Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 4788–4792, May 1995
Biochemistry

Mimics of the binding sites of opioid receptors obtained by molecular imprinting of enkephalin and morphine

(molecular recognition/synthetic polymers/ligand–receptor interactions/molecularly imprinted sorbent assay)

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Communicated by Pedro Cuatrecasas, Parke–Davis Pharmaceutical Research, Ann Arbor, MI, January 3, 1995

ABSTRACT **Molecular imprinting of morphine and the endogenous neuropeptide [Leu^5]enkephalin (Leu-enkephalin) in methacrylic acid-ethylene glycol dimethacrylate copolymers is described. Such molecular imprints possess the capacity to mimic the binding activity of opioid receptors. The recognition properties of the resultant imprints were analyzed by radioactive ligand binding analysis. We demonstrate that imprinted polymers also show high binding affinity and selectivity in aqueous buffers. This is a major breakthrough for molecular imprinting technology, since the binding reaction occurs under conditions relevant to biological systems. The antimorphine imprints showed high binding affinity for morphine, with K_d values as low as 10^{-7} M, and levels of selectivity similar to those of antibodies. Preparation of imprints against Leu-enkephalin was greatly facilitated by the use of the anilide derivative rather than the free peptide as the print molecule, due to improved solubility in the polymerization mixture. Free Leu-enkephalin was efficiently recognized by this polymer (K_d values as low as 10^{-7} M were observed). Four tetra- and pentapeptides, with unrelated amino acid sequences, were not bound. The imprints showed only weak affinity for two D-amino acid-containing analogues of Leu-enkephalin. Enantioselective recognition of the L-enantiomer of phenylalanylglycine anilide, a truncated analogue of the N-terminal end of enkephalin, was observed.**

The study of molecular recognition, with implications for intermolecular chemistry, chemical selectivity, and ultimately in drug design, is at present a rapidly developing field of research (1, 2). Numerous model systems have evolved that mimic the interaction between a substrate (the guest) and a receptor (the host). Molecular imprinting (3) has, particularly during the last few years, developed into a powerful technique for the preparation of highly substrate- and enantioselective polymers (4–7). The technique entails polymerization around a print species using monomers with chemical functionalities complementary to those of the print molecule (4–7). The interactions developed between complementary functionalities present in the imprint molecule and the monomer(s) prior to the initiation of polymerization are conserved in the resultant polymer. Subsequent removal of the print species exposes recognition sites within the polymer possessing a "memory" for this compound in terms of complementarity of both shape and chemical functionality. Antitheophylline and antidiazepam molecularly imprinted polymers (MIPs) have been applied as antibody mimics in an immunoassay-like technique, molecularly imprinted sorbent assay (MIA), for drug determinations in human serum (8). The imprinted antibody mimics showed high binding affinities and selectivities comparable to those demonstrated by the corresponding antigen–antibody systems. Assay results showed excellent correlation with those obtained by using a traditional immunoassay technique. Another important application of MIPs is their use in HPLC separations. In particular, when the print molecule is one of the optical antipodes of a chiral compound, the resultant MIP shows a very high enantioseparative capability. Chiral stationary phases have, to date, been developed for resolution of amino acid derivatives (9–13), sugar derivatives (14, 15), and drug compounds, such as β-blocking agents (16).

Opioid receptors are a family of receptors involved in pain perception (for comprehensive state-of-the-art reviews of opioid research, see ref. 17). At least three classes of opioid receptors, μ, δ, and κ, are present in brain that differ in their ligand selectivity and in their pharmacological effect. Structural and functional characterization of the receptors is still preliminary. This is due to experimental difficulties in purification of the receptors, reconstitution of them in a membrane environment, and isolation and cloning of their genes (18). The advancement of opioid receptor knowledge has, to a large extent, relied on the identification and characterization of selective opioid agonists and antagonists. Several studies of ligand–receptor interactions have used antibodies against enkephalins (19–23) or morphine (24–29) as mimics of the binding site of opioid receptors. Here we report the imprinting of the endogenous neuropeptide [Leu^5]enkephalin (Leu-enkephalin), and of morphine, and the characterization of the binding properties of the resultant imprints. MIPs have several distinct advantages that make them attractive for this kind of study, such as simple and rapid preparation, the stability of imprinted structures, and the wide variety of substances amenable to imprinting. By the choice of the functional monomer(s) employed, it may be possible to undertake systematic studies of particular binding interactions and contributions to binding. In this study, radioligand displacement (MIA) was used as the diagnostic tool to evaluate the selective binding abilities of the imprints. Strong ligand–imprint interaction requires the use of apolar organic solvents, which is a disadvantage for the object of this present study. Hence, this particular issue was addressed and some basic protocols for the optimization of ligand binding to MIPs in both organic and aqueous media were developed. A comparison is made among the binding properties of imprints, natural opioid receptors (17), and several anti-enkephalin and anti-morphine monoclonal antibodies.

