Big Dynorphin, a Prodynorphin-Derived Peptide Produces NMDA Receptor-Mediated Effects on Memory, Anxiolytic-Like and Locomotor Behavior in Mice

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Effects of big dynorphin (Big Dyn), a prodynorphin-derived peptide consisting of dynorphin A (Dyn A) and dynorphin B (Dyn B) on memory function, anxiety, and locomotor activity were studied in mice and compared to those of Dyn A and Dyn B. All peptides administered i.c.v. increased step-through latency in the passive avoidance test with the maximum effective doses of 2.5, 0.005, and 0.7 nmol/animal, respectively. Effects of Big Dyn were inhibited by MK 801 (0.1 mg/kg), an NMDA ion-channel blocker whereas those of dynorphins A and B were blocked by the κ-opioid antagonist nor-binaltorphimine (6 mg/kg). Big Dyn (2.5 nmol) enhanced locomotor activity in the open field test and induced anxiolytic-like behavior both effects blocked by MK 801. No changes in locomotor activity and no signs of anxiolytic-like behavior were produced by dynorphins A and B. Big Dyn (2.5 nmol) increased time spent in the open branches of the elevated plus maze apparatus with no changes in general locomotion. Whereas dynorphins A and B (i.c.v., 0.05 and 7 nmol/animal, respectively) produced analgesia in the hot-plate test Big Dyn did not. Thus, Big Dyn differs from its fragments dynorphins A and B in its unique pattern of memory enhancing, locomotor- and anxiolytic-like effects that are sensitive to the NMDA receptor blockade. The findings suggest that Big Dyn has its own function in the brain different from those of the prodynorphin-derived peptides acting through κ-opioid receptors.

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INTRODUCTION

Processing of prodynorphin gives rise to dynorphin A (Dyn A), dynorphin B (Dyn B), and α-neoendorphin and also to the intermediately sized big dynorphin (Big Dyn), which consists of Dyn A and Dyn B sequences (Fischli *et al*, 1982; Xie and Goldstein, 1987; Day and Akil, 1989). Dyn A, Dyn B, and α-neoendorphin are endogenous ligands for κ-opioid receptors. They have a wide distribution in the CNS including the hippocampus, amygdala, striatum, and spinal cord (Fallon and Leslie, 1986; Khachaturian *et al*, 1982; Mansour *et al*, 1994; Pierce *et al*, 1999; van Bockstaele *et al*, 1994). Consistent with the anatomical localization, these peptides play a role in memory acquisition, modulation of reward induced by intake of addictive substances, motor control and pain processing (see Costigan and Woolf, 2002; de Vries and Shippenberg, 2002; Kreek, 2001; Solbrig and Koob, 2004). Big Dyn is present at substantial levels in the pituitary gland, brain and spinal cord (Fischli *et al*, 1982; Xie and Goldstein, 1987; Day and Akil, 1989). In addition to serving as a precursor for Dyn A and Dyn B this peptide may also have its own function.

Several actions of dynorphins have been found to be insensitive to the general opioid antagonist naloxone and the selective κ-antagonist nor-binaltorphimine 2HCl (nor-BNI) while they are blocked by antagonists of the NMDA receptors (Walker *et al*, 1982; Trujillo and Akil, 1991; Tang *et al*, 1999; reviewed in Hauser *et al*, 2005). The nonopioid effects of Dyn A are apparently relevant for pathophysiological processes such as chronic neuropatic pain and spinal cord and brain injury (Trujillo and Akil, 1991; Vanderah *et al*, 1996; Wang *et al*, 2001; Xu *et al*, 2004; Tan-No *et al*, 2005). Big Dyn administered intrathecally (i.t.) induces characteristic nociceptive behavior insensitive to naloxone but blocked by NMDA receptor antagonists (Tan-no *et al*, 2002). Experiments with inhibitors of dynorphin degradation and anti-Big Dyn antibodies suggested that also

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endogenous Big Dyn is pronociceptive via NMDA receptors (Tan-No et al, 2005).

In the hippocampus dynorphins inhibit Ca^{2+}-dependent glutamate secretion acting through κ-opioid receptors (Drake et al, 1994; Wagner et al, 1992; Terman et al, 2000; Solbrig and Koob, 2004). These effects appear to be relevant for processing of information associated with learning and memory. Behavioral data obtained with synthetic dynorphins and with prodynorphin deficient mice support this notion (Kameyama et al, 1994; Ukai et al, 1996; Hiramatsu et al, 1998, 2000; Wall and Messier, 2000; Nguyen et al, 2005).

The aims of the present work were to assess the effects of Big Dyn on emotional learning and memory, to compare these effects with those of Dyn A and Dyn B, and to evaluate whether effects of these peptides are mediated through κ-opioid or NMDA receptors. Since the κ-opioid system has been linked to aversive behavior (Kreek et al, 2002), the passive avoidance (PA) test based on classical (Pavlovian) fear conditioning and instrumental learning was used. In the course of the experiments it became apparent that Big Dyn increases locomotor and exploratory activities and also produces anxiolytic-like behavior. These effects were further investigated in the open field (OF) and elevated plus maze (EPM) tests. Thereafter, the effects of Big Dyn and the dynorphins on the nociceptive thresholds were studied in hot-plate test for acute thermal nociception.

MATERIALS AND METHODS

Animals

Male adult NMRI mice, weighing 25–30 g at the time of testing, were obtained from B&K Universal AB (Sollentuna, Sweden). The animals were housed in groups of six in standard cages (A3, $42 \times 26 \times 20 \, cm^3$, Macrolon®) in a temperature—and humidity-controlled room with a 12-h light/dark cycle (lights on at 0600), and had free access to standard lab chow (Ewos R36, Ewos AB, Södertälje, Sweden) and tap water. Animals were allowed to habituate to the maintenance facilities and were handled by the same experimenter daily, for a period of at least 5 days before the beginning of the experiments. The cages were changed twice a week. Animal housing and experimental procedures followed the provisions and recommendations of the Swedish animal protection legislation. The experimental procedures were approved by the local Animal Ethics Committee. All animal procedures were carried out by the same experimenter.

Experimental Procedures

PA test. PA is an aversive learning task based on classical (Pavlovian) fear conditioning that allows analysis of both facilitation and impairment of memory function (Bammer, 1982; Misane and Ogren, 2003). PA testing was performed during the light cycle between 1000 and 1300. Prior to testing, the animals were brought to the experimental room where they were allowed to habituate for 30–60 min. The test was conducted using a modified shuttle-box with two communicating compartments of equal size with $4 \times 4 \, cm^2$

sliding door built into the separating wall and a stainless-steel bar floor (Ugo Basile, Italy). The electrical shock compartment was painted black, whereas the light compartment was illuminated by an electric bulb (24 V, 5 W) installed on the top of a plexiglass cover.

Before training, on day 1 mice were allowed to explore the light compartment for 60 s and then the sliding door was opened allowing the animals to enter the black compartment which they could explore for another 60 s. After the exploration session the animals were placed back into their home cages. PA training was conducted in a single session. The animals were treated with test compounds in the home cages, at various time intervals placed in the light compartment with sliding door closed, and allowed to explore the compartment for 60 s. During the exploration period rearing frequency, motility, and defecation were scored. When 60 s expired, the sliding door was automatically opened and the mouse was allowed to crossover into the dark compartment. Once the mouse had entered the dark chamber with all four feet, the sliding door was automatically closed and an scrambled electrical current (0.3 mA, duration 2 s) was delivered through the grid floor. Latency to crossover into the dark compartment (training latency) was recorded. After been conditioned, the mouse was kept for 30 s in the dark compartment and then placed in the home cage. Retention latencies were tested 24 h later (day 2). The animals were placed in the light compartment, with free access to the dark one for a period of 300 s. The latency to crossover into the dark compartment with all four feet was measured (retention latency). Animals who failed to enter the dark compartment within 300 s were assigned a maximum test latency score of 300 s.

