Amphipathic α-Helical Structure Does Not Predict the Ability of Receptor-derived Synthetic Peptides to Interact with Guanine Nucleotide-binding Regulatory Proteins*

(Received for publication, July 31, 1992)

Tilman Vosß, Eva Wallner, Armin P. Czernilofsky, and Michael Freissmuth§
From the Ernst Boehringer Institut für Arzneimittelforshung, Department of Protein Chemistry, Fa. Bender+Cô, Dr. Boehringer-Gasse 5–11, A-1121 Vienna, Austria and the §Institute of Pharmacology, University of Vienna, Währinger Strasse 13a, A-1090 Vienna, Austria

In guanine nucleotide-binding regulatory protein- (G protein) coupled receptors, an amphipathic α-helix has been postulated to be the common structural determinant in the NH2- and COOH-terminal portions of the third intracellular loop representing the major interaction site with the G proteins. Here we assessed the ability of six peptides derived from these sites of the human dopamine D2-, D3-, and β,-adrenergic receptors to either activate G proteins directly or to uncouple the activity of their respective receptors in a native membrane environment. In addition, the cross-reactivity was analyzed. Nonspecific effects occurring at high concentrations were differentiated from G protein-specific effects. The peptide D2N derived from the NH2-terminal part of the third intracellular loop of the dopamine D2 receptor was the only one capable of specifically reversing the action of its receptor, the dopamine-mediated inhibition of the adenyl cyclase. Furthermore, only D2N stimulated pertussis toxin-sensitive G proteins. However, D2N as the only peptide exhibiting specific effects did not exhibit the predicted amphipathic α-helix observed for mastoparan (Higashijima, T., Burnier, J., and Ross, E. M. (1990) J. Biol. Chem. 265, 14176–14186) as demonstrated by circular dichroism spectroscopy. In contrast, a peptide for which a certain degree of helicity was verified spectroscopically (D1C) was neither active in GTPase and adenyl cyclase determinations, nor did it block the receptor-mediated cyclase activation. Hence, the amphipathic α-helix does not represent the main structural determinant for the receptor-G protein interaction site.

Guanine nucleotide-binding regulatory protein- (G protein)3 coupled receptors share the characteristic motive of seven hydrophobic domains which are thought to represent transmembrane domains. While these hydrophobic domains are highly conserved among various receptors the connecting intra- and extracellular loops are fairly variable in their sequence (Strader, et al., 1989). Several molecular biological studies with chimeric receptors and deletion mutants indicate the involvement of the intracellular loops in the interaction with the G proteins. In particular the NH2- and COOH-terminal ends of the third intracellular loop were demonstrated to form the major, but not the sole, interaction sites with the G proteins (O’Dowd et al., 1989; Strader et al., 1989; Strosberg et al., 1991).

A lack of sequence homology in the third intracellular loop does not allow prediction of the consensus sequences for coupling to specific G proteins. Secondary structure predictions, however, suggest that the NH2- and COOH-terminal regions of this loop may form amphipathic α-helical extensions of the transmembrane helices 5 and 6 (Cheung et al., 1988). Therefore, this α-helical structure was proposed as the main determinant in the interaction of the receptor with G proteins (Strader et al., 1989).

Synthetic peptides corresponding to these regions were used to study the rhodopsin-transducin (König et al., 1989) and in particular the β-adrenergic G protein interaction (Palm et al., 1989; Cheung et al., 1991; Münch et al., 1991). These studies demonstrated the ability of the peptides to interfere with the receptor-G protein interaction resulting in an uncoupling of the receptor and to at least partially activate G proteins.

In order to better understand the specificity of the sequences as well as their structural determinants involved in the interaction, we compared the effect of peptides from different receptors. Human embryonal kidney cells expressing the transfected human receptors were used in order to provide a defined molecular system in a native membrane environment. The peptide activities were correlated with the structural properties of the respective peptides.

MATERIALS AND METHODS

Peptide Synthesis—Peptides were synthesized by the solid-phase method using the fluorenlymethoxycarbodiimide chemistry. The identity and purity of the high performance liquid chromatography-purified peptides was verified by plasma-desorption mass spectrometry (Bio Ion BIN-10K). Mastoparan was purchased from Sigma.

