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20. Amino acids 147 to 155 of influenza nucleoprotein; the sequence is TYQRTRALV [O. Rötzschke *et al.*, *Nature* **348**, 252 (1990); (*30*)].
21. Purified K^d was diluted tenfold with buffered 6 M guanidine hydrochloride, concentrated with a Centricon-10 (Amicon), and passed over a fast protein liquid chromatography (FPLC) column (Superose 12; Pharmacia). Heavy and light chain peaks were pooled and dialyzed at 4°C against 8 M urea, 20 mM tris (pH 7.6), 150 mM NaCl, 1 mM EDTA, and 0.2 mM phenylmethylsulfonyl fluoride, then diluted to 0.1 mg/ml with buffered urea and dialyzed against two changes of buffer without urea, first at 4°C, then at room temperature. For renaturations with peptide, a 30-fold molar excess of peptide (*20*) was added at the time of dilution, and dialysis was performed in 500-dalton cutoff dialysis tubing. For renaturations of empty molecules, a 30% excess of human β_2M (Sigma) was added. Renatured material was concentrated eight- to tenfold in a pressure cell (10-kD cutoff; Filtron Omegacell, Northboro, MA) and passed over an FPLC column (Superdex; Pharmacia). Fractions corresponding to heterodimer were pooled and concentrated.
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24. For a two-state unfolding process, the equilibrium constant $K(T)$ can be evaluated at a particular temperature from the limiting ellipticity values of the pure native (N) and denatured (D) states, θ_N and θ_D, respectively, and the ellipticity at T, $\theta(T)$, as follows:
$$K(T) = \frac{\theta_N - \theta(T)}{\theta(T) - \theta_D}$$
ΔG, the change in enthalpy (ΔH), and the change in entropy (ΔS) can then be derived from $K(T)$ by standard thermodynamic relationships. $\Delta G = -RT\ln K$, where R is the gas constant, and T is the temperature in kelvin. $\Delta H = RT^2(\delta \ln K/\delta T)$, and $\Delta S = (\Delta H - \Delta G)/T$. For these and the following equations, T is in kelvin. To evaluate ΔG at temperatures below the transition region where $K(T)$ cannot be directly determined experimentally, we used the following equation [W. J. Becktel and J. A. Schellman, *Biopolymers* **26**, 1859 (1987)]:
$$\Delta G = \Delta H_m \left(\frac{T_m - T}{T_m}\right) - \Delta C_P \left\{T_m - T\left[1 - \ln\left(\frac{T}{T_m}\right)\right]\right\}$$
where the heat capacity change, ΔC_P, is assumed to be temperature-independent [P. L. Privalov and S. J. Gill, *Adv. Protein Chem.* **39**, 191 (1988)] and derived as $\Delta C_P = (\delta \Delta H/\delta \Delta T)_P$ (pressure held constant); T_m is the transition temperature for unfolding during heat denaturation (the temperature at which $\Delta G = 0$); and ΔH_m is the enthalpy change at T_m. We used the following values for these parameters to calculate ΔG(37°C) for empty and peptide-filled K^d from the melting curves shown in Fig. 3A: $\Delta C_{P(empty)} = 0.3$ kcal/mol per degree; $T_{m(empty)} = 45$°C; $\Delta H_{m(empty)} = 48$ kcal/mol; $\Delta C_{P(filled)} = 0.9$ kcal/mol per degree; $T_{m(filled)} = 56$°C; and $\Delta H_{m(filled)} = 107$ kcal/mol. In principle, the standard free energy change for any dissociation reaction is dependent on the concentration at which the equilibrium is measured. Therefore, all calculated values in this paper are strictly applicable only in the concentration ranges reported here. For the large ΔH_m values derived, however, ΔG is relatively independent of concentration.
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31. We thank M. Raghavan for doing the acid-elution experiments; D. C. Rees and T. Arakawa for helpful discussions; C. Bebbington for the glutamine synthetase amplification vector; J. Moore for help with CD analysis; D. Penny for assistance with tissue culture; M. Blum and R. Strong for help with graphics; and D. C. Rees, N. Davidson, and M. Raghavan for critical reading of the manuscript. Supported by NIH (AI28931 to P.J.B.), and an NIH postdoctoral fellowship (M.L.F.). P.J.B. is a scholar of the Pew Charitable Trusts and the Cancer Research Institute.

