and ventricular tissues showed 68 to 94% and 43 to 71% of the H2 receptor transcript levels in cerebral tissue, respectively.

Histamine Receptor Protein Expression. Immunological detection of the histamine H1 receptor was performed using a anti-human H1 receptor polyclonal antibody, which recognized a 57-kDa band in mammalian cerebral and myocardial tissues (Fig. 6). In the guinea pig, the band was evidently lighter in ventricle than in atrium and cerebrum (Fig. 6A). Densitometric quantification of the signal showed that the H1 receptor protein level was 86% in atrium and 65% in ventricle compared with the cerebral level (Fig. 7A). In the rabbit, the atrial band was very faint, and the ventricular band was modest but clearly evident (Fig. 6B). Quantitative analysis revealed that the atrial and ventricular levels of the H1 receptor protein were 10 and 38% of the cerebral level, respectively (Fig. 7B). In the rat, the protein bands were intensely labeled in both atrial and ventricular tissues (Fig. 6C). Thus, the relative amount of immunodetectable H1 receptor, as determined by densitometric scanning, was more than 70% of the cerebral level in these tissues (Fig. 7C). In the human, the H1 receptor protein was much less abundant in myocardial tissues compared with cerebral tissue (Fig. 6D). Quantitative analysis of immunoblots showed that the amounts of atrial and ventricular H1 receptor protein were only 4 and 15% of the cerebral level, respectively (Fig. 7D).

Immunoblot analysis using anti-human H2 receptor polyclonal antibody showed a single protein band with a molecular mass of 69 kDa in mammalian cerebral and myocardial tissues (Fig. 8). In the guinea pig, the band was markedly less in atrium than in ventricle and cerebrum (Fig. 8A). Quantification of the H2 receptor protein indicated that the protein levels in atrial and ventricular tissues were 21 and 64% of the cerebral level, respectively (Fig. 9A). In the rabbit, the atrial band was apparently darker than the ventricular band, although it was not so marked in comparison with the cerebral band (Fig. 8B). On scanning these bands, the atrial and ventricular levels were 67 and 30% of the cerebral level, respectively (Fig. 9B). In the rat, the H2 receptor protein signal was marginally visible in myocardial tissues (Fig. 8C). Densitometric analysis revealed that the H2 receptor protein levels in atrial and ventricular tissues were 13 and 15% of the cerebral level, respectively (Fig. 9C). In the human, immunodetectable H2 receptor was found at high levels in both atrial and ventricular tissues (Fig. 8D). Compared with the cerebrum, the atrial and ventricular expression levels of the H2 receptor protein were 79 and 70%, respectively (Fig. 9D).

As can be seen in Fig. 10, immunofluorescence studies confirmed the results obtained from Western blotting. Thus, immunofluorescence staining for H1 receptor protein in the guinea pig heart showed that its expression was high in the atrial tissue and low in the ventricular tissue. About 83% of cells in the atrium and 50% of cells in the ventricle showed positive immunoreactivity for the H1 receptor. In contrast, positive staining for H2 receptor protein was strong in the ventricle, but weak in the atrium: 90% of ventricular cells and only 45% of atrial cells showed positive staining for H2 receptors.

Identification of Histamine Receptors on the Specialized Conductive Tissue of the Heart. Immunohistochemical studies showed abundant expression of both H1 and H2 receptor proteins in the SA node in the guinea pig heart (Fig. 11). Furthermore, the AV node seemed to equally express H1 and H2 receptor proteins (Fig. 12).

Discussion

Using Northern blotting, immunoblotting, and immunohistochemical techniques, we uncovered the presence and distribution pattern of histamine H1 and H2 receptor mRNA
transcripts and proteins in the heart of various mammals, including humans. On Western blots, we detected H1 and H2 receptor proteins in every mammalian cardiac and brain tissue studied. H1 and H2 receptor proteins migrated as two single bands at ~57 and 69 kDa, respectively.