Cell Culture—Human embryonal kidney cells (293 cells) were grown in Dulbecco’s modified Eagle’s modified/F-12 medium (GIBCO) supplemented with 10% fetal calf serum (GIBCO), 100 units/ml penicillin, 100 µg of streptomycin, and 2 mM L-glutamine. 293 cells were transiently transfected with human dopamine D2 (Deary et al., 1990) and β2 receptor (Frielie et al., 1987) using the calcium phosphate method (Sambrook et al., 1989). The human dopamine D2 receptor (subtype D2A) was stably transfected into 293 cells (DalToso et al., 1989). For pertussis toxin treatment 293 cells were cultivated for 12–16 h in medium containing 100 ng/ml pertussis toxin (Calbiochem).

Membrane Preparation—PBS-washed cells were detached from the plates by incubation with EDTA buffer (145 mM NaCl, 10 mM Tris-HCl, 1.5 mM EDTA, pH 7.4) at 37°C. The harvested cells were
pelletted at 1,000 × g for 5 min and washed once with warm PBS. Cells were resuspended in cold PBS and disrupted with an Ultra Turrax homogenizer at setting 4 for 20 s. The debris was removed by centrifugation at 2,000 × g for 5 min. The membranes were pelletted by centrifugation in a SW 50 rotor (Beckman Instruments) at 100,000 × g for 20 min. The shock-frozen membrane pellets were stored in liquid nitrogen until used. Membrane protein concentration was determined by the method of Bradford.

Adenylyl Cyclase Assay—The enzymatic activity was determined at 32 °C for 20 min in a final volume of 155 µl containing 20 mM HEPES, pH 7.5, 0.75 mM creatine phosphate, 30 units/ml creatine kinase, 2.5 mM MgCl2, 0.1 mM dithiothreitol, 0.1 mM EGTA, 100 µM 1-methyl-3-isobutyl-xanthine, 50 µM GTP, and 50 µM ATP, using 50 µg of membrane protein. The membranes were preincubated with the peptides for 40 min at 0 °C in 20 mM HEPES buffer. The receptors were stimulated with the indicated amounts of the respective agonists. The reaction was stopped by the addition of 100 µl of MeOH/CHCl3 (1:1 mixture). The samples were vortexed and centrifuged in a benchtop centrifuge (Sigma TK20) at 13,000 revolutions/minutes for 5 min. The MeOH/CHCl3-extracted material was analyzed for the cAMP content using a cAMP-labeled assay tracer and a specific cAMP-binding protein (Amersham Corp., cAMP assay kit) using either 25 or 50 µl of the cAMP containing aqueous phase (see above) following the supplier’s protocol.

GTPase Assay in Membranes—GTPase activity was determined in the absence and presence of synthetic peptides. In the latter case membranes were first incubated with the synthetic peptides for 1 h at 0 °C. The enzymatic activity was determined at 32 °C for 5 min in a final volume of 100 µl containing 20 µg of membranes according to Hausleitner et al. (1985). A GTP isotope dilution experiment was carried out to verify the K0, the low and high affinity GTPase (data not shown). A biphasic curve was observed with a plateau between 10 and 40 µM. Hence the GTPase activity obtained at 10 µM GTP was subtracted from the obtained data to correct for the contribution of the high K0 GTPase activity (Hausleitner et al., 1985).

GTPγS Binding to Purified Gα/Gβ Proteins—A Gα protein fraction was purified from bovine brain membranes according to Sternweis and Robishaw (1984). The fractions containing pertussis toxin subunit were pooled and no attempt was made to resolve Gα from Gβ. As judged from Coomassie Blue-stained gels, the preparation contained about equimolar α- and β-subunits. Immunoblotts with subtype-specific antibodies (McCue et al., 1992) showed that 80% of the α-subunits were Gαs, roughly equivalent amounts of Gαi, and Gαq, and traces of Gαo. The kinetics of GTPγS binding to this purified G protein preparation were determined as described by Higashijima et al. (1988) with the following modifications: the concentration of [32P]GTPγS was 0.5 µM (20,000 cycles/min pmol), and the final Lubrol concentration was 0.01%. Parallel experiments were also conducted in which the protein was reconstituted into phosphatidyl choline vesicles. These experiments gave results that were qualitatively similar to those observed at low Lubrol concentrations (data not shown).