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Localization of Targets for Anti-Ulcer Drugs in Cells of the Immune System

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The gastric mucosa consists of the epithelium, which lines the lumen, the lamina propria, and the muscularis mucosae. The targets of drugs used to treat stomach and duodenal ulcers are thought to be the acid-secreting parietal cells of the epithelium. However, immune cells in the lamina propria are the only cells that showed detectable messenger RNAs for histamine, muscarinic, gastrin, and dopamine receptors by in situ hybridization histochemistry. None of the epithelial cells expressed any of these messenger RNAs. Thus, the targets of antiulcer drugs seem to be cells of the immune system in the gut and not parietal cells, as generally believed. This conclusion may revise the thinking about ulcer formation and may shed light on the etiology of such chronic small intestinal diseases as Crohn's disease.

Acid-secreting parietal cells are located among the epithelial cells of the gastric mucosa. Dysfunction of these cells has been thought to be the leading cause of ulcer disease. For centuries agents that neutralize acid were used to treat dyspepsia and gastric ulcers. In the 1950s vagotomy (severing the parasympathetic innervation of the stomach, the vagus nerve) was introduced as a method to treat ulcer disease unresponsive to antacids. Drugs that block the action of the vagal neurotransmitter, acetylcholine, at muscarinic receptors were also employed. Unfortunately, side effects limited the utility of these muscarinic antagonists (*1*).

In the early 1970s histamine H2 receptor antagonists began to be used to heal and protect against peptic ulcers (*2*, *3*). Dopamine also modulates gastric acid secretion (*4*), and dopamine antagonists prevent ulcer relapse (*5*). Administration of tyrosine, which is converted into dopamine by the actions of tyrosine hydroxylase and dopa decarboxylase, also prevents experimental ulcer formation (*6*).

Because the parietal cells of the stomach secrete acid, it has been assumed that all of the drugs mentioned above act on these cells. Gastrin, a peptide hormone produced by antral cells in the stomach, is also thought to stimulate gastric acid secretion by acting on parietal cells (*7*). We have used in situ hybridization histochemistry to visualize histamine (H2), muscarinic acetylcholine (M1 to M5), gastrin (GR), and dopamine (D1 to D5) receptors in the stomach (*8*).

There were numerous cells in the lamina propria of the stomach containing mRNA for H2 (Fig. 1, A to C), M1 (Fig. 1D) to M5, GR (Fig. 1E), and D1 to D5 (Fig. 1F) receptors. The probes directed to D3, D4,

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D5, and M3 receptor mRNAs gave the strongest signals; those directed to H2, M1, M2, M4, M5, and gastrin mRNAs gave moderately strong signals; and those directed to D1 and D2 receptor mRNAs gave the weakest signals.

In a cross section of the gastric mucosa (Fig. 2A) the mucus-filled gastric glands, the epithelium, and the lamina propria (which contains immune cells) can be clearly distinguished. Cells that contain the mRNA for the M1 receptor are shown in a similar cross section in Fig. 2B. Unexpectedly, from the data in Figs. 1 and 2, it appeared that the cells visualized with the receptor mRNA probes were immunocytes in the lamina propria of the stomach and that the epithelial cells contained no mRNA for these receptors. This seemed to be the case in the duodenum as well (*9*). Some of the positive cells seemed very close to the epithelial cells and appeared to be attached to the epithelial cells at their bases. The number of positive immunocytes increased markedly when we induced ulcer formation with cysteamine (*10*) or restraint stress (*11*); this number did not seem to be affected by subdiaphragmatic vagotomy prior to stress (*12*).

The lamina propria consists of a network of connective tissue in which blood vessels, lymphatic vessels, nerve fibers, and cells of the immune system are embedded (*13*). The positive cells described above in the tunica propria could be any one or a combination of lymphocytes, plasmocytes, mast cells, and macrophages. To identify these cells we used immunostaining to visualize plasmocytes [with antibodies to immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin E (IgE)], macrophages, and T cells (with specific monoclonal antibodies directed against macrophage and T cell–specific epitopes) (*14*). Cells that reacted with these antibodies were homogeneously distributed throughout the lamina propria.

