Nonopioid Motor Effects of Dynorphin A and Related Peptides: Structure Dependence and Role of the N-Methyl-D-aspartate Receptor

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ABSTRACT

Dynorphin (Dyn) A and related opioid and nonopioid peptides were tested for their ability to produce motor effects in mice. Central (intracerebroventricular) administration of Dyn A in mice produced marked motor effects characterized by wild running, jumping, circling and/or barrel rolling with an ED$_{50}$ value of 14.32 (95% confidence limits, 10.09–20.32) nmol/mouse. The order of potency of the various Dyn A-related peptides and fragments in producing motor effects was Dyn A = Dyn A-(1–13) > [Ala$_1$]Dyn A-(1–13) = Dyn A-(2–13) > α-Neo-End > Dyn A-(1–8) = Dyn B = Dyn A-(2–8) >> Dyn A-(3–8), Dyn A-(1–5) (or Leu-Enk) and Dyn A-(6–10) displayed no motor effect at doses up to 100 nmol/mouse. The potencies of Dyn A and Dyn A-(2–13) were not affected by preadministration of naloxone (5 mg/kg s.c.), but the motor effects of Dyn A-(1–13) (20 nmol/mouse i.c.v.) were significantly reduced by coadministration of low doses (0.2–0.6 nmol/mouse) of the N-methyl-D-aspartate (NMDA) receptor antagonists dextrorphan, MK-801 and CPP. Dyn A was also a potent inhibitor of the binding of the phenylethylcaine receptor ligand, [3H]MK-801, to rat brain membranes, with a $K_i$ value of 0.41 μM. However, the order of potency of the various Dyn A-related peptides and fragments in inhibiting [3H]MK-801 binding did not correlate with their ability to produce motor effects. On the other hand, Dyn A and related peptides produced a significant potentiation of the binding of the competitive NMDA antagonist [3H]CPP-39653 to rat brain membranes, an effect that correlated well ($r = 0.91$) with their potency in producing motor effects. These results indicate that the nonopioid motor effects of Dyn A and related peptides are structure dependent, with Dyn A-(2–8) being the minimal core peptide for motor activity. In addition, these effects most likely involve the participation of the excitatory amino acid binding domain on the NMDA receptor complex.

Dyn A is an endogenous peptide possessing high binding affinities for all opioid receptor types and some binding selectivity for the kappa site (Garzon et al., 1984). Dyn A-related peptides (see fig. 1 for structures) also bind to nonopioid sites (Dumont and Lemaire, 1993; Smith and Lee, 1988). Various pharmacological and/or pathophysiological effects of Dyn A and related peptides are not antagonized by the opiate antagonist naloxone and are mimicked by nonopioid fragments of the peptide (Dumont and Lemaire, 1997; Shukla and Lemaire, 1994). In rat heart synaptosomal preparations, Dyn A and related peptides inhibit the uptake of [3H]norepinephrine, and a good correlation exists between the ability of the peptides to inhibit [3H]norepinephrine uptake and compete with the binding of [3H]Dyn A-(1–13) to nonopioid sites (Dumont and Lemaire, 1994a).

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ABBREVIATIONS: i.c.v., intracerebroventricular; s.c., subcutaneous; CL, confidence limit; EEG, electroencephalographic; Dyn A, dynorphin A; Leu-Enk, Leu-enkephalin; α-Neo-End, α-Neo-endorphin; NMDA, N-methyl-d-aspartate; AMPA, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate; PCP, phencyclidine.
motor effects in mice, and such effects were blocked by the noncompetitive NMDA receptor antagonists metaphit, dex-
tromethorphan and ketamine (Shukla et al., 1992).