GTPase Activity in Purified Gα/Gβ Proteins—The assay medium corresponded to that employed for GTPγS binding except that 0.5 µM [γ-32P]GTP (7,000 cpn/pmol) was used instead of GTPγS. Unhydrolyzed GTP was removed by adsorption to activated charcoal.

Circular Dichroism Spectroscopy—CD spectra of peptides were measured with a JASCO 600 spectrometer at 32 °C with 10-mm pathlength. Spectra were scanned in 0.2-nm intervals at 50 nm/min. Peptides were dissolved at 20 µM in 5 mM Tris-HCl, pH 7.4, in the presence of sonicated 0.5 mM 1-palmitoyl-2-oleoyl-phosphatidylcholine vesicles (Sigma).

Structural Predictions—Chou-Fassman calculations (Chou and Fassman, 1978) were carried out using the PepPlot program of the GCG-software package (Devereux et al., 1984).

Statistics—All data are expressed as the mean of two to three individual experiments ± S.E. Each experiment was carried out in duplicates. Significance calculations (paired t test) were carried out using the InStat program (GraphPad Software).

RESULTS

Membrane preparations of untransfected and transfected 293 cells expressing the human D1, D2, and β2 receptors were tested for adenylyl cyclase activity in response to the respective agonists. A 2-4-fold stimulation of adenylyl cyclase activity was observed by the transiently transfected dopamine D1- and β2-adrenergic receptors as well as for the intrinsically PGE1 receptor (Fig. 1, left panel). The functional coupling of the stably transfected dopamine D2 receptor was verified by agonist-dependent inhibition of the PGE1-stimulated adenylyl cyclase. The enzymatic activity was usually reduced to about 30% of the maximal activity in the presence of 1 µM PGE1 (Fig. 1, right panel).

We have tested the ability of receptor-derived peptides to (i) inhibit the response elicited by the homologous (uncoupling activity) or a heterologous receptor (cross-reactivity); (ii) the ability to mimic the effect of receptor activation on adenylyl cyclase activity and (iii) the direct activation of G proteins; (iv) we attempted to separate specific receptor- and G protein-dependent effects of the peptides from nonspecific effects.

The sequences of the peptides (Table I) were selected from the NH2 and the COOH terminus of the third intracellular loop of the human D1 receptor (Dearry et al., 1990), D2 receptor (DalToso et al., 1990), and the β2-adrenergic receptor (Frielle et al., 1987). These sequences were chosen to allow a direct comparison with the effect of the homologous β2- and turkey β2-adrenoceptor peptides studied earlier (Cheung et al., 1991; Münch et al., 1991). All peptides contain 3 residues of the predicted transmembrane domains. These sequences are thought to be crucial for membrane interaction and the formation of a biologically active conformation (Okamoto et al., 1990; Münch et al., 1991).

In membranes prepared from cells expressing D2 receptors, the D2N peptide displayed a bell-shaped curve. At low concentrations (1–10 µM), the dopamine-mediated inhibition of adenylyl cyclase activity was partially reversed. In the presence of high D2N concentrations (>10 µM), adenylyl cyclase

![Fig. 1. Adenylyl cyclase activity in membranes from 293 cells expressing the respective receptors. The left panel demonstrates the adenylyl cyclase stimulation by the transfected dopamine D1 receptor (100 µM dopamine), the β2-adrenoceptor (10 µM isoproterenol), and the intrinsically PGE1 receptor (1 µM PGE1), relative to the background activity (basal). Basal activities were 11.7 ± 2, 28.7 ± 1, and 8.5 ± 0.2 pmol cAMP/mg/min, respectively. The D2 receptor response (right panel), stimulation by 100 µM dopamine in the presence of 1 µM PGE1, basal activity 2.1 ± 0.5 pmol cAMP/mg-min is expressed as percentage of PGE1 (1 µM) stimulated adenylyl cyclase activity (PGE1).