OF test. The video camera-based OF test assesses locomotor and exploratory activity and also basic anxiety levels (File, 2001). In the brightly lit open area animals tend to stay near the walls of the OF rather than to enter the central region, a behavior often used as a measure of 'anxiety scores' (Griebel et al, 1993).

The experimental box was made from black glacial polyvinyl chloride and illuminated by 4×60 W lamps mounted 1.5 m above the box (100 lux in the center of the arena). Mice were individually placed in the center of an open-field box (50×50 cm, 25 cm high) for 5 min. The time spent in each of predefined zones (central area and near wall area, 10 cm from walls), total distance travelled during the experiment (locomotion index) and number of crossings between zones (exploration index, the whole area was virtually divided into 16 equal size zones) were automatically recorded using the TSE videotracking system (TSE GmbH, Homburg, Germany). The 'anxiety index', the ratio between time spent near walls and the time spent in the center, was used as the operational measure of 'anxiety level'.

EPM test. Conceptually, the EPM test is based on the natural aversion of rodents for heights and open spaces (Lister, 1987) and on the conflict between exploration of a novel environment and the avoidance of a brightly lit open elevated area. The apparatus consisted of four arms (each $30 \times 5 \, cm^2$) and a central area ($5 \times 5 \, cm^2$) elevated 1 m

above the floor and was made of dark gray glacial polyvinyl chloride (TSE GmbH, Homburg, Germany). Two arms were open whereas the other two were supplied with 10 cm-high walls. The animal was gently released in the center of the apparatus facing an open arm and allowing entrance either into the closed or open arms. The behavior of the mouse was monitored by the TSE videotracking system for 5 min. The main parameters recorded were: time spent in open arms, closed arms and central region, total distance traveled during the experiment, and the number of entries into closed and opened arms. As an operational measures of anxiety-like behavior we used open time ratio ($OTR = 100 \times (T_{op}/T_{tot})$, where T_{op} is the time spent in closed branches of the apparatus in seconds and T_{tot} the total time of experiment) and the number of entries into the open arms (open entries (OE)). Increases in OE and OTR were interpreted as anxiolytic-like indices of drug treatment relative to vehicle-treated animals, and decreases in closed arm entries (CE) and closed time ratio (CTR) were interpreted as reduced protected exploration activity.

Antinociceptive activity. The hot-plate test was performed at 57°C as described elsewhere (Kuzmin *et al*, 2000). The basal nociceptive threshold was measured prior to peptide administration as the latency to paw-licking or lifting of the hindlimbs or jumping, the behavior that was exhibited first. Dynorphins or CSF vehicle, 2 µl/mouse were injected i.c.v. 25 min later and the nociceptive reaction was measured 5 and 10 min after injection. The cutoff time for non-responders was set at 30 s.

Surgery and cannulation. The mice were anesthetized with a combination of Ketalar$^\circledR$ (ketamine hydrochloride 50 mg/ml, 1 : 10, 0.2 ml, subcutaneously (s.c.), Parke Davis, Barcelona, Spain) and Hypnorm$^\circledR$ (fluanisonum 10 mg/ml + fentanylum 0.2 mg/ml; 1 : 10, 0.2 ml, intraperitoneally (i.p.), Janssen, Beerse, Belgium). The body temperature was maintained at 37°C using a thermostat regulated heat pad (CMA/105, CMA/Microdialysis, Stockholm, Sweden). Permanent steel guide cannulae with an outer diameter of 0.4 mm (Plastics One, Roanoke, VA, USA) were implanted into the right lateral ventricle using coordinates based on the stereotaxic plates (AP(Bregma) –2.5 mm, L 3 mm, V 4.25 mm, mouse brain atlas: www.mousebrain.com). The cannula was fixed to the skull by dental carboxylic cement (Durelon ESPE, Germany). The animals were allowed to recover for 5 days in the colony room (two to three mice per cage) before the start of the experiment. I.c.v. injections (2 µl) were made manually using a 10 µl syringe. Mice were partially restrained during injection (about 15–20 s). After completion of the behavioral experiments the position of the cannula was verified histologically using injection of 2 µl of methylene blue in saline.

Drugs

Big Dyn (human prodynorphin 207–238; see Table 1), Dyn A (Big Dyn1–17) and Dyn B (Big Dyn20–32), were synthesized in the Department of Medical Biochemistry and Microbiology, University of Uppsala, Uppsala, Sweden,

Table 1 Amino-Acid Sequences of Dynorphins

Peptide	Sequence
Big dynorphin	YGGFLRRIRPKLKWDNQKRYGGFLRRDFKVVT
Dynorphin A	YGGFLRRIRPKLKWDNQ
Dynorphin B	YGGFLRRDFKVVT

and purified by two consecutive reverse phase chromatography steps on Vydac C18 218 TP 1022 and Sephasil C8 columns in 0.1% TFA and acetonitrile. Peptide identity was confirmed with matrix-assisted laser desorption/ionization time-of-flight spectrometry, and homogeneity evaluated by analytical reverse phase chromatography. Purity was 99%. Peptides freshly dissolved in artificial CSF were infused i.c.v. in a volume of 2 µl/mouse. Peptides were injected in doses ranging from 1 or 10 ng/mice to 10 µg/mice; a range of doses reported to modify behavior after i.c.v. administration of a variety of neuropeptides (for review, see Bodnar and Klein, 2004; Yu *et al*, 2004).

Artificial CSF contained (g/l): NaCl (7.20), NaHCO$_3$ (1.96), KCl (0.18), KH$_2$PO$_4$ (0.068), CaCl$_2$ (0.16), MgCl$_2 \times$ 6H$_2$O (0.17), Na$_2$SO$_4$ (0.07), glucose (1.0). The pH of the solution was adjusted to 7.4 with 10 mM NaOH and HCl.

(5R,10S)-(+)-5-methyl-10,11-dihydro-5H-dibenzo(a,b)-cyclohepten-5,10-imine hydrogen maleate (MK 801; Sigma Chemical Co., St Louis, MO, USA) was dissolved in saline and injected s.c. Nor-binaltorphimine 2HCl (17,17'-(dicyclopropylmethyl)-6,6',7,7'-6,6'-imino-7,7'-bimorphinan-3,4',14,14'-tetrol, nor-BNI; Tocris, UK) was dissolved in saline and injected s.c. 48 h before the experiment at the dose of 6 mg/kg; 5 mg/kg. Buspirone HCl was obtained from Sigma (St Louis, MO) and diazepam from AB Fermenta Läkermedel (Göteborg, Sweden). Buspirone was dissolved in physiological saline and diazepam in 40% solution of propylene glycol in sterilized water. Buspirone and diazepam were administered i.p. (5 ml/kg) 30 min before testing. Doses were calculated as the salt.

Statistics

PA data were analyzed (Statistica 6.0) using two-way ANOVA with treatment as between group factor and training/retention latencies as repeated measurement factor. In the case of a significant treatment $\times$ time interaction one-way ANOVA with treatment as between group factor was performed separately for training and retention latencies followed when appropriately by the Student–Neuman–Keuls *post hoc* test. The data from the OF and EPM tests were analyzed using one-way ANOVA followed by the Student–Neuman–Keuls *post hoc* test. Nociceptive latencies were analyzed using repeated measurement ANOVA with treatment as between group factor and three nociceptive tests as repeated factor followed by the *post hoc* Newman–Keul's test. The level of significance was set at the 0.05 level. The study was designed as a between subjects (independent groups) experiment, that is each animal was used only once.

RESULTS

PA Test (Figures 1 and 2)

Dyn A. When examined 24 h after training, the retention latency in the CSF-treated control group was close to 50 s with a small distribution in the responses, indicating that animals had acquired the task. Two-way ANOVA revealed significant time × treatment interaction in latencies (for F- and P-values see legend for Figure 1). Subsequent one-way ANOVA revealed that Dyn A (0.0005–5.0 nmol i.c.v.) injected 5 min before PA training caused a facilitation of PA

retention (F(5,24) = 3.1, $P < 0.05$) with a significant effect at the 0.005 nmol dose ($P < 0.05$ vs CSF control group) (Figure 1a). Training latencies were not affected by Dyn A: F(5,24) = 0.45, not significant (n.s.) Visual examination of the behavior in the PA apparatus during the 1-min exploration period indicated that locomotor activity was slightly decreased. Based on the dose–response experiments, the 0.005 nmol dose of Dyn A was selected for the subsequent interaction studies with NMDA and κ-opioid antagonists.