A particular role for the NMDA receptor in the nonopioid effects of Dyn A was first suggested by Faden (1992). The
NMDA receptor is an inotropic excitatory amino acid recep-
tor involved in central key functions that include synaptic
plasticity, learning and memory processes as well as neuro-
degeneration (Collingridge and Bliss, 1995; Malenka and
Nicoll, 1993; Wong and Kemp, 1991). This receptor comprises
various binding domains for compounds that modulate its
activity (MacDonald and Mowak, 1990; Wong and Kemp,
1991). Glycine and polyamines exert positive modulatory ac-
tions on the stimulation of the receptor by the excitatory
amino acids glutamic acid and aspartic acid, whereas PCP,
Mg$^{2+}$ and Zn$^{2+}$ negatively modulate the receptor. Compe-
titive NMDA antagonists, such as CGP 39653 (Sills
et al., 1991), interact directly on the excitatory amino acid
binding domain and compete with glutamic acid and aspartic acid to
block their activity. On the other hand, noncompetitive
NMDA antagonists, such as MK-801 \[(\text{1H})-5\text{-methyl-10,11-
dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate}\]
(Sircar et al., 1987), bind to the PCP site located inside the
NMDA receptor-linked ion channel and block its activity in a
use-dependent manner (MacDonald and Mowak, 1990). Dyn
A and related peptides have already been shown to interact
with both excitatory amino acid and PCP binding domains on
the NMDA receptor complex by their potentiation of $[\text{3H}]$CGP
39653 binding (Dumont and Lemaire, 1994b) and inhibition
of $[\text{3H}]$MK-801 binding (Hunter et al., 1994; Shukla et al.,
1992), respectively. The possible involvement of any one of
these two sites in the nonopioid motor effects of these pep-
tides remains to be established.

The present study was aimed at investigating (1) the struc-
ture-activity relationship of the motor effects of Dyn A and
related peptides in mice, (2) the blockade of the motor effects
with competitive and noncompetitive NMDA receptor antag-
onists and (3) the structural requirement for the interaction
of Dyn A and related peptides with the glutamic acid and
PCP binding domains on the NMDA receptor complex. A
close correlation was made between the abilities of the pep-
tides to enhance $[\text{3H}]$CGP 39653 binding and to produce
motor dysfunction.

**Methods**

**Animals.** Male Swiss Webster mice [(SW)f BR] weighing between
20 and 25 g were housed five per cage in a room with controlled
temperature (22 ± 2°C), humidity and artificial light (6:30 a.m. to
7:00 p.m.). A minimum of 10 animals were used per group. The
animals had free access to food and water and were used after a
minimum of 4 days of acclimation to the housing conditions. To avoid
the diurnal variations, all experiments were conducted between 9:00
a.m. and 5:00 p.m. All experiments were authorized by the animal
care committee of the University of Ottawa in accordance with the
guidelines of the Canadian Council on Animal Care.

**Drugs.** Dyn A and related peptides used in the study were syn-
thesized by solid-phase procedure (Merrifield, 1963) as described
previously (Lemaire et al., 1986). The peptides were cleaved from the
resin and deprotected with liquid hydrogen fluoride at 0°C in the
presence of anisole (10% v/v). The synthetic material was then pu-
rified by chromatography on Sephadex G-10 (medium size) and
HPLC on Nucleosil C18. The product (280 nm detection) obtained
from HPLC was lyophilized to give a final recovery of 20% to 25%
(based on the starting Boc amino acid-resin). The identity and purity
of the synthetic peptides were verified by thin-layer chromatogra-
phy, analytical HPLC on Zorbax ODS C18 (0.3 × 25 cm column,
Dupont) and amino acid analysis of acid hydrolysates.

Naloxone hydrochloride (Endo Laboratories, Garden City, NY)
dissolved in sterile water just before use and injected s.c. in a
volume of 10 ml/kg. Dextromethorphan (Research Biochemicals, Natick,
MA); MK-801 (Research Biochemicals) and CPP \[(\text{1H})-3-(2-carboxy-
piperazine-4-yl)-propyl-1-phosphonic acid; Tocris Neuramin, Essex,
UK\] were dissolved in water and coinjected i.c.v. with Dyn A-(1–13).

**Motor dysfunction assay.** The procedure used to assay motor
dysfunction is the same as that described previously (Shukla et al.,
1992). Groups of 10 mice each were injected i.e.v. with different doses
of Dyn A or related peptides. The animals were observed during 30
min for motor effects, characterized by wild running, popcorn jump-
ing, circling, barrel rolling and ataxia. The motor activity began
within 5 min and lasted for 1 to 2 min. In some animals, particularly...
at higher doses of the peptides, episodes of motor activity were recorded intermittently during the observation period. The animals were scored as showing motor activity when one or more of the above-mentioned responses were present. In each group, the number of animals showing these behavioral signs of motor activity was recorded. The dose producing motor dysfunction in 50% mice (ED$_{50}$) and the potency ratios with 95% CL values were calculated according to the method of Litchfield and Wilcoxon (1949) using procedure 47 of the computer program of Tallarida and Murray (1987). The protective effects of dextrophan, MK-801 and CPP against Dyn A-(1–13) (20 nmol/mouse)-induced motor effects were analyzed by Fisher’s exact test using the GraphPAD INSTAT program (GraphPAD Software, San Diego, CA).

