Ethanol Antagonist Peptides: Structural Specificity without Stereospecificity

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ABSTRACT
Increasing evidence suggests that ethanol damages the developing nervous system partly by disrupting the L1 cell adhesion molecule. Ethanol inhibits L1-mediated cell adhesion, and compounds that antagonize this action also prevent ethanol-induced embryotoxicity. Two such compounds are the small peptides NAPVSIPQ (NAP) and SALLRSIPA (SAL). We showed previously that NAP and SAL antagonize ethanol inhibition of L1 adhesion at femtomolar to picomolar concentrations. Here we demonstrate that, despite this extraordinary potency, both NAP and SAL lack stereospecificity. D-NAP, a peptide composed entirely of D-amino acids, was an effective ethanol antagonist in NIH/3T3 cells transfected with human L1 and in the NG108-15 neural cell line. Interestingly, Ala-substituted derivatives of D-NAP demonstrate the same structure-activity relation as the corresponding derivatives of L-NAP. The Ser-Ile-Pro motif was important for the ethanol antagonist activity of D-NAP, L-NAP, and L-SAL, with Ile being the most critical element in all three. Like L-NAP, D-NAP effectively reduced ethanol-induced growth retardation in mouse whole embryo culture. The potential resistance of D-peptides to proteases makes D-NAP a potentially attractive agent for the prevention of fetal alcohol syndrome.

Increasing evidence suggests that ethanol (ETOH) damages the developing nervous system partly by disrupting the function of the neural cell adhesion molecule L1. The neuropathological lesions in fetal alcohol syndrome resemble those of children with mutations in the gene for L1 (Charness et al., 1994; Ramanathan et al., 1996). Ethanol potently inhibits cell adhesion mediated by human L1 (L1 adhesion) in transfected fibroblasts and by rodent L1 in NG108-15 neuroblastoma x glioma and rat cerebellar granule cells (Charness et al., 1994; Ramanathan et al., 1996; Wilkemeyer and Charness, 1998; Wilkemeyer et al., 1999). Moreover, ethanol also inhibits L1-mediated neurite extension in rat cerebellar granule cells plated on an L1 substrate (Bearer et al., 1999).

The inhibition of L1 adhesion by a series of straight, branched, and cyclic alcohols shows remarkable structural specificity, consistent with a ligand-receptor interaction (Wilkemeyer et al., 2000). We refer to those alcohols that inhibit L1 adhesion as alcohol agonists. Selective alcohols that closely resemble the alcohol agonists but do not themselves inhibit L1 adhesion abolish the actions of the alcohol agonists (Wilkemeyer et al., 2000, 2002b). We refer to these compounds as alcohol antagonists. The antagonist activities of some of these alcohols are surmounted by increasing concentrations of alcohol agonists, whereas the antagonist activities of other alcohol antagonists are not, suggesting that there are at least two mechanisms of antagonist action (Wilkemeyer et al., 2002b). Importantly, at least one alcohol antagonist, 1-octanol, potently inhibits ethanol-induced apoptosis and growth retardation in mouse whole embryo culture (Chen et al., 2001).

We have identified a second class of compounds that potently antagonizes ethanol inhibition of L1 adhesion: the peptides NAPVSIPQ (NAP) and SALLRSIPA (SAL) (Wilkemeyer et al., 2002a). These peptides are active fragments of the glial-derived activity-dependent neuroprotective protein and activity-dependent neurotrophic factor, respectively (Brenneman and Gozes, 1996; Bassan et al., 1999; Gozes and Brenneman, 2000; Brenneman et al., 2000b). NAP and SAL...
protect neural cells and intact animals from a diverse array of insults (NAP neuroprotection), including fetal alcohol exposure (Gressens et al., 1997; Brenneman et al., 1998; Glazner et al., 1999, 2000; Gozes et al., 2000; Beni-Adani et al., 2001; Spong et al., 2001; Lekter et al., 2002). Neuroprotection occurs at femtomolar concentrations and is disrupted by amino acid substitutions in the Ser-Ile-Pro (SIP) region of the peptides, consistent with a high-affinity, structurally specific interaction with a target molecule (Brenneman et al., 1998; Wilkemeyer et al., 2003).