Dyn B. Two-way ANOVA revealed significant time × treatment interaction in latencies (for F- and P-values see legend for Figure 1). Subsequent one-way ANOVA revealed that Dyn B (0.007–7.0 nmol i.c.v.) injected 5 min before PA training caused a facilitation of PA retention (F(4,24) = 6.54, $P < 0.01$) with a significant effect at the 0.7 and 7 nmol doses ($P < 0.05$ vs CSF control group) (Figure 1b). Training latencies were not affected by Dyn B: F(4,24) = 0.32, n.s. Visual examination of the behavior in the PA apparatus during the 1-min exploration period indicated that locomotor activity was slightly increased. Based on the
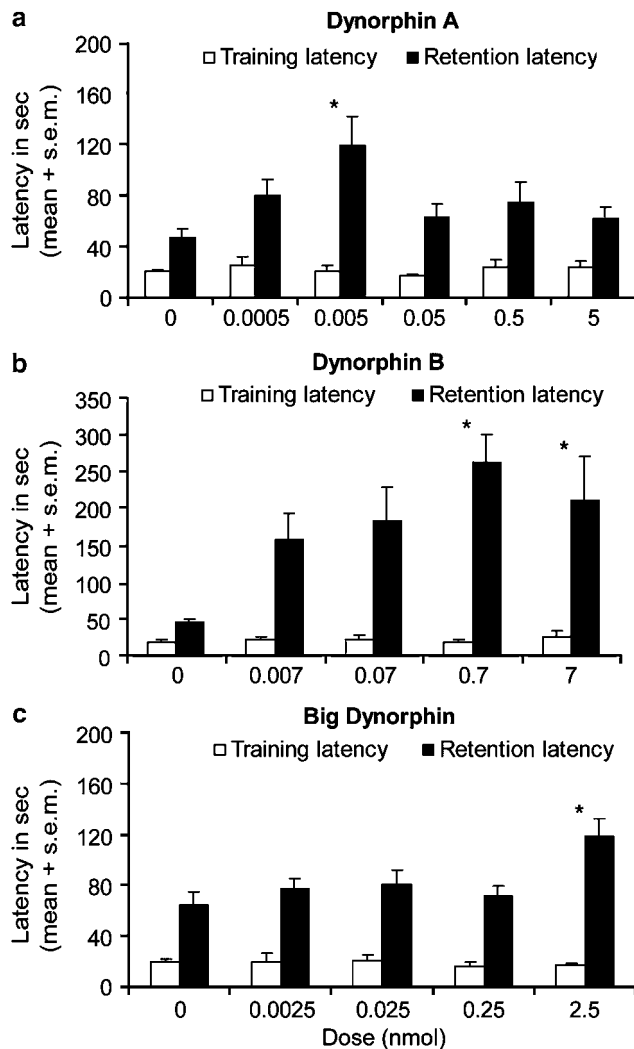

Figure 1 The dose–response effects of dynorphins on PA retention in mice. (a) Effects of Dyn A: mice were injected i.c.v. with Dyn A (0.0005, 0.005, 0.05, 0.5, and 5.0 nmol) 5 min prior to training. Two-way ANOVA: treatment effect F(5,24) = 2.66, $P < 0.05$; time effect F(1,24) = 83.5, $P < 0.01$; interaction F(5,24) = 3.11, $P < 0.05$. (b) Effects of Dyn B: mice were injected i.c.v. with Dyn B (0.007, 0.07, 0.7, and 7.0 nmol) 5 min prior to training. Two-way ANOVA: treatment effect F(4,24) = 6.35, $P < 0.01$; time effect F(1,24) = 94.8, $P < 0.01$; interaction F(4,24) = 6.42, $P < 0.01$. (c) Effects of Big Dyn: mice were injected i.c.v. with Big Dyn (0.0025, 0.025, 0.25, and 2.5 nmol) 5 min prior to training. Two-way ANOVA: treatment effect F(4,24) = 3.71, $P < 0.05$; time effect F(1,24) = 124.6, $P < 0.01$; interaction F(4,24) = 6.31, $P < 0.01$. The CSF (0) control group was run concurrently. The retention test was performed 24 h after training. Verticals bars represent mean (±SEM) of training/retention latency in seconds. *$P < 0.05$ vs CSF group; $n = 6$–10/group.
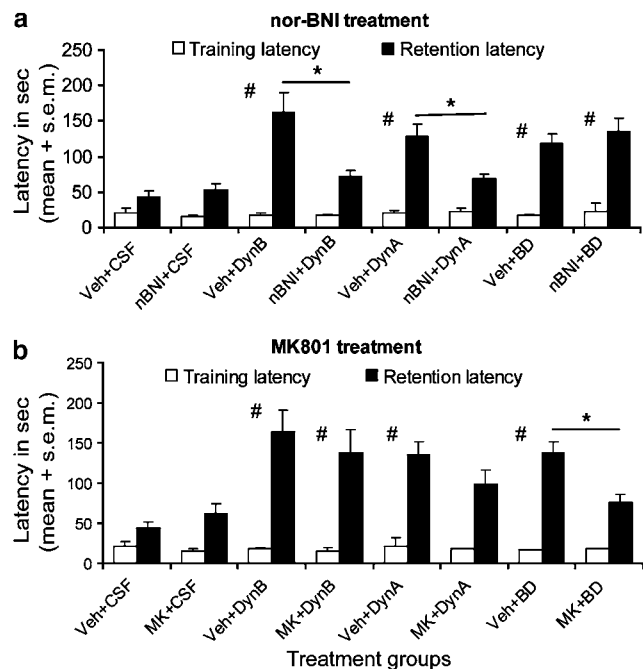

Figure 2 Effects of nor-BNI and MK 801 on the memory facilitation produced by dynorphins in PA task. (a) Experiments with nor-BNI: 48 h before training session mice were injected with nor-BNI (6 mg/kg; s.c.) or vehicle (s.c.), and 5 min before training with Dyn A (i.c.v.; 0.005 nmol), Dyn B (0.7 nmol), Big Dyn (2.5 nmol), or CSF (control group). Two-way ANOVA: treatment effect F(7,38) = 19.8, $P < 0.01$; time effect F(1,38) = 301.4, $P < 0.01$; interaction F(7,38) = 23.0, $P < 0.01$. (b) Experiments with MK 801: Mice were injected with MK 801 (0.1 mg/kg; i.p.) or vehicle (i.p.) 5 min before i.c.v. administration of Dyn A (0.005 nmol), Dyn B (0.7 nmol), Big Dyn (2.5 nmol) or CSF. Training session was performed 5 min after i.c.v. injection. Two-way ANOVA: treatment effect F(6,33) = 13.9, $P < 0.01$; time effect F(1,33) = 303.4, $P < 0.01$; interaction F(6,33) = 16.4, $P < 0.05$. The retention test was performed 24 h after training. Vertical bars represent mean (±SEM) of training/retention latency in seconds. *$P < 0.05$, comparison of the vehicle- and drug-treated groups; #$P < 0.05$, comparison of the CSF and peptide-treated groups; $n = 8$–10/group.

dose–response experiments, the 0.7 nmol dose of Dyn B was selected for the subsequent interaction studies with NMDA and κ-opioid antagonists.

Big Dyn. Two-way ANOVA revealed significant time-treatment interaction in latencies (for F- and P-values see legend for Figure 1). Subsequent one-way ANOVA revealed that Big Dyn (0.0025–2.5 nmol i.c.v.) injected 5 min before PA training enhanced PA retention (F(3,21) = 3.87; $P < 0.05$) with a significant effect at the 2.5 nmol dose ($P < 0.05$ vs CSF control group) (Figure 1c). Training latencies were not significantly affected by Big Dyn: F(3,21) = 0.2, n.s. Visual examination of the behavior in the PA apparatus during the 1-min exploration period indicated that locomotor and exploratory activity was increased. Based on the dose–response experiments, the 2.5 nmol dose of Big Dyn was selected for the subsequent interaction studies with NMDA and κ-opioid antagonists.

