Effects of Systemically Administered Dynorphin A(1–17) in Rhesus Monkeys

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ABSTRACT

The effects of i.v. dynorphin A(1–17) and its main nonopioid biotransformation fragment, dynorphin A(2–17), were compared in rhesus monkeys with those of the selective κ-opioid agonist, U69,593, in assays of operant behavior, thermal antinociception, and neuroendocrine function (prolactin release). Dynorphin A(1–17) (0.1–3.2 mg/kg i.v.) and U69,593 (0.001–0.032 mg/kg s.c.) decreased rates of schedule-controlled (fixed ratio 20) food-reinforced responding, whereas dynorphin A(2–17) (1–3.2 mg/kg i.v.) was ineffective. Pretreatment studies with the opioid antagonist quadazocine (0.32 mg/kg i.v.) revealed that the operant effects of dynorphin A(1–17) were not mediated by κ- or μ-opioid receptors. A different profile was observed in the warm water tail withdrawal assay of thermal antinociception, where both dynorphin A(1–17) and A(2–17) (0.032–3.2 mg/kg i.v., n = 4) were modestly effective in 50°C water, and both were ineffective in 55°C water. By comparison, U69,593 (0.032–0.18 mg/kg s.c.) was maximally effective in 50°C water and partially effective in 55°C. κ-opioid agonists increase serum levels of prolactin in animals and humans. Dynorphin A(1–17) (ED50 = 0.0011 mg/kg i.v.), similar to U69,593 (ED50 = 0.0030 mg/kg i.v.), was very potent in increasing serum prolactin levels in follicular phase female rhesus monkeys, whereas dynorphin A(2–17) (0.32 mg/kg i.v.) was ineffective. The effects of dynorphin A(1–17) and U69,593 on serum prolactin were both antagonized by quadazocine (0.32 mg/kg s.c.) in a surmountable manner, consistent with opioid receptor mediation. The present studies show that serum prolactin levels are a sensitive quantitative endpoint to study the systemic effects of the endogenous opioid peptide, dynorphin A(1–17), in primates.

Dynorphin A(1–17) is an endogenous agonist at κ-opioid receptors, although at high concentrations in vitro it can also produce agonist effects at μ- and δ-opioid receptors (e.g., Goldstein et al., 1981; Alt et al., 1998; Zhang et al., 1998). This peptide has relative binding selectivity for κ-over μ- and δ-receptors in rhesus monkey brain and in cloned rodent and human receptors (e.g., Raynor et al., 1994; Butelman et al., 1998; Zhang et al., 1998). Dynorphin A(1–17) also exhibits efficacy similar to that of arylacetamide κ-agonists such as U50,488 in cloned human κ-receptors, as measured by the accumulation of [35S]GTPγS (Zhu et al., 1997; Remmers et al., 1999).

The effects of a shorter fragment, dynorphin A(1–13), have been studied after systemic administration in humans and experimental animals (e.g., Gilbeau et al., 1986; Aceto and Bowman, 1992; Takemori et al., 1993; Specker et al., 1998). This research focus on the shorter dynorphin fragment is partly due to its early identification by sequencing, but dynorphin A(1–13) is not thought to be a major endogenous fragment in vivo (Goldstein et al., 1979, 1981; Chavkin et al., 1982). Several dynorphin fragments, including nonopioid des-Tyr1 fragments, caused antinociceptive effects in mice after systemic administration; however, these effects were not mediated by opioid receptors, as shown by their insensitivity to naloxone (Hooke et al., 1995).

Full-length, natural sequence dynorphin A(1–17) has not been studied extensively after systemic administration in nonhuman primates or humans, and may be of further interest for several reasons. First, this endogenous opioid is more resistant to biotransformation in blood relative to dynorphin A(1–13), and second, it may give rise to different, possibly active biotransformation fragments (Chou et al., 1994; Muller and Hochhaus, 1995; Yu et al., 1996; Gambus et al., 1998). The main nonopioid biotransformation fragment, dynorphin A(2–17), is also studied here for comparison, because this fragment is active in behavioral assays after systemic administration in mice (Takemori et al., 1993; Hooke et al., 1995).