Next we used the antibodies listed above to stain cells and then performed in situ hybridization histochemistry (ISHH) on the same sections (*8*, *14*). For these preliminary studies we chose rat H2, M1, M2, gastrin, D4, and D5 receptor probes for the ISHH because they gave particularly strong signals; immunostaining prior to ISHH results in a loss of signal, but we could readily visualize the receptor mRNAs after immunostaining cells in the lamina propria. The majority of macrophages had H2 (Fig. 3, A and B), GR, M1 (Fig. 3, C and D), D4, and D5 receptor mRNAs, while a smaller number of macrophages was positive for gastrin receptor mRNA. All of the plasmocytes that make IgA seem to express the receptors we studied (Fig. 4). We could not detect any of the receptors on mast cells.

Resident mast cells in the stomach may supply the histamine that acts on H2 receptors. Vagal fibers probably release the acetylcholine that stimulates the muscarinic

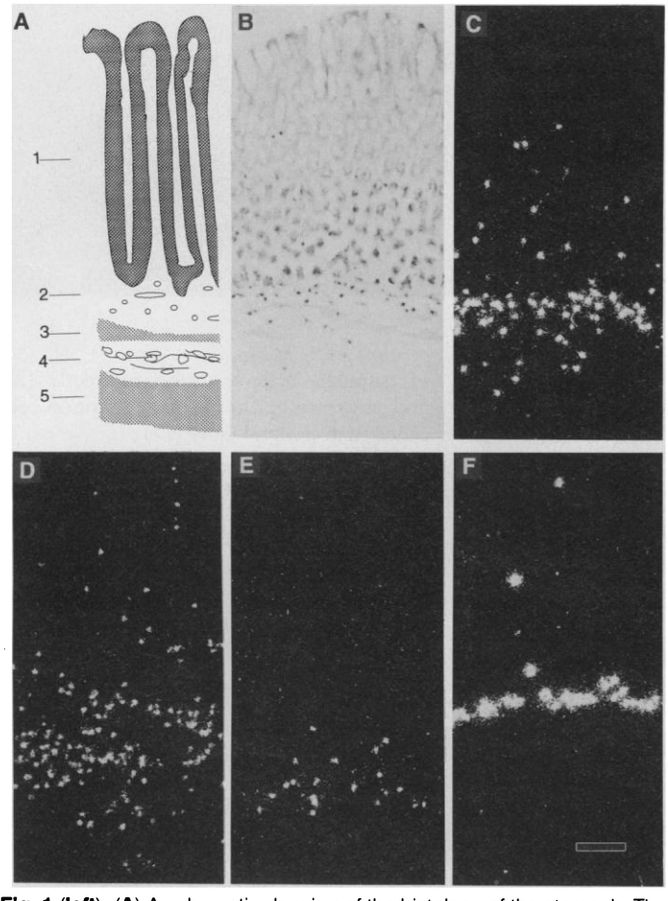


Fig. 1 (left). (**A**) A schematic drawing of the histology of the stomach. The numbered layers are: (1) epithelium (gastric glands), (2) lamina propria, (3) muscularis mucosae, (4) submucosa, and (5) muscularis externa. Visualization of histamine H2 [(**B**) bright-field, (**C**) dark-field], (**D**) gastrin, (**E**) muscarinic M1, and (**F**) dopamine D5 receptor mRNAs by means of ISHH in sections aligned with (A). Bar, 100 μm.