MATERIALS AND METHODS

Polymer Preparation. Morphine MIPs were made by carefully heating 428 mg (1.5 mmol) of morphine free base and 516

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Abbreviations: DADLE, [D-Ala^2,D-Leu^5]enkephalin; MIP, molecularly imprinted polymer; Leu-enkephalin, [Leu^5]enkephalin; MIA, molecularly imprinted sorbent assay; DALE, [D-Ala^2,Leu^5]enkephalin; Met-enkephalin, [Met^5]enkephalin.

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mg (6 mmol) of methacrylic acid until a homogeneous oil was formed. Then, 4.76 g (24 mmol) of ethylene glycol dimethacrylate, 6 ml of acetonitrile, and 56 mg of 2,2'-azobis-(2-methylpropionitrile) were added, and the mixture was sparged with nitrogen and polymerized under a nitrogen atmosphere at 60°C for 16 h. Leu-enkephalin anilide (63 mg, 0.1 mmol) was dissolved in a mixture of 155 mg (1.8 mmol) of methacrylic acid, 1.43 g (7.2 mmol) of ethylene glycol dimethacrylate, and 2.1 ml of acetonitrile. After addition of 17 mg of 2,2'-azobis-(2-methylpropionitrile), the solution was sparged with nitrogen and polymerized under a UV source (366 nm) at 4°C for 16 h. The bulk polymers were ground to particles <25 μm, and fines were removed by sedimentation from ethanol. The particles were carefully washed with large amounts of methanol/acetic acid/water, 4:1:1 (vol/vol), and then methanol and finally dried under vacuum.

MIA. Binding of radiolabeled Leu-enkephalin and morphine in the absence and presence of various concentrations of competing ligands was analyzed under conditions where the number of polymeric binding sites was limited (30). The relative cross-reactivities were calculated after log–logit transformation (30). The equilibrium dissociation constant (K_d) was estimated by nonlinear least square fitting with the EBDA and LIGAND programs (Elsevier–Biosoft) (31, 32).

Nonaqueous assay. Leu-enkephalin anilide MIP (8 mg), 3 ng of [^{3}H]Leu-enkephalin (specific activity, 37.8 Ci/mmol; 1 Ci = 37 GBq), and Leu-enkephalin or other competitors at the indicated concentrations (ranging from 10 nM to 200 μM) were incubated in 1 ml of acetonitrile/acetic acid, 95:5 (vol/vol), for 15 h at room temperature. The morphine MIA was performed in toluene/acetic acid, 49:1 (vol/vol), by using 0.1 mg of morphine MIP and 1 ng of [^{3}H]morphine (specific activity, 70 Ci/mmol). The polymer particles were centrifuged (10,000 × *g*, 5 min), and radioactivity in 200 μl of the supernatant was measured by liquid scintillation counting.

Aqueous assay. Leu-enkephalin anilide MIP (5 mg) was incubated for 15 h at room temperature in 1 ml of buffer containing 3 ng of [^{3}H]Leu-enkephalin (specific activity, 37.8 Ci/mmol) and competing ligands (ranging from 50 nM to 2 mM). The aqueous morphine MIA used 1 ng of [^{3}H]morphine (specific activity, 61.6 Ci/mmol) and 1 mg of morphine MIP. The buffers were 20 mM sodium citrate (pH 3.0, 4.5, or 6.0), 20 mM sodium phosphate (pH 7.3), and 20 mM sodium carbonate (pH 9.2). The buffers contained 0, 1, 10, or 50% ethanol. After centrifugation, radioactivity in 400 μl of the supernatant was measured by liquid scintillation counting.