Effects of nor-BNI pretreatment. Two-way ANOVA revealed significant time × treatment interaction in latencies (for F- and P-values see legend for Figure 2a). Nor-BNI, a selective κ-opioid antagonist exerts long-lasting antagonistic effects that persist for at least 1 month (Horan et al, 1992; Jones and Holtzman, 1992; Broadber et al, 1994; Chang et al, 1994; Kuzmin et al, 2000). In mice, the action of nor-BNI gradually increases reaching a plateau in selective blockage of κ-receptors in 2 days after s.c. administration (Endoh et al, 1992). Nor-BNI injected alone at 6.0 mg/kg, s.c., 48 h prior the PA experiment did not produce any effect on training latencies or PA memory retention: F(7,38) = 0.39, n.s. However, ANOVA revealed significant differences in retention latencies between treatment groups: (F(7,38) = 23.8, $P < 0.01$). Nor-BNI blocked memory facilitation produced by Dyn A or Dyn B; no differences between groups pretreated with nor-BNI and injected with CSF, Dyn A or Dyn B were observed. However, this antagonist did not reverse the facilitation of PA retention (Figure 2a) induced by Big Dyn ($P < 0.01$ as compared with the CSF group).

Effects of MK 801 pretreatment. MK 801 (0.03–0.1 mg/kg, i.p.) injected 5 min before PA training produced no changes in memory retention (100 and 94% of retention latency increase as compared to vehicle group, respectively). However, at the higher 0.3 mg/kg dose MK 801 impaired PA retention ($P < 0.01$, vs vehicle control group; 75% decrease in retention latency) and increased locomotor activity during the 1-min exploration period. ANOVA revealed significant effects of MK801 treatment on retention latencies (F(3,20) = 5.35, $P < 0.01$). Training latencies were not affected by MK 801 (F(3,20) = 1.2, n.s.). Based on these results the 0.1 mg/kg dose of MK 801 was used at in the subsequent interaction studies with dynorphins.

Two-way ANOVA revealed significant time × treatment interaction in latencies (for F- and P-values see legend for Figure 2b). To examine effects on dynorphin-induced facilitation of PA retention, MK 801 was administered 5 min prior to peptide microinjection. ANOVA revealed significant differences in retention latencies between treatment groups (F(7,38) = 15.7, $P < 0.01$). Training latencies did not differ between groups: F(7,38) = 0.14, n.s. MK 801 failed to

reverse the facilitation of PA retention (Figure 2b) induced by Dyn B ($P < 0.01$ as compared to the group CSF + MK 801). In contrast, MK 801 blocked memory facilitation produced by Big Dyn and also nonsignificantly ($P = 0.1$) reduced the effect of Dyn A.

OF Experiments (Figure 3)

Big Dyn administration (2.5 nmol, 10 min prior to behavioral testing) increased locomotor ($P < 0.01$) and exploratory ($P < 0.05$) activity as indicated by the increase in the total distance traveled and number of zone-crossings during 5 min testing (Figure 3a). Big Dyn also increased ($P < 0.05$) the time spent in the central areas of the OF suggesting anxiolytic-like activity of the peptide (Figure 3c). The 'anxiolytic'-like effects of Big Dyn correlated with the locomotor stimulation ($r = 0.89$) and, therefore, may be due to an increase in general locomotor and searching activity induced by this peptide. Indeed, mice treated with Big Dyn demonstrated both horizontal and vertical hyperactivity and sporadic short-lasting 'chaotic' movements and 'bursts' of grooming behavior. The number of crossings between predefined areas was also increased. Pretreatment with MK 801 (0.1 mg/kg; s.c.; 10 min prior to Big Dyn administration) abolished the effects of Big Dyn. Nor-BNI (6 mg/kg; s.c.; 48 h prior peptide injection) did not alter the behavior induced by Big Dyn. Effects of Big Dyn were compared to those of diazepam (1 mg/kg) and buspirone (3 mg/kg). These doses chosen from pilot dose–response experiments produced anxiolytic effect in mice in both OF and EPM tests (data not shown). Diazepam and buspirone similarly to Big Dyn increased time spent in the central areas of the OF (Figure 3d) apparently due to their 'anxiolytic' activity but the number of crossings between predefined areas was unchanged (Figure 3b).

Dyn A (0.005 nmol) and Dyn B (0.7 nmol) had virtually no effect on animal behavior in the OF, no changes in 'anxiety'-like behavior were recorded although locomotor activity tended to increase or decrease, respectively, after peptide administration. MK 801 and nor-BNI did not modify behavior in mice treated with Dyn A or Dyn B.

EPM Experiments (Figure 4 and Table 2)

Anxiolytic-like activity of Big Dyn was further evaluated in the EPM test and peptide effects were compared with those of diazepam (1 mg/kg) and buspirone (3 mg/kg). The operational measures of anxiety-like behavior was time spent in the open arms of the maze (Figure 4a) that was also expressed either as percentage of total time (OTR; Table 2) or the number of entries into the open arms (OE; Figure 4b) (Spine et al, 2000; Lister, 1987). One-way ANOVA revealed difference in the 'anxiety' level between groups, according to 'OTR index' (F(4,50) = 4.6, $P < 0.01$) and 'OE index' (F(4,50) = 15.6, $P < 0.01$). *Post hoc* test revealed that Big Dyn (2.5 nmol) significantly increased time spent in the open arms ($P < 0.01$) and number of entries into the open arms of the apparatus ($P < 0.01$) when compared to the CSF group. Diazepam and buspirone exhibited similar effects when compared with saline-treated animals (increase in OTR and OE, $P < 0.01$). Administration of Big Dyn tended to increase locomotor activity of mice in the EPM test. However, unlike
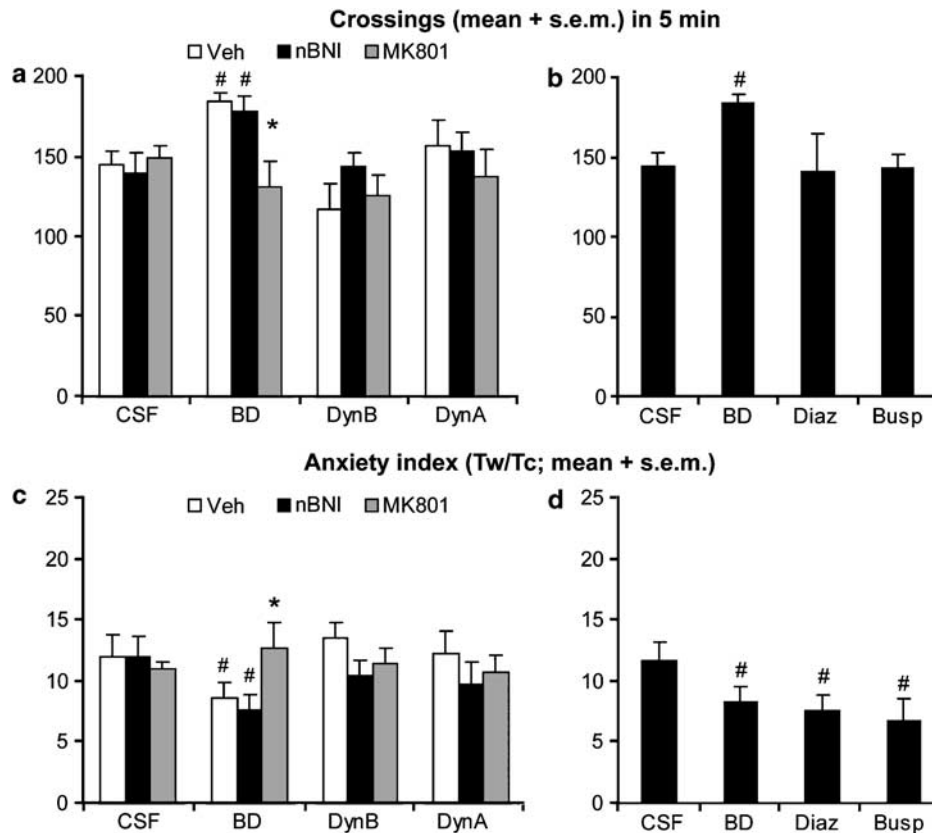
Figure 3 Effects of dynorphins in the open field test. (a and b) Number of crossings between the predefined areas. (a) Peptide effects and their modulation by nor-BNI and MK 801. (b) Comparison of Big Dyn with diazepam and buspirone. (c and d) Anxiety index (T_w/T_c, time spent in the 10 cm near wall zone/time spent in the central area). (c) Peptide effects and their modulation by nor-BNI and MK 801. (d) Comparison of BD with diazepam and buspirone. In (a) and (c) mice pretreated with nor-BNI (3 mg/kg; s.c.), MK 801 (0.1 mg/kg; i.p.) or vehicle (s.c. or i.p., respectively) 48 h (nor-BNI) or 5 min (MK 801, vehicle) later were injected i.c.v. with Dyn A (0.005 nmol), Dyn B (0.7 nmol), Big Dyn (2.5 nmol) or CSF. Testing was performed 10 min after i.c.v injection. *$P < 0.05$, comparison of the vehicle- and drug-treated groups; #$P < 0.05$, comparison of the CSF and peptide-/drug-treated groups; $n = 6$–8/group. In (b) and (d) mice were treated with CSF (i.c.v.; 10 min before test) or Big Dyn (2.5 nmol; 10 min before test) or buspirone (3 mg/kg, i.p.; 30 min before test) or diazepam (1 mg/kg, i.p.; 30 min before test), and were examined in the OF test for 5 min. #$P < 0.05$, comparison to the CSF-treated group; Data are shown as mean ($\pm$SEM); $n = 6$–8/group.