NAP and SAL also antagonize ethanol inhibition of L1 at femtomolar to picomolar concentrations (NAP ethanol antagonism) (Wilkemeyer et al., 2002a); however, NAP neuroprotection and NAP ethanol antagonism show different structure-activity relations (Wilkemeyer et al., 2003). NAP neuroprotection is abolished by Ala substitution of Ser-5 or Pro-7 (P7A-NAP peptide) but is much less sensitive to Ala substitution of Ile-6. In contrast, NAP ethanol antagonism is markedly reduced by Ala substitution of Ile-6 but is less sensitive to Ala replacement of Ser-5 or Pro-7. Notably, P7A-NAP, which lacks neuroprotective activity but retains ethanol antagonist activity, potently prevents ethanol-induced growth retardation in mouse whole embryo culture (Wilkemeyer et al., 2003). These findings suggest that NAP prevents ethanol teratogenesis by antagonizing ethanol inhibition of L1 rather than through its broad neuroprotective actions. The mechanism of NAP-mediated ethanol antagonism is unknown, and much more must be learned before NAP or related compounds can be developed for the prevention of fetal alcohol syndrome.

One common property of high-affinity receptor-ligand interactions is stereospecificity. Almost all proteins are sensitive to Ala replacement of Ser-5 or Pro-7. Notably, P7A-NAP, which lacks neuroprotective activity but retains ethanol antagonist activity, potently prevents ethanol-induced growth retardation in mouse whole embryo culture (Wilkemeyer et al., 2003). These findings suggest that NAP prevents ethanol teratogenesis by antagonizing ethanol inhibition of L1 rather than through its broad neuroprotective actions. The mechanism of NAP-mediated ethanol antagonism is unknown, and much more must be learned before NAP or related compounds can be developed for the prevention of fetal alcohol syndrome.

One common property of high-affinity receptor-ligand interactions is stereospecificity. Almost all proteins are synthesized with L-amino acids, and the potency of the corresponding D-amino acid isomers is often greatly reduced (Fuji, 2002); however, D-amino acids have been introduced within peptides to increase their resistance to proteases, thereby increasing their in vivo stability (Kreil, 1997). In some instances, the presence of a D-amino acid at a specific site also increases biological activity (Kamata et al., 1989; Fujimoto et al., 1991; Das et al., 2003). To explore the mechanism of NAP and SAL ethanol antagonism, we studied a series of peptides in which all of the L-amino acids were replaced with D-amino acids (D-NAP and D-SAL). These experiments were prompted partly by preliminary reports showing that D-NAP and D-SAL retain protective activity against neural insults, including fetal alcohol exposure (Brenneman et al., 2000a; Spong et al., 2000).

Materials and Methods

Materials. Ethanol was purchased from Fisher Scientific Co. (Pittsburgh, PA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or as indicated. Peptides were purchased from Peptide Technologies Corporation (Gaithersburg, MD) and New England Peptide, Inc. (Gardner, MA). Peptide purity (>95%) was assessed by these companies using high-performance liquid chromatography and mass spectrometry. The peptides were dissolved in 10% dimethyl sulfoxide in phosphate-buffered saline (0.13 M NaCl, 0.003 M KCl, 0.01 M Na2HPO4, 0.002 M KH2PO4) and stored at −80°C as 1 mM aliquots. Peptide derivatives of NAP and SAL were named for the single letter amino acids replaced by Ala and their position relative to the N terminus (e.g., SSA-NAP; Ala replaces Ser-5 in NAP).

Cell Cultures. Two subclones of transfected NIH/3T3 cells were used in these studies: 2A2-L1 and Vec-A5. The 2A2-L1 cell line is an ethanol-sensitive subclone derived from a stable transfection of NIH/3T3 cells with the human L1 cDNA, and Vec-A5 is a subclone from a transfection with the empty expression vector (Wilkemeyer and Charness, 1998). NIH/3T3 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% normal calf serum and 400 μg/ml G418 (Invitrogen, Carlsbad, CA). NG108-15 cells were plated in serum-free, defined medium (Charness et al., 1986). Two days before the start of cell adhesion assays, serum-free medium containing bone morphogenetic protein-7 (BMP-7) (Creative BioMolecules, Inc., Hopkinton, MA) (10 ng/ml, final) was added daily to the NG108-15 cells. Both cell lines were cultured at 37°C in an atmosphere of 90% air and 10% CO2.