RESULTS

Polymer Preparations. Two methods can be used for the initiation of polymerization of imprinted polymers: heat (45–120°C) and UV (366 nm). The latter is routinely performed within the range of 0–20°C (for a discussion of polymerization protocols and parameters influencing the imprinting efficiency, see refs. 5 and 33) and is preferred, since it has been demonstrated that polymers made at lower temperatures, by this method, exhibit higher recognition abilities (10). It is believed that weak noncovalent interactions, such as hydrogen bonding, essential for imprint formation and subsequent recognition, are stronger at lower temperatures due to a favorable entropy term. Imprinting of morphine using this method was not possible, since colored side products that inhibited further polymerization appeared. Instead, the safer heat-induced polymerization method was used for morphine. Due to the poor solubility of the free peptide Leu-enkephalin in the polymerization mixture, Leu-enkephalin anilide, a C-terminal-blocked derivative, was used as an alternative print molecule. The molar ratios of methacrylic acid to print molecule were 18 and 4 for Leu-enkephalin anilide and morphine, respectively (not optimized values). As a rule of thumb, a 2- to 3-fold molar excess of methacrylic acid to each polar functionality of the print molecule is used (10). Increased ratios improve the recognition abilities of the imprinted sites, but their number may be reduced since the ratios are varied by addition of differing amounts of print species to an otherwise constant mixture. After preparation and work up of the polymers, the print species could be extracted quantitatively. In both cases, elemental analysis of nitrogen failed to detect any remaining print substance and, hence, it was calculated that ≥98% of the print molecules were removed.

Radioactive Ligand Binding Analysis in Organic Solvents. Ligand binding was investigated in several solvents (8). The binding strength increased with decreased polarity of the solvent used (data not shown). Nonspecific binding, as measured by binding under the same conditions to a nonimprinted reference polymer, could be suppressed by addition of small amounts of acetic acid. A mixture of 2% acetic acid in toluene was found to be optimal for binding of [^{3}H]morphine to the morphine MIP, since this solvent combined high affinity with low nonspecific binding at low polymer concentrations. Acetonitrile/acetic acid, 95:5 (vol/vol), was used in the Leu-enkephalin anilide MIP binding studies, since the peptides were not soluble in other less-polar solvents.

The Scatchard plots were nonlinear (Fig. 1), due to a heterogeneous population of sites with various affinities for the print molecule, and were approximated best by models with two or three apparent K_d values for high- and low-affinity binding sites. The apparent K_d values for Leu-enkephalin binding to the Leu-enkephalin anilide MIP were found to be 0.13 ± 0.041 μM and 43 ± 37 μM, associated with site populations of 0.017 ± 0.0047 μmol/g and 1.0 ± 2.1 μmol/g, respectively. This MIP expressed similarly high affinity for Leu-enkephalin and [Met^5]enkephalin (Met-enkephalin), and almost identical competition curves were obtained for these two compounds (Fig. 2*A*). Other compounds, such as the D-amino acid-containing analogues [D-Ala^2,Leu^5]enkephalin (DALE) and [D-Ala^2,D-Leu^5]enkephalin (DADLE), showed very low cross-reactivity (Table 1). Morphine was found to bind to the morphine MIP with apparent K_d values of 92 ± 52 nM and 8.9 ± 2.1 μM, associated with site populations of 1.2 ± 0.65 and 39 ± 3.4 μmol/g of polymer, respectively. Only the structurally closely related compounds codeine, normorphine, and hydromorphone showed significant cross-reactivity, whereas the cross-reactivity of naloxone and naltrexone was minimal (Table 2).