the OF experiments, this increase (both distance travelled and number of transitions between different branches of apparatus) was not statistically significant. Also, diazepam and buspirone failed to influence locomotor behavior in the EPM test.

Hot-Plate Experiments (Figure 5)

Big Dyn and Dyn A injected i.t. produced pronociceptive effects in mice (Tan-No *et al*, 2002). To test whether the increase in the PA latencies reflects enhanced sensitivity to the foot shock, the effect of dynorphins on pain sensitivity after i.c.v. administration at several doses including those effective in the PA test was evaluated in the hot-plate test.

Effect of Dyn A. Mice were first tested for their nociceptive thresholds, 30 min later given Dyn A (0.005, 0.05, and 0.5 nmol, i.c.v.) and again tested for nociceptive thresholds 5 and 10 min later. A repeated measurement analysis of nociceptive latencies was performed with treatment (four levels: 0 (CSF); 0.005, 0.05, and 0.5 nmol) as between group factors and time (basal, +5 and +10 min values) as

repeated measurement factor. The basal nociceptive reaction was similar in all treatment groups (9–10 s). ANOVA revealed a significant treatment effect ($F(3,26) = 4.3$, $P < 0.05$), a significant time effect ($F(2,56) = 45.2$, $P < 0.01$) and significant treatment × time interaction ($F(6,56) = 12.6$, $P < 0.01$). *Post hoc* analysis revealed that Dyn A (0.05 nmol) significantly elevated nociceptive latencies at both 5 and 10 min tests ($P < 0.01$). Other tested doses of Dyn A did not change nociceptive latencies.

Effect of Dyn B. The same experimental procedure was used as described for Dyn A. Dyn B was injected i.c.v. (0.07, 0.7, and 7 nmol) to separate groups of mice. The basal nociceptive reaction was similar in all treatment groups (9–10 s). ANOVA revealed a significant treatment effect ($F(3,26) = 9.2$, $P < 0.001$), a significant time effect ($F(2,52) = 23.2$, $P < 0.0001$) and significant treatment × time interaction ($F(6,52) = 9.2$, $P < 0.01$). *Post hoc* analysis revealed that Dyn B (7 nmol) significantly elevated nociceptive latencies at 5 and 10 min after peptide administration ($P < 0.01$). Other tested doses of Dyn B failed to influence nociceptive latencies.
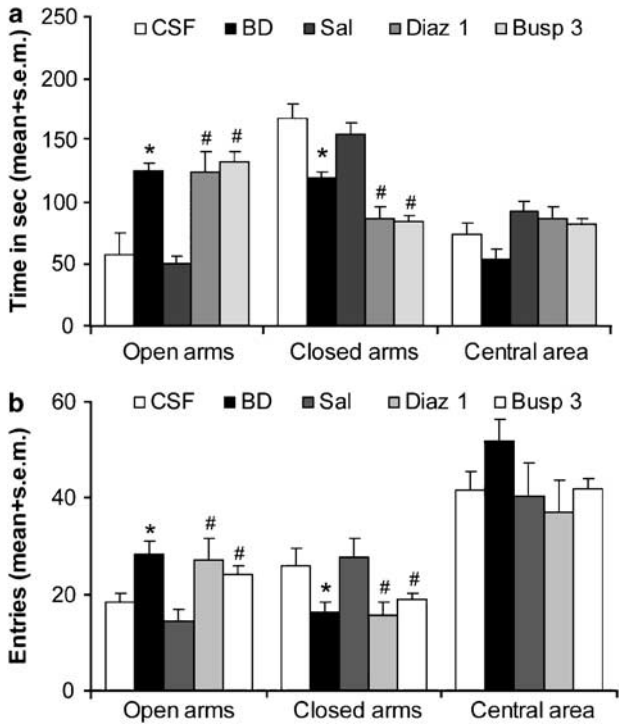
Figure 4 Effects of Big Dyn in the elevated plus maze test. Comparison with diazepam and buspirone. (a) Time (s) spent in the open, closed, and central areas of the EPM apparatus. (b) Number of entries into the open, closed, and central areas of the EPM apparatus. Mice were treated with CSF (i.c.v.; 10 min before test), Big Dyn (2.5 nmol; i.c.v.; 10 min before test), buspirone (3 mg/kg; i.p.; 30 min before test) or diazepam (1 mg/kg; i.p.; 30 min before test) and were examined in the EPM test for 5 min. *,#$P < 0.05$, comparison to the CSF or saline groups, respectively; Data are shown as mean ($\pm$SEM); $n = 8$–10/group.

Table 2 Effects of Big Dyn on Mouse Behavior in the EPM Test. Comparison to Diazepam and Buspirone

Group (dose)	V tot (N)	D_{tot} (cm)	OTR (T_{op}/T_{tot})
CSF	88 ± 3	1898 ± 61	0.22 ± 0.06
Big Dyn (2.5 nmol)	96 ± 4	2058 ± 80	0.42 ± 0.02*
Saline	82 ± 4	1670 ± 89	0.26 ± 0.02
Diazepam (1 mg/kg)	79 ± 4	1581 ± 93	0.42 ± 0.03#
Buspirone (3 mg/kg)	85 ± 4	1872 ± 71	0.44 ± 0.03#

Data are presented as mean ($\pm$SEM); $n = 10$.
V_{tot} (N)—total number of transitions between branches in 5 min experiment.
D_{tot} (cm)—total distance (in cm) traveled during 5-min experiment.
OTR (T_{op}/T_{tot})—anxiety index as the ratio between time (s) spent in the open arms of the apparatus to total time (300 s).
*$P < 0.05$, comparison to CSF group.
#$P < 0.05$, comparison to the saline group.