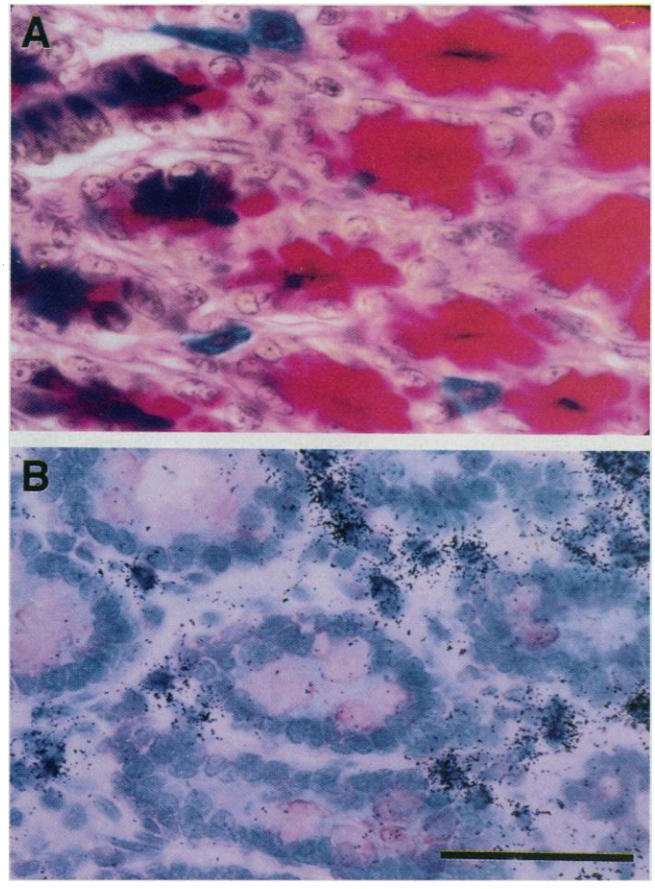


Fig. 2 (right). (**A**) A histological staining (Alcian blue–periodic acid Schiff–haematoxylin) that shows a cross section of the lamina propria in the stomach. The epithelium of the gastric glands surrounds the secretion product (dark blue or red). Connective tissue, blood vessels, and immunocytes (plasmocytes and mast cells are light blue) fill the space in between the gastric glands. Bar, 50 μm. (**B**) Cross section of the lamina propria of a stomach as in (A) hybridized with M1 receptor probe. All the labeled cells are in the space between the gastric glands, and the epithelial cells do not show any labeling. Probes for the histamine receptor, the other muscarinic receptors, the dopamine receptors, and the GR revealed a similar pattern of labeling.

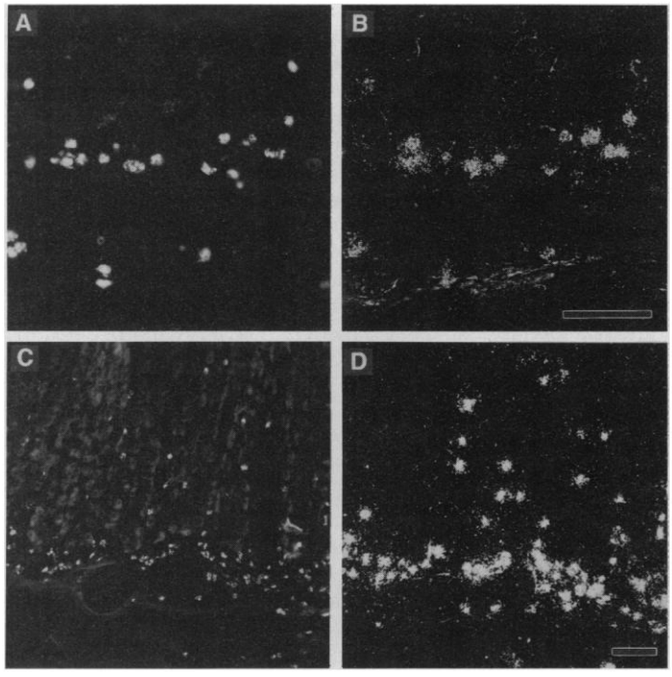


Fig. 3 (**left**). Colocalization of an antigen specific for macrophages (**A** and **C**) and the mRNA encoding the H2 (**B**) and M1 (**D**) receptor (antibody from Chemicon MC1435) (diluted 1:100). Fluorescence (A) and dark-field (B) photographs of the same section indicate that the large majority of the immunostained macrophages are positive for both of the mRNAs. Bar, 100 μm. **Fig. 4** (**right**). Colocalization of the mRNA encoding the H2 (**B**), M1 (**D**), and GR (**F**) receptors with IgA as showed by fluorescence immunostaining (**A, C**, and **E**). The antibody, raised in rabbit and preconjugated to FITC (Nordic Immunologicals), was used at a 1:100 dilution. Bar, 100 μm.