Cell Adhesion Assay. Cell adhesion was measured using a short-term aggregation assay (Wilkemeyer et al., 2002a). Briefly, cells were detached and dissociated to single-cell suspension with calcium- and magnesium-free phosphate-buffered saline supplemented with 2 mM EDTA and 0.1 mg/ml DNase and diluted to 330,000 cells/ml for the NIH/3T3 cells and 250,000 cells/ml for the NG108-15 cells. Peptides and ethanol were mixed together before their addition to the cells, which were then gently rotated for 30 min on ice. Cells were viewed at a final magnification of 200×, and each well was scored for single and adherent cells in 5 or 6 microscopic fields of view. The percentage of adherent cells was calculated for each field and averaged. We define L1-mediated cell adhesion (L1 adhesion) as the difference in the percentage of adherent cells between an L1-expressing cell line (2A2-L1 or BMP-7-treated NG108-15 cells) and a non-L1-expressing cell line (Vec-A5 or NG108-15 cells grown in serum-free medium). Ethanol inhibition of cell adhesion was calculated as 100 × (1 − the ratio of L1 adhesion in the presence and absence of ethanol). We define antagonists as compounds that alone have no effect on L1 adhesion but block the ethanol inhibition of L1 adhesion. Antagonist activity was calculated as 100 × (1 − (% percent inhibition of cell adhesion by ethanol plus peptide)/percent inhibition of cell adhesion by ethanol alone)).

Whole Embryo Culture. On embryonic day 8 (ED8), C57BL/J embryos were explanted under a dissecting microscope with the removal of maternal decidua, trophoblast, parietal yolk sac, and Reichert’s membranes, while the visceral yolk sac, ectoplacental cone, and amnion remained intact (Kotch et al., 1995). The embryos that have 3 to 5 somite pairs were used for culture. Each embryo was placed in a 30-ml vial containing 2.5 ml of medium (75% heat-inactivated rat serum, 25% Tyrode’s solution). The vials were flushed with a mixture of 5% O2, 5% CO2, and 90% N2 and attached to a rotating wheel in an incubator maintained at 37°C. Explanted embryos were exposed to experimental agents for 6 h only, followed by culture for an additional 20 h in control medium. Extraembryonic membranes were removed, and the embryos were examined under a dissecting microscope without knowledge of treatment condition for morphological assessment and to determine the number of somite pairs. Each embryo was cultured separately, constituting an independent experiment.

Statistical Analysis. Using StatView software, the differences among group means were analyzed by analysis of variance. Multiple comparison post tests between groups were conducted using Bonferroni/Dunn comparisons.

Results

D-NAP Is a Potent Ethanol Antagonist in L1-Transfected NIH/3T3 Cells. NAP is a potent and structurally specific antagonist of ethanol inhibition of L1 adhesion (Wilkemeyer et al., 2002a, 2003). To determine the stereospecificity of this interaction, we studied the antagonist properties of a NAP peptide that was composed entirely of D-amino acids (D-NAP). Cell adhesion assays were performed
using human L1-expressing NIH/3T3 cells (2A2-L1) in the absence and presence of 100 mM ethanol or 100 mM ethanol plus various concentrations of either d-NAP or l-NAP. Ethanol reduced L1 adhesion by 48 ± 2% (n = 47). d-NAP and l-NAP each produced a monophasic, dose-dependent antagonism of ethanol inhibition of L1 adhesion (Fig. 1). Antagonism by d-NAP was first apparent at concentrations of 10^−17 M and increased progressively over 10 log orders. The IC_{50} value for d-NAP, derived by linear regression analysis of the dose-response curve, was approximately 2.5 × 10^−14 M (Table 1). The antagonist potency of d-NAP was comparable to that of l-NAP (IC_{50}, 6.5 × 10^−14 M). d-NAP from two different commercial vendors had very similar activity profiles and IC_{50} values (2.5 × 10^−14 M versus 7.3 × 10^−14 M; data not shown). These experiments demonstrate a surprising lack of stereospecificity for NAP antagonism of ethanol inhibition of L1 adhesion.

**d-NAP Does Not Modulate Basal Cell Adhesion.** d-NAP could antagonize ethanol-mediated inhibition of L1 adhesion by increasing basal levels of cell adhesion. Therefore, we measured L1 adhesion in 2A2-L1 cells after a 30-min exposure to 10^−7 M d-NAP. L1 adhesion did not differ significantly in the presence or absence of d-NAP (control, 40 ± 3.5%; d-NAP, 42 ± 2.4%, n = 7; p > 0.05). In contrast, 100 mM ethanol alone decreased L1 adhesion (27 ± 2.7%, n = 7) by an average of 58 ± 6.7% in the same set of experiments. Indirect immunofluorescence with flow cytometry showed that d-NAP or l-NAP did not change the cell surface expression of L1 (data not shown). These results are consistent with previous data showing that the alcohol antagonists 1-octanol, 1-pentanol, and the peptides l-NAP and l-SAL do not modify L1 expression or adhesion (Wilkemeyer et al., 2000, 2002a).