Effect of big Dyn. The experimental procedure was similar to the one described for Dyn A. Big Dyn (0.025, 0.25 and 2.5 nmol) was injected i.c.v. to separate groups of mice. The basal nociceptive reaction was similar in all treatment groups (9–10 s). ANOVA failed to reveal significant changes in nociceptive thresholds indicating neither analgesic nor hyperalgesic responses to Big Dyn administration.
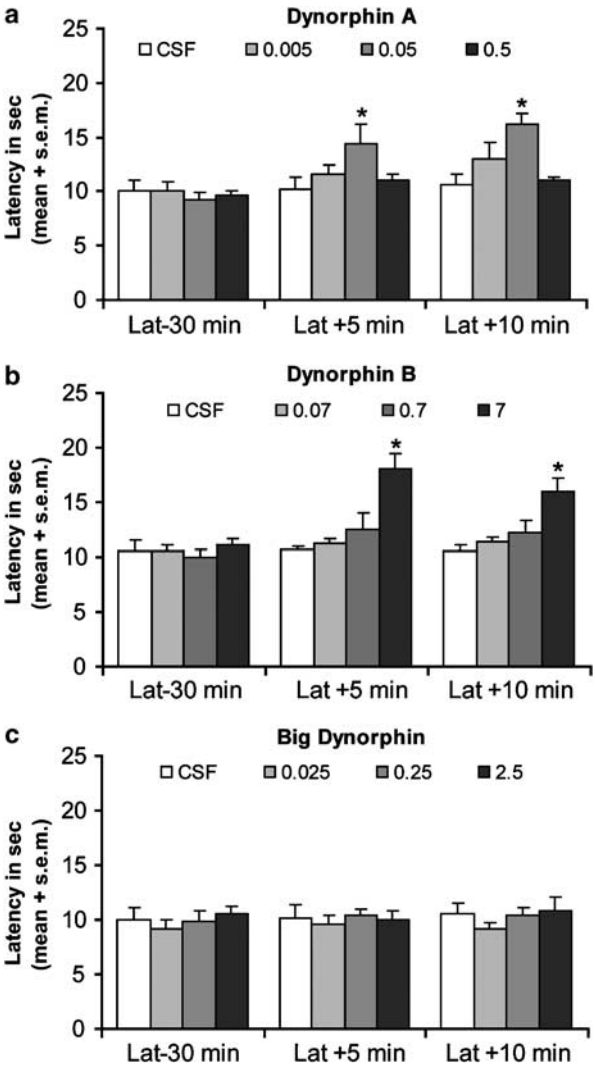
Figure 5 Analgesic activities of Dyn A (a), Dyn B (b), and Big Dyn (c) in the hot-plate test. Shown are basal (measured 30 min prior peptide or CSF injection) and peptide-induced (measured 5 and 10 min after peptide or CSF injection) latencies (s) for the nociceptive reaction. Doses are in nmol. Data are expressed as mean ($\pm$SEM); $n = 10$/group. *$P < 0.01$, significant increase in nociceptive latencies as compared to the basal level of nociceptive reaction.

DISCUSSION

Like other neuropeptide precursors, prodynorphin is multipotent, that is, able to generate several peptide products. The focus has conventionally been on Dyn A, Dyn B and α-neoendorphin as ligands on κ-opioid receptors. Reports in the literature have, however, indicated that Dyn A may be substantially different from the other two with regard to some actions, particularly a neurotoxic effect on the spinal cord (Trujillo and Akil, 1991; Vanderah et al, 1996; Wang et al, 2001; Xu et al, 2004; Tan-No et al, 2005). This effect was blocked by NMDA-receptor antagonists (reviewed in Hauser et al, 2005) indicating a separate pharmacophore in the peptide C-terminus. The much less studied putative precursor to Dyn A and Dyn B, Big Dyn (Table 1) known to exist in brain, might have similar actions on the NMDA receptors. Experiments with prodynorphin-deficient mice have supported the notion on

nonopioid effects of dynorphins (Wang et al, 2001; Tan-No et al, 2005). The present study has indicated behavioral effects of Big Dyn distinct from those of the dynorphins in both quality and separate receptor profile. Thus, prodynorphin produces peptides acting primarily through κ-opioid receptors (Dyn B and α-neoendorphin) or NMDA receptors (Big Dyn) and also through both systems (Dyn A).

It may seem paradoxical, that the behavioral effects of Big Dyn are mediated through the NMDA glutamate receptors but not opioid receptors. The affinity of Big Dyn for κ-, μ- and δ-opioid receptors expressed in cells transfected with the respective cDNA is roughly similar to that of Dyn A (Merg et al, submitted). Thus Big Dyn may also have a dual action in the CNS; it may activate κ-receptors in the areas where they are expressed but target the glutamatergic transmission in other regions. Given the wide representation of NMDA receptors throughout the CNS, effects on these receptors would prevail.

There are several pieces of evidence supporting the notion that changes in behavior induced by Big Dyn were due to the intact peptide and not to its degradation/processing products Dyn A and Dyn B or the enkephalins. Big Dyn and its fragments produced different patterns of behavioral effects in each of four tests employed and these effects were differentially sensitive to antagonists of the NMDA glutamate and κ-opioid receptors. Nonetheless, the possibility that some effects of Big Dyn may not represent actions of its own but a combination with the effects elicited by its fragments Dyn A and Dyn B, can not be ruled out.

Peptidase inhibitors that prevent Big Dyn degradation along with pharmacokinetic analysis of peptide biotransformation in the targeted brain structures may be used to separate effects of Big Dyn from those of its fragments. Nothing is known, however, about mechanisms of Big Dyn degradation and selective inhibitors of its processing/conversion have not yet been designed. Biotransformation of synthetic Dyn A in vivo has recently been investigated using microdialysis and mass spectrometry (Reed et al, 2003; Klintenberg and Andren, 2005) whereas data on Big Dyn degradation are limited to our own mass-spectroscopy study showing slow (3–5% in 20 h) conversion of Big Dyn to dynorphins and rapid Dyn B degradation in extracts of the rat hippocampus, striatum and substantia nigra (Sandin et al, 1997). These data support the notion that changes in behavior after administration of Big Dyn are produced by the parent peptide and not by its fragments. The kinetics of the behavioral response may also help to discriminate effects of Big Dyn from those of its fragments. Peptide effects were investigated at different time points after i.c.v. administration in the hot-plate test (see Figure 5). Whereas Dyn A and Dyn B increased latency, no effects were observed after Big Dyn injection. This suggests that Big Dyn is not converted to Dyn A and Dyn B, or that the levels of these products are much lower than those required to produce behavioral effects.

Big Dyn injected prior to the training session enhanced memory retention in the PA test and enhanced locomotor and exploratory activities and produced an anxiolytic-like effect in the OF test. The anxiolytic-like activity of Big Dyn was also evident in the EPM test; the magnitude of the activity was similar to that exhibited by diazepam and buspirone. In contrast to effects of Dyn A and Dyn B

mediated through κ-opioid receptors, the Big Dyn-induced behavior was blocked by pretreatment with MK 801, an NMDA receptor antagonist. This is in line with our previous report that this peptide injected i.t. at low doses, from 1 to 10 fmol induces a characteristic nociceptive behavior consisting of biting and licking of the hindpaw and the tail along with a hindlimb scratching, a behavior also blocked by NMDA receptor antagonists. Dyn A was 100-fold less potent whereas Dyn B was inactive (Tan-No et al, 2002). In this study, Big Dyn administered i.c.v. had no effects on nociceptive thresholds in the hot-plate test, whereas Dyn A or Dyn B had analgesic effects, although with low amplitude and duration, an observation consistent with previous reports (Kaneko et al, 1983).