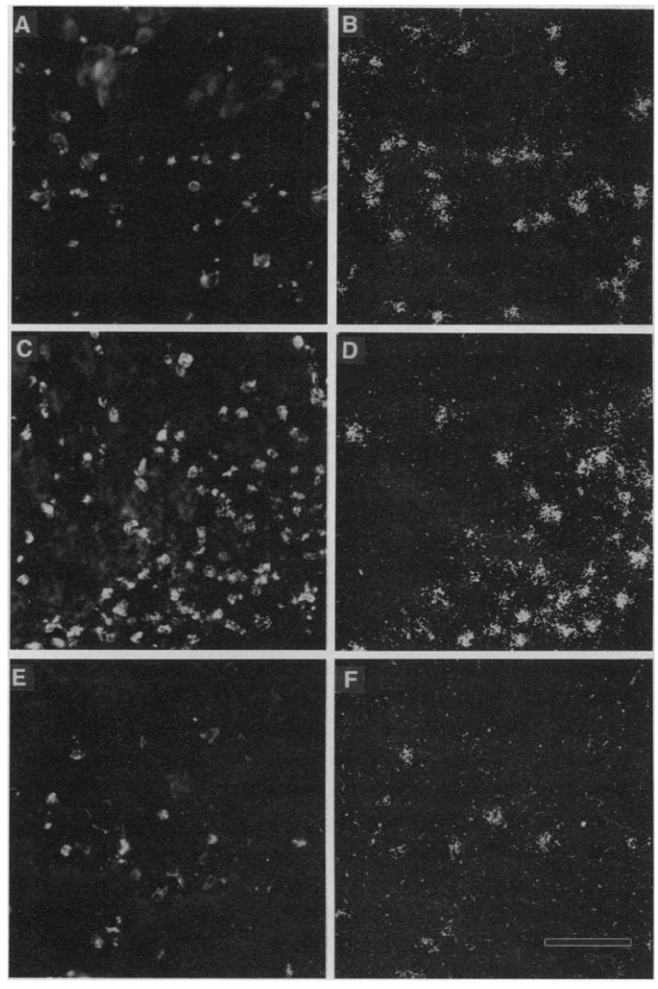
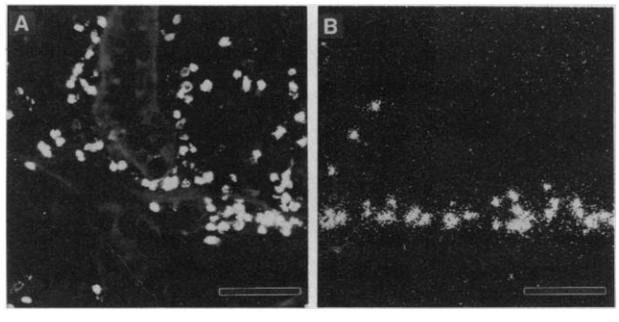


Fig. 5. Immunohistochemical (**A**) and ISHH (**B**) localizations of TH and its mRNA, respectively, in stomach. No hybridization signal was seen with probes complementary to DBH or PNMT.



M1 to M5 receptors. The source of dopamine, however, that acts on dopamine receptors in the lamina propria was not clear. Thus, we used immunostaining and ISHH to visualize tyrosine hydroxylase (TH), the rate-limiting enzyme in the pathway leading to dopamine synthesis. The vast majority of what appeared to be immunocytes in the lamina propria proved to be positive for TH itself and its mRNA (Fig. 5, A and B). The absence of dopamine β-hydroxylase (DBH) and phenylethanolamine-*N*-methyl-transferase (PNMT) mRNAs suggests that neither noradrenaline or adrenaline is made in these cells.