<table>
<thead>
<tr>
<th>Table I Sequences of the synthesized peptides</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2N(208-227)</td>
</tr>
<tr>
<td>D2C(360-377)</td>
</tr>
<tr>
<td>D1N(135-140)</td>
</tr>
<tr>
<td>D1C(360-377)</td>
</tr>
<tr>
<td>β1N(246-251)</td>
</tr>
<tr>
<td>β1C(518-528)</td>
</tr>
<tr>
<td>Mastoparan</td>
</tr>
<tr>
<td>VYIYKTVLRRRRKVRNTK</td>
</tr>
<tr>
<td>RKLSSQXKEKATQGNALI</td>
</tr>
<tr>
<td>TRNYIAQKQTKPALAR</td>
</tr>
<tr>
<td>SFKMSFSPFRTEVTKL35TSV</td>
</tr>
<tr>
<td>RVFRAEQQVKKIDCTR</td>
</tr>
<tr>
<td>REQKALKTGLII</td>
</tr>
<tr>
<td>INLKALALLAKKIL</td>
</tr>
</tbody>
</table>
activity was reduced to levels below those observed in the absence of the peptide (see Fig. 2A, top). The D1C and the β1N peptide inhibited the adenylyl cyclase activity at concentrations above 20 μM. All other peptides were unable to interfere with the effect of dopamine in a statistically significant manner.

The adenylyl cyclase stimulation by the D1 receptor was inhibited by both D1 peptides (Fig. 2B, center). The effect of D1C was nearly quantitative, while that of D1N was less pronounced. In addition, the D2N peptide exhibited a nearly quantitative inhibitory effect at concentrations above 10 μM. A 50% inhibition was observed for the β1N peptide.

The β1 peptides are essentially inactive when tested in membranes prepared from cells transfected with the β1-adrenoceptor (Fig. 2C, bottom). A slight stimulation of enzyme activity by the D2N peptide (10 μM) was observed in these cells. (Fig 2C, top). This, however, was not statistically significant.

We note that several peptides (D2N, D1N, D1C, and β1N) displayed strong inhibitory effects at concentrations exceeding 10 μM. These inhibitory effects are almost certainly non-specific (i.e. unrelated to G protein coupling, see below). For unknown reasons, these effects were more pronounced in cells expressing the D1 receptor than those transfected with the β1 receptor or the D2 receptor.

Membranes from untransfected cells were used to differentiate between specific, i.e. receptor and G protein-dependent, and nonspecific effects of the inhibitory peptides. D2N revealed its inhibitory effect on PGE1-stimulated adenylyl cyclase at concentrations >10 μM (Fig. 3, Δ). The enzymatic activity was reduced to less than 20% of the maximal value thus paralleling the effects observed in the membranes containing the D2 receptor (Fig. 2A, top). In membranes in which G1-dependent adenylyl cyclase stimulation was activated by GTPγS, a slight, although statistically significant, stimulation of adenylyl cyclase up to 10 μM (p < 0.006) preceded the inhibition at high concentrations (Fig. 3, ○). This pattern of stimulation (p < 0.006) at lower peptide concentration and inhibition above 10 μM was also observed upon PGE1 stimulation when 293 cells were pretreated with pertussis toxin to disrupt G1-linked pathways (Fig. 3, *). The effects of the D1 peptides and β1N were similar to those shown in Fig. 2B, center and bottom, respectively (data not shown).

These observations show that the inhibition of adenylyl cyclase by D2N, D1N, D1C, and β1N observed at higher concentrations (>10 μM) are nonspecific, i.e. receptor- and G protein-independent. From the data summarized in Figs. 2 and 3, we conclude that with the exception of D2N, the peptides are essentially inactive with respect to specific receptor uncoupling. In addition, D1N, D1C, β1N, β1C, and D2C failed to further enhance the stimulation of adenylyl cyclase by GTPγS. Hence, these peptides are not capable of mimicking receptor-mediated activation of the enzyme via G1. The peptide D2N, in contrast, seems to exert dual effects: a specific reversal of D1 receptor-mediated adenylyl cyclase inhibition at concentrations ≤10 μM and a nonspecific inhibitory effect at higher concentrations.

The experiments described so far were carried out to identify peptides capable of acting as specific receptor antagonists. In the following we analyzed the ability of the peptides to act as agonists, i.e. to directly stimulate G proteins. The release of prebound GDP is the receptor-controlled rate-limiting step in the activation-deactivation cycle of G proteins (Freissmuth et al., 1989). Hence, stimulation of low Km GTPase activity in the native membrane environment directly reflects the ability of the peptides to mimic receptor-mediated activation of G proteins.