Radioactive Ligand Binding Analysis in Aqueous Buffers. Ligand binding was analyzed at five pH values ranging from 3 to 9.2, each in the presence of four ethanol concentrations. At all pH values, addition of 1% and 10% ethanol slightly decreased the binding strength and a greater decrease was

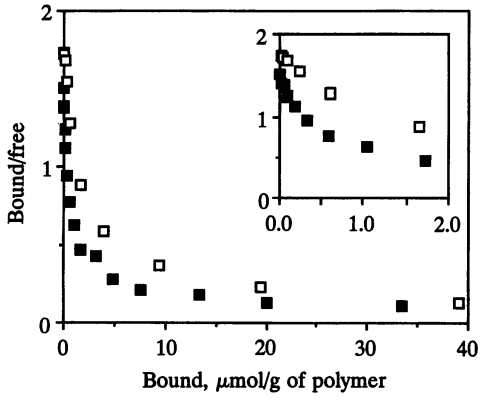


FIG. 1. Scatchard plot of the binding of morphine to the morphine MIP in toluene/acetic acid, 49:1 (vol/vol) (□), and 20 mM sodium citrate (pH 6.0) containing 10% ethanol (■).

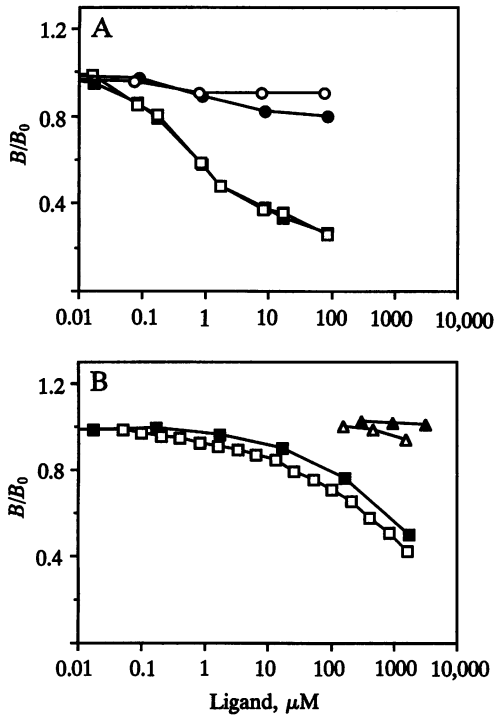


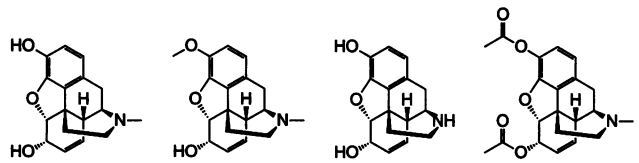
FIG. 2. Displacement of [^{3}H]Leu-enkephalin binding to the Leu-enkephalin anilide MIP in acetonitrile/acetic acid, 95:5 (vol/vol) (*A*), and 20 mM sodium citrate (pH 4.5) containing 10% ethanol (*B*) by increasing concentrations of competing ligand. B/B_0 is the ratio of the amount of radioligand bound in the presence of displacing ligand, *B*, to the amount bound in the absence of displacing ligand, B_0. The displacing ligands are as follows: □, Leu-enkephalin; ■, Met-enkephalin; ○, DADLE; ●, DALE; △, Lys-Phe-Glu-Lys; ▲, Gly-Gly-Gly-Gly-Gly.

observed in the presence of 50% ethanol (data not shown). For practical reasons, some ethanol was necessary in the incubation mixture to keep the polymer particles in a uniform suspension, since the ethanol increased the solvent wettability of the mainly hydrophobic polymer. An ethanol concentration of 10% was found to be a good compromise. This low concentration did not significantly affect either the affinity or specificity of the MIPs. The effect of pH was more complex: morphine binding was stronger at high pH, whereas low pH was favorable for binding of Leu-enkephalin. The same trends were true for binding to the corresponding nonimprinted reference polymers. In both cases, the ligand binding to both imprinted and nonimprinted polymers were recorded and the pH where the maximal difference between specific and non-specific binding occurred was calculated. For the Leu-enkephalin MIP, optimal ligand binding occurred at pH 4.5, whereas pH 6 was found to be optimal for to the morphine MIP.