Our results seem to contradict data in the literature indicating that histamine H2 and acetylcholine receptors are present on isolated parietal cells purified from the stomach (*15*). However, parietal cell preparations used in these previous studies (*15, 16*) were 65 to 90% pure. The remaining 10 to 35% of the cells could have been from the tunica propria and accounted for the response to H2 and acetylcholine agonists. Consistent with our results, gastrin has been suggested to stimulate acid secretion indirectly (*2*), and gastrin receptors may be present on cells other than parietal cells (*17*).

Our findings indicate that the agents used to treat ulcer disease may act on immunocytes in the lamina propria and not on parietal cells as previously thought. Thus, their effects on acid secretion may be indirectly transmitted to the epithelial (parietal) cells via the immunocytes. A factor such as nitric oxide (*18*) might mediate the interaction between immunocytes and epithelial cells of the stomach.

It is possible that epithelial cells have H2, GR, muscarinic, or dopamine receptors, but that their respective mRNAs are present in such low abundance that we failed to detect them. On the basis of our data, however, we suggest that current models describing the regulation of gastric acid secretion may need revision.

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8. Adult male Sprague-Dawley rats were used for these studies. They were decapitated, or anesthesized and subjected to transcardiac perfusion with fixative, and their stomachs and the upper portions of their duodenums were removed and frozen on dry ice. Twelve-micrometer-thick longitudinal sections were cut, and ISHH or immunostaining (or both) were performed. For ISHH alone, sections from ten rats were cut in a cryostat and thaw-mounted onto gelatin-coated slides. The slides were kept at −80°C until used. The procedure for ISHH was as described [W. S. Young III, E. Mezey, R. E. Siegel, *Mol. Brain Res.* 1, 231 (1986)]. Forty-eight nucleotide oligodeoxyribonucleotide probes were used. The probes were complementary to the mRNA nucleic acid sequences: H2: 645–692 and 936–983 [M. Ruat, E. Traiffort, J.-M. Arrang, R. Leurs, J.-C. Schwartz, *Biochem. Biophys. Res. Commun.* **179**, 1470 (1991)]; GR: 135–182 [A. S. Kopin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 3605 (1992)]; D1: 810–857 [F. J. Monsma, L. C. Mahan, L. D. McVittie, C. R. Gerfen, D. R. Sibley, *ibid.* **87**, 6723 (1990)]; D2: 783–787 [J. R. Bunzow *et al.*, *Nature* **336**, 783 (1988)]; D3: 67–114 [P. Sokoloff, B. Giros, M.-P. Martres, M.-L. Bouthenet, J.-C. Schwartz, *ibid.* **347**, 146 (1990)]; D4: 733–774 and 829–882 [H. H. M. Van Tol *et al.*, *ibid.* **350**, 610 (1991)]; D5: 1–48 and 967–1011 [R. K. Sunahara *et al.*, *ibid.*, p. 614]; M1: 9–57 [T. I. Bonner, N. J. Buckley, A. C. Young, M. R. Brann, *Science* **237**, 527 (1987)]; M2: 255–303 [J. Gocayne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 8296 (1987)]; M3: 6–51, M4: 9–57 [T. I. Bonner, N. J. Buckley, A. C. Young, M. R. Brann, *Science* **237**, 527 (1987)]; M5: 4–51 [T. I. Bonner, A. C. Young, M. R. Brann, N. J. Buckley, *Neuron* **1**, 403 (1988)]; TH: 1441–1488 [B. Grima, A. Lamouroux, F. Blanot, N. F. Biguet, J. Mallet, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 617 (1985)]; DBH: 115–162 [A. Lamoroux *et al.*, *EMBO J.* **6**, 3921 (1987)]; and PNMT: 183–233 [E. Mezey, *Nucleic Acids Res.* **17**, 2125 (1989)]. The sections were coated with Kodak NTB3 emulsion and developed after 2 to 3 days (D3 to 5, M1 to 5, and TH) or 1 to 2 weeks (GR, D1, and D2). In all these cases, when two different oligonucleotides were used to probe the same mRNA, both probes showed the same pattern of labeling. In addition, sense strand probes based on the sequences of the D4 and D5 receptor mRNAs gave no cellular labeling.
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14. For immunocytochemistry, three rats were anesthesized with ether and perfused through the ascending aorta with 300 ml of 4% paraformaldehyde and 1% picric acid in 0.1 M sodium phosphate buffer (pH 7.4). The stomachs were quickly removed, postfixed in the same fixative for 2 hours at 4°C, and soaked in a cryoprotectant solution [20% sucrose and 3% polyethylene glycol (400 MW) in physiological saline] overnight. The tissues were then frozen in isopentane at −80°C. Longitudinal 10-μm-thick sections were cut in a cryostat (Reichert-Jung Frigocut 2800E) and stored at −80°C until they were stained. Sections were immunostained and then processed for ISHH. The slides were first incubated with the primary antibody overnight at 4°C. The sections were then washed in 0.1 M phosphate-buffered saline (PBS) (pH 7.2) and transferred into a 1:100 dilution of an antibody to mouse IgG conjugated to fluorescein isothiocyanate (FITC) (Sigma) for 1 hour at room temperature. At the end of this incubation, the slides were transferred into PBS and the ISHH procedure was performed. The antibodies used to stain immunocytes included mouse monoclonal antibody to rat macrophage (1:400, Chemicon 1435, raised against peritoneal macrophages); antibody to rat mast cells conjugated to FITC (1:100, Accurate Chemicals); antibody to rat IgA conjugated to rhodamine or FITC (1:50, Nordic Immunology); antibody to rat IgE and antibody to rat IgM (1:400, Pierce); and antibody to rat IgG conjugated to phycoerythrin (1:100, Jackson Immunologicals). The secondary antibodies were antibody to mouse IgG or antibody to goat IgG conjugated to FITC or rhodamine (1:100, Sigma).
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Solution Structure of the SH3 Domain of Src and Identification of Its Ligand-Binding Site