**Preparation of rat brain membranes.** Six male Wistar rats were decapitated, and their whole brains were rapidly removed and homogenized in ice-cold Tri-s-HCl (5 mM, pH 7.4; buffer A) with a glass-Teflon homogenizer. The homogenate was centrifuged at 27,000 g for 30 min. The pellet was resuspended in buffer A and centrifuged at 27,000 g for 30 min. The resulting pellet was homogenized and incubated on ice in a total volume of 1.0 liter of buffer A supplemented with 0.3 M KCl for 60 min (Lee et al., 1982). The suspension was then centrifuged at 27,000 g for 30 min, and the pellet was resuspended in buffer A. This washing procedure was repeated an additional three times, and the final membrane pellet was resuspended in buffer A at a concentration of 2.0 mg protein/ml (Lowry et al., 1951) and kept frozen at −90°C.

**$^3$H]MK-801 binding assay.** Rat brain membranes (0.8 mg) were incubated in 2 ml of buffer A and enzyme inhibitors bestatin (30 μM), bacitracin (25 μM), captopril (10 μM) and thiopran (0.3 μM) and 5.0 nM $^3$H]MK-801 at 22°C for 30 min in the absence and presence of the indicated concentration of Dyn A or related peptides. Binding was terminated by rapid filtration over Whatman GF-93488 filters. The filters were washed four times with 3 ml of ice-cold buffer A, placed in vials containing 10 ml of Ecolume and counted in a Beckman Beta counter LS 7800 at 40% efficiency. The specific binding was evaluated using the difference between the counts in the presence and absence of 10 μM MK-801. The concentrations of the peptides that produced 50% inhibition of the binding of tritiated ligand (IC$_{50}$) were derived using the nonlinear regression curve-fitting program GraphPAD INPLOT. $K_i$ values were calculated using the equation of Cheng and Prusoff (1973), and results are expressed as the mean ± S.E.M. of five duplicated sets of experiments. Statistical significance was measured by the Student’s t test.

**$^3$H]CGP-39653 binding assay.** The ability of Dyn A and its analogs to potentiate the binding of $^3$H]CGP-39653 to rat brain membranes was measured as described previously (Dumont and Lemaire, 1994b). The rat brain membranes were prepared as described by Sills et al. (1991). $^3$H]CGP-39653 (5 nM) binding was performed in 2 ml of Tris-HCl (5 mM, pH 7.7; buffer B) at 4°C for 60 min, containing 30 μM bestatin, 25 μM bacitracin, 10 μM captopril, 0.3 μM thiopran and 0.8 mg of rat brain membranes. The binding was stopped by filtration as described above. Specific binding was defined as the difference between the total radiolabel bound and that bound in the presence of 10 μM CPP. The potentiation of $^3$H]CGP-39653 binding by Dyn A and its analogs (10 μM) was expressed as the percent increase over the control binding activity in the absence of the peptide. Experiments were repeated five times in duplicate, and results are the mean ± S.E.M. values. Statistical significance was determined using one-way analysis of variance followed by the Newman-Keuls test.

**Results**

**Motor effects of Dyn A and related peptides.** The i.c.v. administration of Dyn A (5–30 nmol/mouse), Dyn A-(1–13) (5–30 nmol/mouse), Dyn A-(1–8) (10–60 nmol/mouse), Dyn B (20–100 nmol/mouse) and α-Neo-End (5–100 nmol/mouse) showed dose-dependent motor effects characterized by wild running, jumping, circling and barrel rolling (fig. 2A). Dyn A (ED$_{50}$ = 14.32 nmol/mouse) and Dyn A-(1–13) (ED$_{50}$ = 14.40 nmol/mouse) were equipotent in producing motor effects (table 1). α-Neo-End (ED$_{50}$ = 32.97 nmol/mouse), Dyn A-(1–8) (ED$_{50}$ = 42.08 nmol/mouse) and Dyn B (ED$_{50}$ = 45.01 nmol/mouse) were significantly less potent compared with Dyn A in producing motor effects. Leu-Enk (20–100 nmol/mouse) did not show any motor activity (table 1).