Dyn A and Dyn B enhanced PA retention albeit with different potency (see Figure 1) by acting through κ-opioid receptors. Different stability of these peptides may account for differences in dose–responses. Indeed, Dyn A degrades slower than Dyn B in extracts of several brain structures (Sandin et al, 1997). The facilitatory effects of Dyn A on memory retention in the PA task, sensitivity to the κ-antagonist and the dose dependence are in an agreement with previous reports (Hiramatsu and Inoue, 2000; Hiramatsu et al, 1996, 1998; Ukai et al, 1997). κ-Opioid agonists induce dysphoria in humans and endogenous dynorphins mediate aversive reactions in animals (Kreek et al, 2002; Shippenberg et al, 2001). This property may be essential for the ability of dynorphins to facilitate learning and memory based on aversive stimulation, for example associated with stress, pain, and tissue injury. These forms of memory often have importance for survival. This notion is supported by observations that dynorphins or κ-opioids enhance memory retention in the PA test (i.c.v. administration) and spontaneous memory in anxiety-provoking situations (intracortical administration; Wall and Messier, 2000). Big Dyn may also facilitate memory and learning in the PA task, however, effects of this peptide are mediated through NMDA receptors. It is then logical to suggest that dynorphins may be neutral or impair forms of memory based on positive reinforcement. Indeed, injection of synthetic dynorphin into the hippocampus impairs memory in the Morris water maze task (Sandin et al, 1998). Consistently, prodynorphin knockout mice demonstrated diminished age-associated impairment in spatial water maze performance suggesting adverse actions of endogenous dynorphins. Endogenous dynorphins are upregulated with age and, consistently, less impairment was observed in aged prodynorphin-deficient mice (Nguyen et al, 2005).

The localization of dynorphins in the striato-nigral pathway (Graybiel, 1990; Vincent et al, 1982) may be associated with their role in regulation of locomotor behavior. In rodents agonists of κ-opioid receptors inhibit locomotion and produce ataxia and sedation (Jackson and Cooper, 1988). κ-Opioid-induced effects on motor activity are apparently mediated through the regulation of the mesolimbic and nigrostriatal dopaminergic neurotransmission. A complex dose- and time-dependent influence of κ-opioids on locomotor activity in mice was shown (Kuzmin et al, 2000). However, no significant effects of Dyn A and Dyn B on locomotor behavior in the OF were observed at doses that produced maximal effects in the PA test. In contrast, Big Dyn increased both horizontal and vertical

locomotor activity and also sporadically induced chaotic movements and bursts of grooming behavior that all were blocked by MK 801. This observation corroborates the notion that NMDA receptors modulate locomotor behavior (Gimenez-Llort *et al*, 1995; Melani *et al*, 1999).

Selective κ-opioid agonists have been found to exhibit anxiolytic properties in rodents (Privette and Terrian, 1995). Moreover, the anticonflict effect of diazepam was abolished by pretreatment with naloxone or nor-BNI suggesting that endogenous dynorphins play a role in the regulation of anxiety (Tsuda *et al*, 1996). The κ-opioid effects on memory in anxiety-provoking situations (Wall and Messier, 2000) may involve modulation of dopaminergic function (Itoh *et al*, 1993). Big Dyn-induced anxiolytic-like activity was evident in both the OF and EPM tests. These effects apparently involved NMDA receptors but not κ-opioid receptors. Interaction of Big Dyn with NMDA receptors may be more complex than a simple stimulation since NMDA agonists generally produce anxiogenic rather than anxiolytic effects (for review, see Berginck *et al*, 2004).

In conclusion, Big Dyn produces a unique profile of behavioral effects including memory facilitation, locomotor activation and reduction of anxiety-like behavior. These effects are different from those of Dyn A and Dyn B, which primarily act on κ-opioid receptors whereas Big Dyn effects are mediated through NMDA receptors. The questions whether the NMDA receptors are the primary targets for Big Dyn and whether the endogenous dynorphin system is involved in the regulation of memory and anxiety under normal and pathophysiological conditions should be addressed in future studies.

REFERENCES

Bammer G (1982). Pharmacological investigations of neurotransmitter involvement in passive avoidance responding: a review and some new results. *Neurosci Biobehav Rev* **6**: 247–296.

Berginck V, van Megen HJ, Westenberg HG (2004). Glutamate and anxiety. *Eur Neuropsychopharmacol* **14**: 175–183.

Bodnar RJ, Klein GE (2004). Endogenous opiates and behavior. *Peptides* **25**: 2205–2256.

Broadber JH, Negus SS, Butelman ER, deCosta BR, Woods JH (1994). Differential effects of systemically administered nor-binaltorphimine (nor-BNI) on kappa-opioid agonists in the mouse writing assay. *Psychopharmacology* **115**: 311–319.

Chang AC, Takemori AE, Portoghese PS (1994). 2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1S)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide: an opioid receptor affinity label that produces selective and long-lasting kappa antagonism in mice. *J Med Chem* **37**: 1547–1549.

Costigan M, Woolf CJ (2002). No DREAM, No pain. Closing the spinal gate. *Cell* **108**: 297–300.

Day R, Akil H (1989). The posttranslational processing of prodynorphin in the rat anterior pituitary. *Endocrinology* **124**: 2392–2405.

De Vries TJ, Shippenberg TS (2002). Neural systems underlying opiate addiction. *J Neurosci* **22**: 3321–3325.

Drake CT, Terman GW, Simmons ML, Milner TA, Kunkel DD, Schwartzkroin PA *et al* (1994). Dynorphin opioids present in dentate granule cells may function as retrograde inhibitory neurotransmitters. *J Neurosci* **14**: 3736–3750.

Endoh T, Matsuura H, Tanaka C, Nagase H (1992). Nor-binaltorphimine: a potent and selective kappa-opioid receptor antagonist with long-lasting activity *in vivo*. *Arch Int Pharmacodyn Ther* **316**: 30–42.

Fallon JH, Leslie FM (1986). Distribution of dynorphin and enkephalin peptides in the rat brain. *J Comp Neurol* **249**: 293–336.

File SE (2001). Factors controlling measures of anxiety and responses to novelty in the mouse. *Behav Brain Res* **125**: 151–157.

Fischli W, Goldstein A, Hunkapiller MW, Hood LE (1982). Isolation and amino acid sequence analysis of a 4000-dalton dynorphin from porcine pituitary. *Proc Natl Acad Sci USA* **79**: 5435–5437.

Gimenez-Llort L, Ferre S, Martinez E (1995). Effects of the systemic administration of kainic acid and NMDA on exploratory activity in rats. *Pharmacol Biochem Behav* **51**: 205–210.

Graybiel AM (1990). The basal ganglia and the initiation of movement. *Rev Neurol (Paris)* **146**: 570–574.

Griebel G, Belzung C, Misslin R, Vogel E (1993). The free exploratory paradigm: an effective method for measuring neophobic behavior in mice and testing potential neophobia reducing drugs. *Behav Pharmacol* **4**: 637–644.

Hauser KF, Aldrich JV, Anderson KJ, Bakalkin G, Christie MJ, Hall ED *et al* (2005). Pathobiology of dynorphins in trauma and disease. *Front Biosci* **10**: 216–235.

Hiramatsu M, Inoue K (2000). Des-tyrosine(1) dynorphin A-(2–13) improves carbon monoxide-induced impairment of learning and memory in mice. *Brain Res* **859**: 303–310.

Hiramatsu M, Inoue K, Kameyama T (2000). Dynorphin A-(1–13) and (2–13) improve beta-amyloid peptide-induced amnesia in mice. *Neuroreport* **11**: 431–435.

Hiramatsu M, Mori H, Murasawa H, Kameyama T (1996). Improvement by dynorphin A (1–13) of galanin-induced impairment of memory accompanied by blockade of reductions in acetylcholine release in rats. *Br J Pharmacol* **118**: 255–260.

Hiramatsu M, Murasawa H, Mori H, Kameyama T (1998). Reversion of muscarinic autoreceptor agonist-induced acetylcholine decrease and learning impairment by dynorphin A (1–13), an endogenous κ-opioid receptor agonist. *Br J Pharmacol* **123**: 920–926.

Horan P, Taylor J, Yamamura HI, Porreca F (1992). Extremely long-lasting anatagonistic actions of nor-binaltorphimine (nor-BNI) in the mouse tail-flick test. *J Pharm Exp Ther* **260**: 1237–1243.

Itoh J, Ukai M, Kameyama T (1993). Dopaminergic involvement in the improving effects of dynorphin A-(1–13) on scopolamine-induced impairment of alternation performance. *Eur J Pharmacol* **241**: 99–104.

Jackson A, Cooper SJ (1988). Observational analysis of the effects of kappa opioid agonists an open field behaviour in the rat. *Psychopharmacology (Berlin)* **94**: 248–253.