**d-NAP Is an Effective Ethanol Antagonist in Neural Cells.** We have used the neuroblastoma x glioma cell line NG108-15 to model ethanol inhibition of cell adhesion in the developing nervous system (Charness et al., 1994; Wilkemeyer et al., 1999, 2000, 2002a). Treatment of NG108-15 cells with BMP-7 for 48 h increases cell adhesion by inducing the expression of endogenous L1 and the neural cell adhesion molecule (Perides et al., 1992, 1993). The antagonist potency and efficacy of l-NAP is similar in BMP-7-treated NG108-15 and L1-transfected NIH/3T3 cells (Wilkemeyer et al., 2002a). To determine whether d-NAP is an ethanol antagonist in neural cells, we studied its effects in BMP-7-treated NG108-15 cells. As shown in Fig. 2, NG108-15 cells cultured in the presence of 10 ng/mL BMP-7 had significantly higher cell adhesion (43 ± 3%, n = 5) than cells cultured in serum-free medium (13 ± 1%, n = 5; p < 0.001). Ethanol decreased cell adhesion (25 ± 2%, n = 5; p < 0.01) in BMP-treated NG108-15 cells but not in cells exposed to 10^−7 M d-NAP (42 ± 3%, n = 5). These experiments show that d-NAP is an effective ethanol antagonist, both in neural cells and transfected fibroblasts.

**d-SAL Is Also a Potent Ethanol Antagonist.** The unexpected observation that d-NAP was as potent as l-NAP prompted us to ask whether this property would be shared by the related peptide SAL. We chose 2A2-L1 cells for these experiments because the L1-mediated component of cell adhesion can be defined more accurately than for NG108-15 cells, in which BMP-7 induces both L1 and the neural cell adhesion molecule. As with NAP, d-SAL and l-SAL exhibited dose-dependent ethanol antagonist activity over many log orders (Fig. 3). Antagonism by d-SAL and l-SAL was first apparent at concentrations of about 10^−13 M and maximal at 10^−6 M (91 ± 5 and 90 ± 5%, respectively). Linear regression analysis yielded IC_{50} values of approximately 100 × 10^−12 M for d-SAL and 8.2 × 10^−12 M for l-SAL (Table 1). Together, these data demonstrate that although NAP and SAL are highly potent ethanol antagonists, they lack stereospecificity.

**Structure-Activity Relation for d-NAP.** Next we asked whether d-NAP would exhibit the same structural specificity as l-NAP. The Ser-Ile-Pro sequence is critical for l-NAP-mediated ethanol antagonism (Wilkemeyer et al., 2003) and l-SAL-mediated neuroprotection (Brenneman et al., 1998).

We therefore evaluated the antagonist activity of a series of d-NAP derivatives in which d-Ala replaced single amino acids in the Ser-Ile-Pro sequence. As a control, we also tested an Ala substitution that had little effect on l-NAP activity (Wilkemeyer et al., 2003). d-N1A-d-NAP (Table 1). Cell adhesion assays were performed using 2A2-L1 cells in the absence and presence of 100 mM ethanol and various concentrations of these peptides. In these experiments, d-NAP produced a maximal antagonist effect of 86 ± 11% (n = 17) (Table 1 and Fig. 4).