The nonopioid fragments [Ala$^1$]Dyn A-(1–13) (10–60 nmol/mouse), Dyn A-(2–13) (10–60 nmol/mouse) and Dyn A-(2–8) (10–100 nmol/mouse) also showed dose-dependent motor effects after i.c.v. administration (fig. 2B). [Ala$^1$]Dyn A-(1–13) (ED$_{50}$ = 26 nmol/mouse), Dyn A-(2–13) (ED$_{50}$ = 28.82 nmol/mouse) and Dyn A-(2–8) (ED$_{50}$ = 47.01 nmol/mouse) were significantly less potent than Dyn A-(1–13) and Dyn A in producing motor effects (table 1). Dyn A-(3–8) showed motor effects in 33% of mice at the dose of 100 nmol/mouse. Dyn A-(6–10) (20–100 nmol/mouse) did not show any motor effect (table 1). The overall rank order of potency of the different Dyn A-related peptides was Dyn A = Dyn A-(1–13) > [Ala$^1$]Dyn A-(1–13) = Dyn A-(2–13) > α-Neo-End > Dyn A-(1–8) = Dyn A-(2–8) > > > Dyn A-(3–8). Pretreat-
was previously shown to potentiate the binding of the NMDA antagonist dextrorphan, MK-801 or CPP against the motor effects of Dyn A-(1–13). NMDA antagonists were coadministered at the indicated doses with Dyn A-(1–13) (20 nmol/mouse i.c.v.), which alone produced motor effects in 90% of tested mice. *P < .05 was considered significant.

Table 1: Relative potencies of Dyn A and related peptides in producing motor effects in mice

<table>
<thead>
<tr>
<th>Peptide</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (95% CL) (nmol/mouse)</th>
<th>Potency ratio (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioid peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyn A</td>
<td>14.32 (10.09–20.32)</td>
<td>1</td>
</tr>
<tr>
<td>Dyn A-(1–13)</td>
<td>14.40 (11.39–18.20)</td>
<td>0.99 (0.87–1.12)</td>
</tr>
<tr>
<td>Dyn A-(1–8)</td>
<td>42.08 (32.20–54.98)</td>
<td>0.34 (0.31–0.37)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu-Enk [Dyn A-(1–5)]</td>
<td>&gt;100&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dyn B</td>
<td>45.01 (29.57–68.52)</td>
<td>0.32 (0.29–0.34)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>α-Neo-End</td>
<td>32.97 (20.65–58.29)</td>
<td>0.43 (0.35–0.49)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nonopioid peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[Ala&lt;sup&gt;8&lt;/sup&gt;]Dyn A-(1–13)</td>
<td>26.00 (16.84–40.16)</td>
<td>0.55 (0.45–0.68)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dyn A-(2–13)</td>
<td>28.82 (20.25–41.04)</td>
<td>0.49 (0.44–0.56)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dyn A-(2–8)</td>
<td>47.01 (34.33–64.37)</td>
<td>0.31 (0.33–0.28)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dyn A-(3–8)</td>
<td>&gt;100&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Dyn A-(6–10)</td>
<td>&gt;100&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> No animal showed any motor effect at the tested dose range of 25 to 100 nmol/mouse (i.c.v.).
<sup>b</sup> Only 33% of animals showed motor effects at a dose of 100 nmol/mouse (i.c.v.).
<sup>c</sup> *P < .05 compared with Dyn A.

Fig. 3. Percent protection of different doses of the NMDA antagonists dextrorphan, MK-801 or CPP against the motor effects of Dyn A-(1–13).

Fig. 4. Concentration-dependent inhibition of [3H]MK-801 binding with Dyn A-(1–13) in the absence (○) or presence of 10 μM of naltrexone (●). The results are expressed as the mean ± S.E.M. of five experiments conducted in duplicate.