Photoaffinity binding studies using [125I]iodoazidophenylpyramine and subsequent SDS-polyacrylamide gel electrophoresis analysis had previously indicated that the H1 receptor protein has a molecular mass of 56 kDa under reducing conditions in rat, guinea pig, and mouse brain (Ruat and Schwartz, 1989). Similarly, studies in bovine adrenal medulla membranes with the photoaffinity ligand [3H]azidobenzamide had found labeled peptides in the size range of 53 to 58 kDa (Yamashita et al., 1991b). Moreover, when the bovine adrenal medulla H1 receptor had been expression-cloned in Xenopus oocytes, it was deduced to correspond to a 491-amino acid protein with a calculated molecular mass of 56 kDa (Yamashita et al., 1991a). Thus, the molecular mass for the H1 receptor estimated in this study is comparable with those noted in the previous reports.

On the other hand, the molecular mass of 69 kDa obtained for the H2 receptor is considerably larger than the previously calculated molecular masses (40.2–40.5 kDa) for the cloned H2 receptors (Gantz et al., 1991a; Ruat et al., 1991; Traiffort et al., 1995; Kobayashi et al., 1996). This difference could be explained by glycosylation of the native H2 receptor. Indeed, cloned H2 receptor protein carries N-glycosylation sites in the N terminus region (Gantz et al., 1991a,b; Ruat et al., 1991; Traiffort et al., 1995).

We found that the expression levels of H1 and H2 receptor proteins in cardiac tissues from each mammalian species correlated with their mRNA expression levels, as determined by Northern blot analysis. This correlation implies that the expression of H1 and H2 receptors in the mammalian heart is regulated in a transcriptional manner. Northern blot analysis of cardiac and cerebral tissues from the four mammals,
including human, revealed two hybridizing forms of mRNA for the \( H_1 \) receptor, 3.3- and 3.9-kb transcripts. These transcripts may correspond to the use of distinct start sites in the 5′ region of the gene or distinct polyadenylation sites or, alternatively, to products of distinct genes (Traiffort et al., 1994). Northern blot analysis of \( H_2 \) receptor mRNA identified a single transcript of 3.2 kb in guinea pig and rabbit cardiac and cerebral tissues, whereas the second higher molecular mass species of 7 kb was clearly evidenced in rat and human tissues. Although hybridization in most previous studies indicated the presence of a single \( H_2 \) receptor transcript of 4.6 to 6 kb (Ruat et al., 1991; Traiffort et al., 1995; Karlstedt et al., 2001), it is interesting to note that, although not addressed by the authors, an earlier study had shown the presence of several \( H_2 \) receptor transcripts on Northern blots from various canine tissues (Gantz et al., 1991b).

In guinea pigs, we found \( H_1 \) receptors to be expressed in the heart at a relatively high level, mainly in the atrium and less in the ventricle, compared with their abundant expression in the brain. In contrast, \( H_2 \) receptors were abundant in the ventricle and slightly expressed in the atria. Conversely, in rabbits, \( H_2 \) receptors seemed to be the predominant histamine-receptor subtype in the atrium, with much less \( H_1 \) receptor expression; whereas the ventricle expressed an abundance of \( H_1 \) receptors and much less \( H_2 \) receptors.

Indeed, previous pharmacological studies had indicated a marked difference between guinea pig and rabbit hearts in terms of which receptor subtypes mediate the positive inotropic effect of histamine in left atrium and ventricle. Although the positive inotropic effect of histamine in the left atrium of the rabbit is exclusively mediated by \( H_2 \) receptors (Hattori et al., 1988), in the guinea pig, the positive inotropic effect in the left atrium is mediated entirely by \( H_1 \) receptors (Steinberg and Holland, 1975; Verma and McNeill, 1977). On the other hand, in guinea pig ventricular muscle, the positive inotropic effect of histamine is mediated predominantly by \( H_2 \) receptors (Hattori et al., 1994), whereas \( H_1 \) receptors almost exclusively mediate the inotropic effect in the rabbit ventricle (Hattori et al., 1990, 1994). Thus, the particular distribution of \( H_1 \) and \( H_2 \) receptors in guinea pig and rabbit myocardial tissues that we report here, well explains previous pharmacological findings.