Table 2. Cross-reactivity of opiates for binding of [^{3}H]morphine to the antimorphine MIP and anti-morphine antibodies

Ligand	Relative cross-reactivity, %					
	MIP in buffer	MIP in toluene	MAb1	MAb2	MAb3	MAb4
Morphine (**1**)	100	100	100	100	100	100
Codeine (**2**)	25	4.7	18	104	36	<0.1
Normorphine (**3**)	9.9	8.3				
Hydromorphone	15	6.0		112	9.8	
Heroin (**4**)	8.3	2.3				
Naloxone	0.4	<0.1	<0.5	0.1	0.7	0.1
Naltrexone	0.3	<0.1		≪0.1	0.2	≪0.1
Leu-enkephalin	≪0.1					
Met-enkephalin	≪0.1			≪0.1		≪0.1

Cross-reactivities are expressed as the molar ratio (in percent) of morphine to ligand giving 50% inhibition of radiolabeled morphine binding. MAb1, data for clone 12D4 in ref. 25; MAb2, data for clone 8.2.1 in ref. 26; MAb3, data for clone 368-21 in ref. 28; and MAb4, data for clone MO-3 in ref. 35.



1, Morphine **2**, Codeine **3**, Normorphine **4**, Heroin

The apparent K_d values for binding of Leu-enkephalin were 0.10 ± 0.059 μM, 12 ± 3.8 μM, and 440 ± 91 μM, associated with binding site populations of 3.8 ± 1.8 nmol/g, 0.72 ± 0.24 μmol/g, and 36 ± 6.4 μmol/g, respectively. In this instance, a three-site model was used, since this gave better agreement with experimental data. In general, the cross-reactivity of structurally related compounds was higher in water than in acetonitrile (Fig. 2*B* and Table 1). Competitive binding of four tetra- and pentapeptides (Ala-Ala-Tyr-Ala-Ala, Gly-Gly-Gly-Gly-Gly, Leu-Leu-Val-Phe, and Lys-Phe-Gly-Lys), with totally unrelated amino acid sequences, could not be detected. For morphine binding, the apparent K_d values were determined to be 1.2 ± 0.21 and 24 ± 4.9 μM, associated with site populations of 0.78 ± 0.17 and 6.9 ± 0.65 μmol/g of polymer, respectively. The cross-reactivities recorded in water were slightly higher than those obtained in toluene (Table 2).

Table 1. Cross-reactivity of enkephalin derivatives for binding of marker ligand to the anti-Leu-enkephalin anilide MIP, anti-Leu-enkephalin antibodies, and δ, μ, and κ opioid receptors

Ligand	Cross-reactivity relative to marker ligand, %						
	MIP in water	MIP in acetonitrile	MAb1	MAb2	δ sites	μ sites	κ sites
Leu-enkephalin (H-Tyr-Gly-Gly-Phe-Leu-OH)	100	100	100	100	175	10	≪0.1
Met-enkaphalin (H-Tyr-Gly-Gly-Phe-Met-OH)	68	101	122	1.4	221	20	≪0.1
DALE (H-Tyr-D-Ala-Gly-Phe-Leu-OH)	86	≪0.1	13	36			
DADLE (H-Tyr-D-Ala-Gly-Phe-D-Leu-OH)	55	≪0.1	<1	0.036	100	14	≪0.1
α-Endorphine	47						
Gly-Gly-Phe-Leu	5.2		<1	0.011			
Morphine (**1**)	5.2	≪0.1		≪0.1	2.3	106	0.16

Cross-reactivities are expressed as the molar ratio (in percent) of Leu-enkephalin to ligand giving 50% inhibition of radiolabeled Leu-enkephalin binding. Column MAb1 shows data for clone NOC1 from ref. 21 and column MAb2 shows data for clone RLE1 from ref. 23. Data for δ, μ, and κ sites are from ref. 34. Cross-reactivities are expressed as the ratio (in percent) of the inhibition constant for DADLE to the inhibition constant for ligand on binding of [^{3}H]DADLE to the δ sites. For μ and κ sites, the cross-reactivities are expressed relative to [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and [^{3}H]ethylketazocine, respectively, in an analogous manner.