Table 2: Effect of preadministration (15 min) of naltrexone (5 mg/kg s.c.) on the potencies of Dyn A-(1–13) and Dyn A (2–13) in producing motor effects in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED&lt;sub&gt;50&lt;/sub&gt; (95% CL) (nmol/mouse)</th>
<th>Potency ratio (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyn A</td>
<td>14.32 (10.09–20.32)</td>
<td>1</td>
</tr>
<tr>
<td>Naltrexone + Dyn A</td>
<td>15.97 (12.68–20.13)</td>
<td>0.90 (0.80–1.01)</td>
</tr>
<tr>
<td>Dyn A-(2–13)</td>
<td>28.82 (20.25–41.04)</td>
<td>1</td>
</tr>
<tr>
<td>Naltrexone + Dyn A</td>
<td>27.02 (18.94–38.55)</td>
<td>1.07 (1.06–1.07)</td>
</tr>
</tbody>
</table>

Amount of mice with naloxone (5 mg/kg s.c.) did not affect the potencies of Dyn A and Dyn A-(2–13) (table 2).

To verify the possible involvement of the NMDA receptor in the motor effects of Dyn A-(1–13), the peptide was coadministered with competitive or noncompetitive NMDA antagonists (fig. 3). Coinjection of Dyn A-(1–13) (20 nmol/mouse i.c.v.) with dextrorphan (0.2, 0.3 and 0.6 nmol/mouse i.c.v.) or MK-801 (0.3 and 0.4 nmol/mouse i.c.v.) or CPP (0.3 and 0.4 nmol/mouse i.c.v.) produced significant (P < .05) blockades of its motor effects. Coinjection of the peptide with higher doses of the NMDA antagonists showed some decrease in protective activities (fig. 3).

Competition with [3H]MK-801 binding to rat brain membranes. Various Dyn A-related peptides were also tested for their ability to compete with the binding of [3H]MK-801 to rat brain membranes (fig. 4; table 3). Dyn A-(1–13) was 2-fold more potent than Dyn A in inhibiting the binding of [3H]MK-801 (5 nM), with a K<sub>i</sub> value of 0.21 μM compared with 0.41 μM for Dyn A. The order of potency of the various Dyn A-related peptides in the binding assay was distinct from that mentioned above for the production of motor effects: Dyn A-(1–13) > Dyn A > [Ala<sup>1</sup>]Dyn A-(1–13) > Dyn A-(1–9) > Dyn B > α-Neo-End > Dyn A-(1–8). Dyn A-(2–13), Dyn A-(3–13), Dyn A-(2–8) and Dyn A-(1–5) were inactive at concentrations up to 10 μM. In addition, the ability of Dyn A-(1–13) to displace [3H]MK-801 binding was not affected by 10 μM naltrexone (fig. 4).

Potentiation of [3H]CGP-39653 binding. Dyn A-(1–13) was previously shown to potentiate the binding of the NMDA receptor antagonist [3H]CGP-39653 to rat brain membranes (Dumont and Lemaire, 1994b). Figure 5 illustrates the effects of Dyn A and various related peptides on such binding activity. Among the opioid peptides, Dyn A and Dyn A-(1–13) (10 μM) were equipotent and caused a 2.9-fold increase in [3H]CGP-39653 binding, followed by α-Neo End and Dyn A-(1–8), which showed 2.3- and 1.4-fold increases, respectively. Dyn B, Dyn A-(3–8) and Leu-Enk showed no significant potentiation (fig. 5). The nonopioid Dyn A-related peptides Dyn A-(2–13) and [Ala<sup>1</sup>]Dyn A-(1–13) were also able to enhance the binding of [3H]CGP-39653, displaying 2.4- and 2.3-fold stimulations, respectively. However, Dyn A-(2–8) was less potent and showed only a 1.4-fold increase in the binding activity. Comparison between the potencies of the various peptides in producing motor effects (table 1) and potentiating the binding of [3H]CGP-39653 (fig. 4) provided a good correlation with an r value of 0.91 (fig. 6). The potentiation of [3H]CGP-39653 binding by Dyn A-(1–13) (10 μM) was nonopioid, being not affected by naltrexone (10 μM; data not shown).
Fig. 5. Effect of Dyn A and related peptides (10 μM) on the binding of \[^{3}H\]GCP-39653 (5 nM) to rat brain membranes. The results represent the mean ± S.E.M. of five experiments conducted in duplicate. **, P < .01, and *, P < .05 compared with control.