In the rat heart, \( H_2 \) receptors were present at a rather low level both in the atrium and ventricle; in contrast, the expression of \( H_2 \) receptors was significant in both tissues. Yet, in the rat heart, histamine is known to cause a weak positive inotropic effect and only at very high concentrations. Notably, this inotropic effect is attenuated by \( \beta \)-blockers rather than by \( H_1 \) receptor antagonists (Laquer and McNeill, 1980; Levi et al., 1991). Although this suggest that the weak positive inotropic response in the rat heart may result from catecholamine release, it is also conceivable that in the rat heart \( H_1 \) receptors are weakly coupled to their transduction pathways.

In human atrial and ventricular tissue, \( H_1 \) receptor expression was less abundant, whereas \( H_2 \) receptors were richly expressed. This is the first study showing that \( H_2 \) receptors are present as the predominant histamine receptor subtype.
in the human heart at both mRNA and protein levels, using Northern and Western blot analysis. The present data are also in good agreement with the previous functional reports that histamine produces a positive inotropic effect in human atrial and ventricular muscles, likely due to the exclusive activation of H2 receptors (Eckel et al., 1982; Levi et al., 1991; Du et al., 1993).

Our immunohistochemical studies clearly show the presence of both H1 and H2 receptors in the SA and AV nodes of the guinea pig heart. Indeed, pharmacological evidence for the presence of H2 receptors in the SA node was already strong. In fact, a sinus rate increase in response to histamine had been demonstrated in isolated hearts from guinea pigs, rabbits, and cats, and in isolated right atria from guinea pigs, rabbits, and monkeys (Levi et al., 1991; Hattori, 1999). In these preparations, the histamine-induced increase in sinus rate was selectively and competitively antagonized by H2 receptor antagonists such as cimetidine, indicating that H2 receptors that are present in the SA node primarily mediate the positive chronotropic effect of histamine. On the other hand, it is not clear at this time what role, if any, is played by the H1 receptors present in the SA node.

In the guinea pig heart, histamine is known to impair AV conduction (Levi, 1972; Levi and Kuye, 1974). This negative dromotropic effect is effectively abolished by H1 receptor antagonists and is mimicked by selective H1 receptor agonists (Levi et al., 1975), suggesting that H1 receptors mediate the histamine-induced slowing of AV conduction. However, histamine may also enhance automaticity in the AV node. Electrophysiological experiments using AV node preparations from the rabbit and guinea pig have indicated that H2 receptors promoting an increase in automaticity are likely to exist in the AV node (Borchard and Hafner, 1986; Sanchez-Chapula and Elizalde, 1987). Therefore, our immunohistochemical data demonstrating the presence of both H1 and H2 receptors in the AV conduction system are in good agreement with the results of previous pharmacological studies.

In conclusion, we provide here for the first time evidence from Northern and Western blot studies that histamine H1 and H2 receptors are variably distributed not only among different mammalian species but also in different regions of the heart. Notably, these novel molecular findings agree with previous pharmacological results obtained in whole animal and isolated tissue experiments. With the exception of the rat heart, where pharmacological responses are apparently dissociated from the abundant expression of H1 receptors, and where adrenergic mechanism may also play a role, we find that in guinea pig, rabbit, and human heart the expression level of H1 and H2 receptors clearly determines the functional response to histamine. The fact that a highly abundant expression of H2 receptors was found in human atrial and ventricular myocardium, substantiates our previous evidence of histamine arrhythmogenicity in the human heart in various disease states (Levi et al., 1981; Wolff and Levi, 1986; Levi, 1988). Moreover, we recently reported that endotoxin-induced sepsis results in a dramatic increase in the expression of H1 and H2 receptor and histidine decarboxylase genes in the cardiovascular system, causing a marked increase not
only in the production of histamine but also in its effects (Matsuda et al., 2002). These changes may well contribute to the cardiovascular deterioration associated with sepsis in humans. The new knowledge of a differential distribution of...
histamine receptor subtypes in the human heart will foster a better understanding of histamine roles in cardiovascular pathophysiology and possibly contribute to new therapeutic approaches to histamine-induced cardiac dysfunctions.

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