DISCUSSION

In acetonitrile, Met-enkephalin is the only significant cross-reactant for radiolabeled Leu-enkephalin binding to the Leu-enkephalin anilide MIP (Fig. 2*A*) and the structurally related DALE and DADLE cross-react very weakly. These findings demonstrate that the imprints possess the ability to recognize small structural differences in the substrate. It is well established that the opioid receptors bind ligands in a highly stereospecific manner (17, 36, 37). Since the all-D-configured enkephalin molecule was not available, binding of D- and L-Phe-Gly anilides was measured, where the L-enantiomer constituted a mimic of the N-terminal fragment of the enkephalin molecule. Although radioligand displacement was weak (in both cases a cross-reactivity in the order of <0.1% was recorded), the competition curves clearly showed preferential binding of the L-enantiomer (data not shown). When the polymer particles were packed into HPLC columns and analyzed for their ability to resolve the two enantiomeric forms of Phe-Gly anilide, the Leu-enkephalin anilide MIP was found capable of separating the enantiomers with an α value of 1.7. As expected, the L-isomer was the more strongly retained antipode. Enantioselective binding of L-Phe-Gly anilide was not observed for any nonimprinted reference polymer. These findings demonstrate that information about the chirality of the Leu-enkephalin anilide print molecule is "memorized" by the MIP binding site.

In toluene, the morphine MIPs showed excellent binding affinity and extremely high specificity for morphine. The low cross-reactivities of codeine (**2**) and normorphine (**3**) are especially impressive. The only structural difference between morphine and normorphine is the presence and absence, respectively, of a methyl group on the amino group. Codeine contains an additional methyl group on the 3′-phenolic oxygen compared with the morphine structure. Cross-reactivity of these compounds was, however, expected since even monoclonal antibodies have difficulty in distinguishing between them (Table 2), and in fact, codeine is a notoriously difficult cross-reactant for anti-morphine antibodies (25, 26, 28). Indeed, the MIP cross-reactivity for codeine is significantly lower than that generally reported for monoclonal antibodies (Table 2). Recently, antibodies capable of high levels of discrimination between morphine and codeine have been reported (35).

The ligand binding studies had, up to this point, been performed exclusively in organic solvents (8), and in some instances such solvent systems may have relevance to biologically related studies of molecular recognition. The opioid receptors are membrane bound proteins and the use of organic solvents may mimic the microenvironment of the membrane-surrounded receptor binding site. In fact, the conformational structure of enkephalins in membrane-mimetic environments have been studied and the results obtained have been used to explain the ligand–receptor interaction (38, 39). Nevertheless, we wanted to extend our MIP system to studies of imprint binding in aqueous systems. It was far from obvious that this would be possible, as the substitution of water for organic solvents completely alters the relative strengths of polar and hydrophobic interactions. Water molecules strongly interfere with polar interactions, including hydrogen bonding, whereas hydrophobic effects are very strong in water. It was found that to achieve optimal specific binding, weak to moderately strong buffers and low concentrations of the organic additive (ethanol) should be used. The optimal pH had to be analyzed for each MIP. For morphine MIPs, maximal specific binding was found at pH 6. The nonspecific binding increased at higher pH values possibly due to ion-pair formation between randomly distributed carboxylate ions of the polymer and amino groups of morphine. In aqueous buffer, the binding strength of morphine is only a factor of 10 weaker than in an optimized organic solvent combination. Less-pronounced specificities were obtained—i.e., structurally similar compounds cross-react to a higher degree in water than in organic solvents. This is probably due to interference with the hydrogen-bonding interactions between ligand and imprint by water molecules. It must, however, be emphasized that the morphine MIP still showed similar levels of specificity to those generally reported for monoclonal antibodies (Table 2) (25, 26, 28). For the Leu-enkephalin anilide MIP, the strongest ligand binding was recorded at low pH, as was the nonspecific binding to a nonimprinted reference polymer, and the maximal difference between specific and nonspecific binding was found at pH 4.5. The zwitterionic nature of the peptide may inhibit binding to the carboxylate containing imprints under basic conditions. Again weaker affinity and higher cross-reactivities were obtained in water than in acetonitrile. Very important, however, is the observation that peptides of equal length, with unrelated amino acid sequences, did not bind at all. This fact demonstrates that the binding is not based solely on a pure ion-exchange interaction but that the polymer recognizes amino acid side chains of the peptide. The observation that Gly-Gly-Phe-Leu (an enkephalin derivative lacking the N-terminal tyrosine residue) binds with significant cross-reactivity (Table 1) further supports this notion.