Fig. 6. Correlation between the abilities of Dyn A and related peptides to produce motor effects and potentiate \[^{3}H\]GCP 39653 binding. The potency ratio obtained in table 1 was plotted in comparison with the potency ratio derived from figure 5, using Dyn A for maximal effect.

Discussion

Dyn A and related peptides, when administered i.c.v. or into other brain regions, produce motor dysfunction such as wild running, jumping, circling, barrel rolling, ataxia and unusual contorted posture (Nakazawa et al., 1989; Walker et al., 1982). In the cortical EEG recordings, Dyn A (i.c.v.) or \([\text{des-Tyr}^1]\)Dyn A induced large-amplitude slow-wave EEG activity in rats (Walker et al., 1982). These Dyn-induced motor effects and changes in cortical EEG activity were not antagonized by pretreatment of naloxone, suggesting the involvement of some nonopioid mechanism (Walker et al., 1982). Here, we found that i.c.v. injections of Dyn A and related peptides in mice produce dose-dependent motor effects. The nonopioid nature of the motor effects of these peptides was demonstrated by their insensitivity to naloxone pretreatment and the high potencies of the nonopioid Dyn A analogs or fragments, such as \([\text{Ala}^1]\)Dyn A-(1–13), Dyn A-(2–13) and Dyn A-(2–8). Dyn A-(2–8) was found to be the minimal active core of the peptide. The same minimal core peptide was previously reported for the suppression of naloxone-precipitated opioid withdrawal syndrome in morphine-dependent mice (Takemori et al., 1993).

There have been numerous reports concerning the involvement of the NMDA receptor complex in the central nonopioid effects of Dyn A and related peptides (for a review, see Shukla and Lemaire, 1994). We previously found that the motor effects induced by Dyn Ia are altered by preadministration of the noncompetitive NMDA receptor antagonists metaphit, dextromethorphan and ketamine (Shukla et al., 1992). In the present study, the motor effects of Dyn A-(1–13) were almost completely blocked by coadministration of the peptide with a low concentration (0.3 nmol/mouse of the noncompetitive NMDA antagonist dextromethorphan (fig. 2). The competitive and noncompetitive NMDA antagonists CPP and MK-801 displayed significant but only partial blockades of Dyn A-(1–13)-induced motor activity. The decreased protective activity of these compounds at higher doses may be due to their ability to cause motor effects of their own (Koek and Colpaert, 1990).

The NMDA receptor comprises various binding domains that regulate its activity. Thus, in addition to the site for the excitatory amino acid (glutamic acid or aspartic acid), there exists sites for glycine, PCP, polyamines and divalent ions (Mg\(^{++}\) and Zn\(^{++}\)), which modulate the entry of Ca\(^{++}\) and Na\(^{+}\) consecutive to the stimulation-induced opening of the receptor-linked ion channel (Wong and Kemp, 1991). To verify whether the motor effects of Dyn A and related peptides could be due to their interaction with the PCP receptor, we measured their ability to compete with the binding of the specific PCP receptor ligand \[^{3}H\]MK-801 to rat brain membranes (table 3). Dyn A and related peptides did compete with the binding of \[^{3}H\]MK-801, but their order of potency was distinct from that observed for motor activity (table 1). Thus, the nonopioid peptides Dyn A-(2–13) and Dyn A-(2–8), which produced marked motor activity, did not decrease the binding of \[^{3}H\]MK-801. On the other hand, Dyn B, Dyn A-(1–8) and \(\alpha\)-Neo-End displayed <10% of the potency of Dyn A-(1–13) in the \[^{3}H\]MK-801 binding assay while exhibiting >30% of the potency of Dyn A-(1–13) in the motor activity assay.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Ki (μM)</th>
<th>Potency ratio</th>
</tr>
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<tbody>
<tr>
<td>Opioid peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dyn A-(1–13)</td>
<td>0.21 ± 0.04</td>
<td>100</td>
</tr>
<tr>
<td>Dyn A</td>
<td>0.41 ± 0.01(^b)</td>
<td>51</td>
</tr>
<tr>
<td>Dyn A-(1–9)</td>
<td>1.23 ± 0.32(^b)</td>
<td>17</td>
</tr>
<tr>
<td>Dyn B</td>
<td>2.34 ± 0.49(^b)</td>
<td>9</td>
</tr>
<tr>
<td>(\alpha)-Neo-End</td>
<td>3.66 ± 0.42(^b)</td>
<td>6</td>
</tr>
<tr>
<td>Dyn A-(1–8)</td>
<td>6.25 ± 0.27(^b)</td>
<td>3</td>
</tr>
<tr>
<td>Leu-Enk</td>
<td>N.A.*</td>
<td></td>
</tr>
<tr>
<td>Nonopioid peptide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{Ala}^1])Dyn A-(1–13)</td>
<td>0.88 ± 0.11(^b)</td>
<td>24</td>
</tr>
<tr>
<td>Dyn A-(2–13)</td>
<td>N.A.*</td>
<td></td>
</tr>
<tr>
<td>Dyn A-(2–9)</td>
<td>N.A.*</td>
<td></td>
</tr>
<tr>
<td>Dyn A-(2–8)</td>
<td>N.A.*</td>
<td></td>
</tr>
<tr>
<td>Dyn A-(6–10)</td>
<td>N.A.*</td>
<td></td>
</tr>
</tbody>
</table>