In order to discriminate between interactions with either the G1 or the G1/G2 pools, membranes from 293 cells treated with pertussis toxin were compared with membranes from untreated cells. Mastoparan which is known to activate the G1/G2 pool (Higashijima et al., 1988) was used to verify the efficiency of the pertussis toxin treatment (Table II). The D2N peptide is the only peptide other than mastoparan which is capable of stimulating GTPase activity at concentrations up to 10 μM in a statistically significant manner (p < 0.03 for D2N and MP). This stimulation is pertussis toxin-sensitive. An inhibitory effect is observed at higher concentrations. All other peptides do not cause any significant stimulation in either untreated or treated membranes. This was verified over a concentration range of 0.1–50 μM.

The data presented thus far indicate that, in the native cell membrane, D2N acts in a manner analogous to a partial agonist. D2N is capable of reversing D1 receptor-mediated
TABLE II
Effect of peptides on GTPase activity

<table>
<thead>
<tr>
<th>Peptide</th>
<th>-Pertussin toxin</th>
<th>+Pertussin toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>D2N*</td>
<td>1.32 ± 0.19</td>
<td>0.86 ± 0.22</td>
</tr>
<tr>
<td>D2C</td>
<td>1.02 ± 0.17</td>
<td>1.12 ± 0.15</td>
</tr>
<tr>
<td>D1N</td>
<td>1.02 ± 0.24</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>D1C</td>
<td>1.08 ± 0.21</td>
<td>1.11 ± 0.06</td>
</tr>
<tr>
<td>β1N</td>
<td>1.00 ± 0.17</td>
<td>1.21 ± 0.19</td>
</tr>
<tr>
<td>β1C</td>
<td>1.08 ± 0.26</td>
<td>1.15 ± 0.13</td>
</tr>
<tr>
<td>Mastoparan*</td>
<td>1.41 ± 0.12</td>
<td>0.90 ± 0.14</td>
</tr>
</tbody>
</table>

The inhibition of adenyl cyclase activity, but, per se, stimulates guanine nucleotide turnover by Gαi. To further substantiate this interpretation, we measured the effect of D2N on the association rate of GTPγS to a purified pool of Gαi/Gαo proteins. D2N and mastoparan enhanced the binding rate of GTPγS by a factor of 3 and 5, respectively (Fig. 4A). The concentration-response curve of D2N exhibited a biphasic behavior. Maximal rate enhancement (3-fold increase) was obtained at a concentration of 10 μM (Fig. 4B). The decline at concentrations exceeding 10 μM parallels the nonspecific effect observed in 293 membranes (see Figs. 2 and 3) and probably arises from partial denaturation of the protein at high peptide concentrations. The stimulation by mastoparan did not reach saturation at 100 μM, which is consistent with published data (Higashijima et al., 1988). The apparent affinity of D2N under the assay conditions employed exceeded that of mastoparan. This difference in affinity was exploited to test for antagonistic effects of D2N on the mastoparan-promoted guanine nucleotide exchange. At a maximally active concentration of D2N, no additional enhancement was observed at low concentrations of mastoparan, and the rate enhancement at high concentrations of mastoparan (30 and 100 μM) was suppressed by D2N (Fig. 4B, Δ). The other receptor-derived peptides produced only modest effects on the association rate of GTPγS binding to Gαi/Gαo with a maximally 1.5-fold stimulation at 100 μM. The data for D1N as an example for an inactive peptide are shown in Fig. 4B (\( V \)).

Furthermore, we measured the effects of all peptides on the steady-state GTPase activity of the purified pool of Gαi/Gαo proteins. D2N and MP expectedly stimulated the turnover rate about 3- and 4-fold, respectively (Table III). All other peptides did not exhibit any effects.

A correlation has been shown to exist for mastoparan and modified mastoparan peptides between amphipathic α-helicity and their ability to functionally interact with Gαi/Gαo proteins in reconstituted systems (Higashijima et al., 1990).

We therefore analyzed the properties of the peptides by CD spectroscopy. Selected spectra of peptides reconstituted in phospholipid vesicles and recorded at 32 °C, the temperature used for adenyl cyclase and GTPase assays, are shown in Fig. 5. Mastoparan exhibited the expected strong negative peak around 222 nm characteristic for a mostly α-helical structure (Chen et al., 1974; Higashijima et al., 1990). D2N as the only peptide capable of activating G proteins does not display any significant α-helicity. Furthermore, D1C which is neither capable of uncoupling the D1 receptor nor of stimulating G proteins appeared to have a partially helical structure.