Substitution of d-Ala for d-Ile (d-I6A-d-NAP) reduced antagonist efficacy by more than half, but a marked alteration in the shape of the dose-response curve made it difficult to calculate an IC_{50} value (Fig. 4A). Substitution of d-Ala at Ser-5 (d-S5A-d-NAP) had little effect on antagonist efficacy (91 ± 5%, n = 3) but reduced antagonist potency by about 16,600-fold (Table 1). The d-P7A-d-NAP peptide had a slightly decreased efficacy compared with d-NAP (78 ± 7%, n = 11) but only a small reduction in potency (54-fold; Table 1). Replacement of the N-terminal d-Asn residue with d-Ala (d-N1A-d-NAP) resulted in a slight reduction in efficacy (78 ± 10%, n = 3) and a 274-fold reduction in potency (Table 1). These results demonstrate that the structure-activity re-
Ethanol antagonist activity of both NAP and SAL peptides. These data highlight the importance of the Ile residue in the Ser-Ile-Pro motif, in particular Ile, for ethanol antagonist properties of S6A-SAL, L-SAL, and P8A-SAL (note that Ser-Ile-Pro begins with the 6th amino acid from the N-terminal of SAL as opposed to the 5th in NAP). Figure 5 shows that Ala replacement of Ile-7 had the greatest effect on SAL-mediated antagonist activity, reducing efficacy by 50% and potency by 5-fold (Table 1). In contrast, Ala substitution at the Ser-6 or Pro-8 positions had little effect on efficacy. The S6A-SAL peptide had a 2-fold increase in potency over the parent L-SAL peptide, and P8A-SAL showed a small (3–4-fold) reduction in potency (Table 1). These data highlight the importance of the Ile residue in the ethanol antagonist activity of both NAP and SAL peptides.

**Structure-Activity Relation for L-SAL.** To determine whether the Ser-Ile-Pro motif is also critical for L-SAL, we characterized the ethanol antagonist properties of S6A-SAL, I7A-SAL, and P8A-SAL (note that Ser-Ile-Pro begins with the 6th amino acid from the N-terminal of SAL as opposed to the 5th in NAP). Figure 5 shows that Ala replacement of Ile-7 had the greatest effect on SAL-mediated antagonist activity, reducing efficacy by 50% and potency by 5-fold (Table 1). In contrast, Ala substitution at the Ser-6 or Pro-8 positions had little effect on efficacy. The S6A-SAL peptide had a 2-fold increase in potency over the parent L-SAL peptide, and P8A-SAL showed a small (3–4-fold) reduction in potency (Table 1). These data highlight the importance of the Ile residue in the ethanol antagonist activity of both NAP and SAL peptides.

**D-NAP and L-SAL Prevent Ethanol-Induced Embryotoxicity.** Structurally dissimilar compounds that antagonize ethanol inhibition of L1 adhesion also prevent ethanol-induced embryotoxicity (Chen et al., 2001; Wilkemeyer et al., 2003). We therefore asked whether D-NAP and L-SAL would prevent ethanol-induced growth retardation in mouse whole embryo culture. Embryonic day 8.0 mouse embryos (3–5 somite pairs) were cultured for 24 h in the absence or presence of 100 mM ethanol and the indicated concentrations of either L-SAL or D-SAL. Shown are the means ± S.E.M. for the percentage of antagonist activity for each peptide (n = 3–10). Mean values for L1-mediated adhesion were 42 ± 1.1% in the absence of ethanol and 28 ± 2.9% in the presence of ethanol. Note that n-SAL and l-SAL are equally potent ethanol antagonists.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Maximal Effect (%)</th>
<th>IC50 (μM)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NAPSVIPQ</td>
<td>86 ± 6</td>
<td>65</td>
<td>4–11</td>
</tr>
<tr>
<td>d-NAPSVIPQ</td>
<td>86 ± 11</td>
<td>25</td>
<td>4–17</td>
</tr>
<tr>
<td>d-NAPVSIPAQ</td>
<td>78 ± 10</td>
<td>6857</td>
<td>3–12</td>
</tr>
<tr>
<td>5</td>
<td>41 ± 7</td>
<td>415,000</td>
<td>3–8</td>
</tr>
<tr>
<td>d-NAPVSIAQ</td>
<td>78 ± 7</td>
<td>1356</td>
<td>6–24</td>
</tr>
<tr>
<td>L-SALLRSIFP</td>
<td>90 ± 5</td>
<td>8224</td>
<td>3–10</td>
</tr>
<tr>
<td>L-SALLRSAFA</td>
<td>91 ± 5</td>
<td>103,000</td>
<td>3–5</td>
</tr>
<tr>
<td>L-SALLRSAFA</td>
<td>81 ± 11</td>
<td>3952</td>
<td>4–7</td>
</tr>
<tr>
<td>L-SALLRSAFAP</td>
<td>43 ± 7</td>
<td>38,651</td>
<td>5–9</td>
</tr>
<tr>
<td>L-SALLRSAIAP</td>
<td>90 ± 5</td>
<td>28,000</td>
<td>4–9</td>
</tr>
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* p < 0.001 compared with n-NAP.

††† p < 0.001 compared with l-SAL.