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The Src homology 3 (SH3) region is a protein domain of 55 to 75 amino acids found in many cytoplasmic proteins, including those that participate in signal transduction pathways. The solution structure of the SH3 domain of the tyrosine kinase Src was determined by multidimensional nuclear magnetic resonance methods. The molecule is composed of two short three-stranded anti-parallel β sheets packed together at approximately right angles. Studies of the SH3 domain bound to proline-rich peptide ligands revealed a hydrophobic binding site on the surface of the protein that is lined with the side chains of conserved aromatic amino acids.

Mutational analysis of the Src protein kinase suggests that the SH3 domain of this protein, like the SH2 domain, may act as a regulatory element with dual functions—to modulate catalytic activity and to facilitate binding to other cellular proteins. Deletion or substitution of amino acids within the SH3 domain enhances the catalytic activity and oncogenic potential of Src (*1*), and prevents binding of at least one Src substrate (p110) (*2*). Similar results have been observed with the SH3 domain of the related protein tyrosine kinase, Abl (*3*, *4*), and a protein (3BP-1) has been identified that binds to the SH3 domains of Abl and Src in vitro (*5*). The 3BP-1 protein contains a sequence outside of its SH3 binding domain that is similar to the guanosine triphosphatase (GTPase) activation domain of several GTPase activating proteins (GAPs) that regulate the Rho/Rac family of guanine nucleotide binding proteins (*5*). Although the functional role of the SH3–3BP-1 interaction in vivo has not been established, certain SH3 domains appear to participate in signal transduction pathways that include both tyrosine kinases and small guanine nucleotide binding proteins (*6–9*). It is possible that these domains bind to GAPs and guanine nucleotide exchange factors (GEFs) that regulate the activity of guanine nucleotide binding proteins.

Neither the ligand specificities nor structures of SH3 domains have been determined. We report the structure determination of the SH3 domain of Src and the identification of its ligand binding site by multidimensional nuclear magnetic resonance (NMR) methods. The backbone fold and receptor site of Src SH3 are unrelated to those of the SH2 domains in Src, Abl, and the p85α subunit of phosphatidylinositol-3′ kinase (PI3′K) (*10*).

The sequential assignment of a 64-resi-

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