\(^{a}\)N.A., not active at 10 μM.

\(^{b}\)P < .05 compared with Dyn A-(1–13).
dysfunction assay. Hence, the interaction of Dyn A and related peptides with the PCP binding domain on the NMDA receptor complex is more likely not responsible for their motor activity but rather may be involved in some other nonopioid effects of the peptides, such as the nonopioid component of their analgesic activity (Hooke et al., 1995).

Recently, we reported that the binding of the competitive NMDA receptor antagonist \(^3\)H](CGP-39653 is significantly enhanced by Dyn A and related peptides (Dumont and LeMAIRE, 1994b). To verify whether the motor effects of Dyn A and related peptides were due to their interaction with the \(^3\)H]CGP 39653 binding site (i.e., the excitatory amino acid binding domain on the NMDA receptor complex), we studied their ability to potentiate \(^3\)H]CGP 39653 binding to rat brain membranes. The results indicate that Dyn A and some related peptides, including the nonopioid peptides (Ala\(^1\))Dyn A(1–13), Dyn A(2–13) and Dyn A(2–8), markedly increase the binding of \(^3\)H]CGP 39653 (fig. 5), which is in good correlation with their motor activity (table 1). The correlation coefficient that was obtained between the order of potency of these peptides to enhance \(^3\)H]CGP 39653 binding and display motor activity approached unity (\(r = 0.91; \text{fig. 6).} \)) Thus, the motor impairment caused by Dyn A and related peptides may be due to their interaction with the excitatory amino acid binding domain on the NMDA receptor complex, possibly resulting in a potentiation of the action of endogenous glutamic acid or aspartic acid. These data are in accordance with previous results that indicated that the motor effects of thiopeptide analogs of Dyn A(1–9) correlate well with their ability to interact with the glutamic acid site on the NMDA receptor complex (Le et al., 1997).

Dyn A was shown to produce both stimulatory (Walker et al., 1982) and inhibitory (Wagner et al., 1993) effects on the central nervous system. The stimulatory effects are nonopioi- nod, whereas the inhibitory effects involve the participation of kappa opioid receptors. The interaction of Dyn A with the NMDA receptor has been suggested to be responsible of various physiological and pathophysiological phenomena, including alldynia (Nichols et al., 1997; Vanderah et al., 1996), hyperalgesia (Dubner and Ruda, 1992), blockade of morphine tolerance (Takemori et al., 1993) and sensitivity to kindling manifested by seizure (Elmer et al., 1996). The mechanism by which Dyn A may potentiate the action of excitatory amino acids on the NMDA receptor remains to be defined, but our findings of a direct interaction of the peptide with the glutamic acid site and of a correlation between this interaction and its NMDA-mediated motor effects are supported by the previously observed displacement of \(^3\)H]glutamic acid binding by Dyn A(1–13) (Massardier and Hunt, 1989) and potentiation by this same peptide of NMDA-induced biting and scratching behavior (Skilling et al., 1992). Therefore, the interaction of Dyn A with the excitatory amino acid binding domain on the NMDA receptor complex may potentiate the action of endogenous excitatory amino acids and thus participate in various NMDA receptor-mediated behavioral and neuroplastic activities.

References


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