**Fig. 2.** Ethanol antagonist activity of n-NAP in NG108-15 cells. Cell adhesion assays were performed in BMP-7-treated NG108-15 cells in the absence (control) and presence of 100 mM ETOH or 100 mM ethanol plus 1 × 10−7 M n-NAP (n-NAP/ETOH). Shown are the means ± S.E.M. for the percentage of cell adhesion (n = 5). Also shown is the mean ± S.E.M. for the percentage of cell adhesion of NG108-15 cells grown in the absence of BMP-7 (defined medium). d-NAP blocked ethanol inhibition of cell adhesion by 90 ± 7%; **, p < 0.01 comparing control with ETOH; †††, p < 0.001 comparing n-NAP/ETOH with ETOH.

**Fig. 3.** L-SAL and D-SAL antagonism of ethanol inhibition of L1 adhesion. Cell adhesion assays were performed in 2A2-L1 cells in the absence and presence of 100 mM ethanol and the indicated concentrations of either L-SAL or D-SAL. Shown are the means ± S.E.M. for the percentage of antagonist activity for each peptide (n = 3–10). Mean values for L1-mediated adhesion were 42 ± 1.1% in the absence of ethanol and 28 ± 2.0% in the presence of ethanol. Note that n-SAL and l-SAL are equally potent ethanol antagonists.
Discussion

The major finding of this study is the absence of stereospecificity for NAP and SAL antagonism of ethanol inhibition of L1 adhesion. Stereoisomers of NAP showed no difference in potency or efficacy. Although D-SAL was less potent than L-SAL, it retained picomolar potency and full efficacy. The mechanism of action of the stereoisomers of NAP seems to be similar. Neither D-NAP nor L-NAP (Wilkemeyer et al., 2002a) had a primary effect on the cell surface expression of L1 or on L1-mediated cell adhesion; rather, both peptides antagonized the effects of ethanol on the adhesive properties of L1. The NAP stereoisomers had equivalent activity in BMP-7-treated NG108-15 cells and L1-transfected fibroblasts. Hence, the antagonist properties of NAP stereoisomers extend broadly to mesenchymal and neural cells and to both rodent and human L1.

The Ser-Ile-Pro motif is conserved in NAP and SAL and is necessary for NAP antagonism of ethanol inhibition of L1 adhesion (ethanol antagonism) (Wilkemeyer et al., 2003) and NAP- and SAL-mediated neuroprotection (Brenneman et al., 1998; Wilkemeyer et al., 2003). Within this motif, Ile is critical for L-NAP ethanol antagonism and L-NAP protection against ethanol-induced embryotoxicity (Wilkemeyer et al., 2003). Our present data indicate that Ile is also critical for the ethanol antagonist activity of D-NAP and L-SAL. The sequences surrounding Ile are clearly important; peptides composed of the scrambled amino acids of NAP (PNIQVASP and ASPNQPIV) are inactive (Wilkemeyer et al., 2002a). The fact that Ile is a critical residue in both NAP and SAL highlights the importance of the flanking Ser and Pro because there is little homology in the remaining amino acids surrounding the Ser-Ile-Pro motif of NAP (NAPV-SIP-Q) and SAL (SALLR-SIP-A). Hence, the critical determinant of the ethanol antagonist activity of NAP and SAL is an Ile flanked by Ser and Pro. Curiously, this requirement exists irrespective of the chirality of the amino acids.

These studies extend our earlier observation that NAP and SAL are extremely potent ethanol antagonists (Wilkemeyer et al., 2002a). The extraordinary potency of these peptides is consistent with their interaction with a very high-affinity binding site. The lack of stereospecificity under such circumstances is unexpected because a high-affinity binding site should impose tight structural constraints on potential ligands. Even more curious than this lack of stereospecificity is the preservation of the structure-activity relation in peptide derivatives of the NAP stereoisomers. Conceivably, the Ser-Ile-Pro motif on NAP or SAL interacts with a structurally symmetric target site that recognizes two chiral configurations. Alternatively, NAP and SAL could self-aggregate and form membrane pores similar to the antibiotic peptides magainin and cecropin, which also lack stereospecificity (Wade...
Levo- and dextro-amino acids are nonsuperimposable mirror images of each other. The vast majority of receptors and enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997). D-Serine is synthesized in the L-configuration, and most enzymes are synthesized in the L-configuration, and most synthetic D-ligands and D-substrates are inactive or much less effective compared with their L-stereoisomers. There are, however, some notable exceptions (Kreil, 1997).