Jones DNC, Holtzman SG (1992). Long term kappa-opioid receptor blockade following nor-binaltorphimine. *Eur J Pharm* **215**: 345–348.

Kameyama T, Ukai M, Miura M (1994). Dynorphin A-(1–13) potently improves galanin-induced impairment of memory processes in mice. *Neuropharmacology* **33**: 1167–1169.

Kaneko T, Nakazawa T, Ikeda M, Yamatsu K, Iwama T, Wada T et al (1983). Sites of analgesic action of dynorphin. *Life Sci* 33(Suppl 1): 661–664.

Khachaturian H, Watson SJ, Lewis ME, Coy D, Goldstein A, Akil H (1982). Dynorphin immunocytochemistry in the rat central nervous system. *Peptides* 3: 941–954.

Klintenberg R, Andren PE (2005). Altered extracellular striatal *in vivo* biotransformation of the opioid neuropeptide dynorphin A(1–17) in the unilateral 6-OHDA rat model of Parkinson's disease. *J Mass Spectrom* 40: 261–270.

Kreek MJ (2001). Drug addictions. Molecular and cellular endpoints. *Ann NY Acad Sci* 937: 27–49.

Kreek MJ, LaForge KS, Butelman E (2002). Pharmacotherapy of addictions. *Nat Rev Drug Discov* 1: 710–726.

Kuzmin A, Sandin J, Terenius L, Ogren SO (2000). Dose- and time-dependent bimodal effects of kappa-opioid agonists on locomotor activity in mice. *J Pharmacol Exp Ther* 295: 1031–1042.

Lister RG (1987). The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 92: 180–185.

Mansour A, Fox CA, Burke S, Meng F, Thompson RC, Akil H et al (1994). Mu, delta, and κ-opioid receptor mRNA expression in the rat CNS: an *in situ* hybridization study. *J Comp Neurol* 350: 412–438.

Melani A, Corsi C, Gimenez-Llort L, Martinez E, Ogren SO, Pedata F et al (1999). Effect of N-methyl-D-aspartate on motor activity and *in vivo* adenosine striatal outflow in the rat. *Eur J Pharmacol* 385: 15–19.

Misane I, Ogren SO (2003). Selective 5-HT1A antagonists WAY 100635 and NAD-299 attenuate the impairment of passive avoidance caused by scopolamine in the rat. *Neuropsychopharmacology* 28: 253–264.

Nguyen XV, Masse J, Kumar A, Vijitruth R, Kulik C, Liu M et al (2005). Prodynorphin knockout mice demonstrate diminished age-associated impairment in spatial water maze performance. *Behav Brain Res* 161: 254–262.

Pierce JP, Kurucz OS, Milner TA (1999). Morphometry of a peptidergic transmitter system: dynorphin B-like immunoreactivity in the rat hippocampal mossy fiber pathway before and after seizures. *Hippocampus* 9: 255–276.

Privette TH, Terrian DM (1995). Kappa opioid agonists produce anxiolytic-like behavior on the elevated plus-maze. *Psychopharmacology (Berlin)* 118: 444–450.

Reed B, Zhang Y, Chait BT, Kreek MJ (2003). Dynorphin A(1–17) biotransformation in striatum of freely moving rats using microdialysis and matrix-assisted laser desorption/ionization mass spectrometry. *J Neurochem* 86: 815–823.

Sandin J, Nylander I, Georgieva J, Schott PA, Ogren SO, Terenius L (1998). Hippocampal dynorphin B injections impair spatial learning in rats: a κ-opioid receptor-mediated effect. *Neuroscience* 85: 375–382.

Sandin J, Tan-No K, Kasakov L, Nylander I, Winter A, Silberring J et al (1997). Differential metabolism of dynorphins in substantia nigra, striatum, and hippocampus. *Peptides* 18: 949–956.

Shippenberg TS, Chefer VI, Zapata A, Heidbreder CA (2001). Modulation of the behavioral and neurochemical effects of psychostimulants by kappa-opioid receptor systems. *Ann NY Acad Sci* 937: 50–73.

Solbrig MV, Koob G (2004). Epilepsy, CNS viral injury and dynorphin. *Trends Pharmacol Sci* 25: 98–104.

Tang Q, Gandhoke R, Burritt A, Hruby VJ, Porreca F, Lai J (1999). High-affinity interaction of (des-Tyrosyl) dynorphin A(2–17) with NMDA receptors. *J Pharmacol Exp Ther* 291: 760–765.

Tan-No K, Takahashi H, Nakagawasai O, Niijima F, Sato T, Sato S et al (2005). A pronociceptive role of dynorphins in uninjured animals: nociceptive effects of N-ethylmaleimide mediated through inhibition of degradation of endogenous dynorphins. *Pain* 113: 301–309.

Tan-No K, Esashi A, Nakagawasai O, Niijima F, Tadano T, Sakurada C et al (2002). Intrathecally administered big dynorphin, a prodynorphin-derived peptide, produces nociceptive behavior through an N-methyl-D-aspartate receptor mechanism. *Brain Res* 952: 7–14.

Terman GW, Drake CT, Simmons ML, Milner TA, Chavkin C (2000). Opioid modulation of recurrent excitation in the hippocampal dentate gyrus. *J Neurosci* 20: 4379–4388.

Trujillo KA, Akil H (1991). Opioid and non-opioid behavioral actions of dynorphin A and the dynorphin analogue DAKLI. *NIDA Res Monogr* 105: 397–398.

Tsuda M, Suzuki T, Misawa M, Nagase H (1996). Involvement of the opioid system in the anxiolytic effect of diazepam in mice. *Eur J Pharmacol* 307: 7–14.

Ukai M, Itoh J, Kobayashi T, Shinkai N, Kameyama T (1997). Effects of the κ-opioid dynorphin A(1–13) on learning and memory in mice. *Behav Brain Res* 83: 169–172.

Ukai M, Shan-Wu X, Kobayashi T, Kameyama T (1996). Systemic administration of dynorphin A-(1–13) markedly improves cycloheximide-induced amnesia in mice. *Eur J Pharmacol* 313: 11–15.

Van Bockstaele EJ, Sesack SR, Pickel VM (1994). Dynorphin-immunoreactive terminals in the rat nucleus accumbens: cellular sites for modulation of target neurons and interactions with catecholamine afferents. *J Comp Neurol* 341: 1–15.

Vanderah TW, Laughlin T, Lashbrook JM, Nichols ML, Wilcox GL, Ossipov MH et al (1996). Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone. *Pain* 68: 275–281.

Vincent S, Hökfelt T, Christensson I, Terenius L (1982). Immunohistochemical evidence for a dynorphin immunoreactive striato-nigral pathway. *Eur J Pharmacol* 85: 251–252.

Wagner JJ, Caudle RM, Chavkin C (1992). Kappa-opioids decrease excitatory transmission in the dentate gyrus of the guinea pig hippocampus. *J Neurosci* 12: 132–141.

Walker JM, Moises HC, Coy DH, Baldrighi G, Akil H (1982). Nonopiate effects of dynorphin and des-Tyr-dynorphin. *Science* 218: 1136–1138.

Wall PM, Messier C (2000). Concurrent modulation of anxiety and memory. *Behav Brain Res* 109: 229–241.

Wang Z, Gardell LR, Ossipov MH, Vanderah TW, Brennan MB, Hochgeschwender U et al (2001). Pronociceptive actions of dynorphin maintain chronic neuropathic pain. *J Neurosci* 21: 1779–1786.

Xie GX, Goldstein A (1987). Characterization of big dynorphins from rat brain and spinal cord. *J Neurosci* 7: 2049–2055.

Xu M, Petraschka M, McLaughlin JP, Westenbroek RE, Caron MG, Lefkowitz RJ et al (2004). Neuropathic pain activates the endogenous kappa opioid system in mouse spinal cord and induces opioid receptor tolerance. *J Neurosci* 24: 4576–4584.

Yu Y, Jawa A, Pan W, Kastin AJ (2004). Effects of peptides, with emphasis on feeding, pain, and behavior: a 5-year (1999–2003). *Peptides* 25: 2257–2289.