The molar ellipticities (θ) at 222 nm are summarized in Table IV. These data are compared with the charge Δ, the hydrophobic moment μ reflecting the amphipathy and the average hydrophobicity HΦ.

**DISCUSSION**

Previous studies have focused on the interaction of synthetic peptides with G proteins in reconstituted systems (Hi-
Receptors which couple to the G\textsubscript{i}/G\textsubscript{o} group have been shown to discriminate among the subtypes of G\textsubscript{i} and G\textsubscript{o} (Senegles et al., 1990; Kurose et al., 1991; Freissmuth et al., 1991; Bertin et al., 1992). It is interesting to test whether this selectivity is also specified by the third intracellular loop. Experiments are currently underway with D2N to test this question.

A slight stimulation of adenyl cyclase by D2N was observed in membranes stimulated with GDP\textsubscript{S} or in pertussis-treated membranes. The mechanism underlying this activation is not clear at present but may be explained by direct activation of G\textsubscript{i}. Alternatively, it may be compatible with recent observations which indicated a direct activation of adenyl cyclase by \( \beta \gamma \)-subunits (Tang and Gilman, 1991), a phenomenon which has also been observed in cell membranes upon stimulation of the adenosine A\textsubscript{j}- or the D\textsubscript{2}-receptor (Federman et al., 1992).

Since substitutions of amino acids in recombinant receptors by site-directed mutagenesis may produce global effects on protein structure, the use of receptor-derived peptides has been advocated as an alternative, complementary tool to study functionally important regions of receptors. However, in general, only a small fraction of the peptide adopts the correct conformation and, hence, large concentrations are usually employed. It is therefore not surprising that, at these high concentrations, additional, nonspecific effects are observed. An analysis of these nonspecific effects is of particular importance if the activity of the peptides is determined using an effector system in a native cell membrane. We have separated these nonspecific effects from specific effects based on the criterion that the former were independent of the mode of activation of adenyl cyclase (i.e., receptor-type, GDP\textsubscript{S}, forskolin) and resistant to pertussis toxin.

No correlation was found between activity and the physical properties of the peptides. \( \alpha \)-Helicity and average hydrophobicity are not essential for activity as seen for D2N (low helicity and hydrophobicity) and mastoparan (high helicality and hydrophobicity). Furthermore, the concept of amphipathy (Cheung et al., 1988) does not seem to hold any more. All peptides with a high value of \( \mu \) did not exhibit any activity. The active peptides D2N and mastoparan, in contrast, exhibited nearly the lowest \( \mu \) values.

The \( \alpha \)-helicity of mastoparan which is essential for its activity (Higashijima et al., 1990) and predictions derived from Chou-Fasman analysis of the hamster \( \beta \textsubscript{2} \)-adrenoceptor (Cheung et al., 1988) have led to the hypothesis that the common structural theme of NH\textsubscript{2}- and COOH-terminal domains of the third intracellular loop is an amphipathic \( \alpha \)-helix. These helices are believed to represent the structural determinants essential for a functional interaction site with the G proteins.

Similar calculations carried out for the D\textsubscript{2} receptor did not reveal any probability for the occurrence of residues characteristically found at the beginning of or within an \( \alpha \)-helical structure at the NH\textsubscript{2} terminus of the third intracellular domain (Fig. 6).

These data demonstrate that the structural characteristics summarized in Table IV are not useful in identifying parameters essential for active peptides. Hence, our data cast doubt on the currently favored model of G protein-coupled receptors, which assumes that the common structural determinant of the G protein interaction site is an amphipathic \( \alpha \)-helix. Therefore, a new concept has to be developed for the general characteristics of this interaction site since no homologies are observed in either the primary structure or in secondary structure elements.

Acknowledgments—We thank Drs. P. Seeburg and M. Caron for...
Structure-Function Analysis of Receptor-derived Peptides

D2, D1 and β1 receptor cDNAs, Dr. A. Himmler for providing the receptor expression plasmids and Dr. H. Ahorn for the synthesis of the receptor peptides. We are grateful to Prof. L. Mátr for his support of this study.

REFERENCES