The observation that imprinted polymers show high binding affinity and specificity in aqueous buffers is an important breakthrough, since the rebinding can now be performed under conditions compatible with biological systems. The noncovalent interactions, mainly electrostatic and hydrogen bonds, that are employed for imprinting (and subsequent recognition) are highly dependent on the medium used for polymerization. For maximal efficiency of imprint formation, at least for the type of polymers used in this study, the polymerization reaction should be performed in a solvent as apolar as possible without compromising solubility of the imprint species (33). The use of MIP antibody mimics is not restricted in the same manner; as is shown in this report, MIP recognition is efficient in both apolar organic and aqueous media. The total binding to a polymer can be divided into two categories: specific binding to the imprints and nonspecific binding (in aqueous medium mainly of hydrophobic nature) to the polymer. If the nonspecific element dominates, any selectivity shown by the imprints will be obscured. If the nonspecific binding affinity is weak relative to the specific affinity for imprint ligand, then the ratio of specific to nonspecific binding can be made more favorable by lowering the concentrations of polymer and ligand. Previous studies, with few exceptions (8), have used chromatography (4–6, 9–16) to analyze the recognition properties of imprinted polymers. In a packed column, the amount of polymer is ≈400 mg/ml and the effective concentration of ligand is ≈1 μg/ml. In this study, a polymer concentration of 1–5 mg/ml was used and the concentration of radioligand was 1–3 ng/ml. In addition, the competitive assay format used further reduces the interference of nonspecific binding, since the displacement events occur predominantly at the saturated high-affinity sites.

Imprinting of morphine and the anilide derivative of Leu-enkephalin yielded polymers possessing a capacity to mimic the binding site of opioid receptors. At this stage, we wish to avoid overinterpretation of any apparent resemblance between ∂-sites and the Leu-enkephalin MIP, as demonstrated in Table 1. Although in water a relatively strong binding of morphine to the Leu-enkephalin MIP was observed, in acetonitrile, opiates (such as morphine, naloxone, and naltrexone) interacted only very weakly with this polymer (Table 1). In aqueous buffers the morphine MIP showed only very weak affinity for Leu- and Met-enkephalins and the penta and tetrapeptides Ala-Ala-Tyr-Ala-Ala, Gly-Gly-Gly-Gly-Gly, Leu-Leu-Val-Phe, and Lys-Phe-Gly-Lys (in all cases a cross-reactivity of $<<$0.1% was recorded). Binding of the two enkephalins was, however, slightly stronger than binding of any of the other four peptides.

An important element of the opioid receptor binding site is the "anionic site" (40). The use of methacrylic acid as the functional monomer is appropriate for the creation of such a binding site. Further refinement of the binding-site mimic can be achieved by the use of a multitude of monomers, each with a different functionality (12). Polymerization of such a mixture of monomers would produce polymers possessing truly tailor-made recognition properties.

In a wider perspective, the results reported in this study demonstrate the ability to use chemically prepared polymers with preselected specificity as receptor-binding-site mimics. In this context, mention should be made of our previous study of theophylline-specific MIPs (8). Recently, single-stranded RNA obtained from an oligonucleotide combinatorial library showed strong highly specific binding of theophylline (41). Hence, several possibilities, such as the use of antibodies, small RNAs, and imprinted polymers, are available for the preparation of mimics of the binding sites of receptors. The advantages of our approach are the simple, rapid, and cheap preparation of the MIPs and their stability. Besides fundamental studies of molecular recognition, imprinting may be a useful tool in drug development. For instance, imprints against a drug compound or endogenous ligand may facilitate the primary screening for alternative substances (agonists and/or antagonists) that bind to the binding site associated with the known effector molecule. Furthermore, the high binding affinities and selectivities obtained in aqueous buffers may lead to the use of MIPs in enzyme-labeled ligand binding assay formats, such as ELISA and immuno affinity techniques for isolation/separation of water-soluble biologically related compounds.

We thank Drs. A. Mayes and R. Ansell for linguistic advice. This investigation was supported by the National Swedish Board for Technical Development and the Swedish Natural Science Research Council.

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