In the present studies, we characterized the effects of i.v. dynorphin A(1–17) and A(2–17) in rhesus monkeys, in assays of thermal antinociception and operant rate suppression, previously used to study nonpeptide κ-agonists (e.g., Dykstra et al., 1987; Negus et al., 1993; France et al., 1994). We also focused on the effects of these dynorphin peptides on the

ABBREVIATIONS: FR20, fixed ratio of 20; %MPE, percent maximum possible effect.
release of the anterior pituitary hormone prolactin, a neuroendocrine endpoint that is responsive to both \( \kappa \)- and \( \mu \)-opioid agonists. The effects of \( \kappa \)-agonists on serum prolactin levels are probably mediated by hypothalamic opioid receptors, which modulate the dopaminergic tuberoinfundibular system, and are located in areas that may be outside the blood-brain barrier (e.g., Manzanares et al., 1991; Merchenthaler, 1991; Simpkins et al., 1991; Moore and Lookingland, 1995). The effects of the dynorphins were compared to those of the selective ary lacetamide \( \kappa \)-agonist, U69,593, which is expected to cause agonist effects in all three endpoints (antinociception, operant, and neuroendocrine), as previously documented with selective \( \kappa \)-agonists in human and nonhuman primates (Negus et al., 1993; France et al., 1994; Ur et al., 1997).

When possible, the effects of dynorphin A(1–17) were studied after pretreatment with the opioid antagonist, quazadocine (Dykstra et al., 1987), to determine whether the effects of this peptide were mediated by opioid receptors. The operant effects of dynorphin A(1–17) were also studied after pretreatment with the inhibitor of mast cell degranulation, cromolyn. These studies were carried out to determine whether behavioral effects of i.v. dynorphin A(1–17) may be a consequence of its ability to release inflammatory mediators (such as histamine; e.g., Sydbom and Terni- nius, 1985) from mast cells.

Materials and Methods

Subjects

Captive-bred, intact rhesus monkeys (age range: 4–7 years old; weight range: 3.8–6.8 kg), obtained from Biomedical Resources Foundation (Houston, TX), were used. They were housed singly in a room maintained at 20–22°C with controlled humidity, and a 12-h light/dark cycle (lights on at 7:00 AM). Monkeys used in serum prolactin (six females) and antinociception studies (three females, one male) were fed approximately 10 jumbo primate chow biscuits (Purina, Richmond, VA) daily, supplemented by fruit two times per week. Three of the female subjects were used both in the prolactin and the antinociception experiments. Monkeys in the food-reinforced responding studies (four females, one male) were fed appropriate amounts of chow to maintain body weight at levels approximately 90% of free-feeding levels. Water was freely available in home cages, via an automatic water spout.

Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committee of Rockefeller University, and Guidelines of the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Health Council (Department of Health, Education and Welfare, Publication ISBN 0–309-05377–3, revised 1996).

Schedule-Controlled Food-Reinforced Responding

Apparatus and Procedure. The procedure used in the present study was similar to that described by Negus et al. (1993), but adapted to chair-trained rhesus monkeys. Chaired monkeys were trained and tested in melamine operant boxes (MED Associates, Georgia, VT). The boxes contained an operant panel with two levers within easy reach of the monkeys, on either side of a centrally located pellet dispenser (300-mg sucrose pellets were used; P.J. Noyes Co., Lancaster, NH). A 1-inch-diameter white light was placed 1 inch above each lever, and these lights were transilluminated with white light during response periods. The operant boxes were connected to a PC-compatible computer, via a MED Associates interface. The session contingencies and data collection were programmed with a menu-based desktop program (Schedule Manager, MED Associates).

Monkeys were gradually reduced to 90% of their free-feeding weight. This was followed by shaping (by successive approximations) of a lever-press with contingent delivery of a pellet when food availability was signaled by illumination of the lights above the levers. After shaping, the response requirement was gradually increased until a fixed ratio of 20 (FR20) was achieved by pressing on either lever. Monkeys were also gradually trained to respond in five consecutive 15-min cycles. These cycles consisted of an initial 10-min time-out, followed by a 5-min response period, during which 10 (three subjects) or 8 pellets (two subjects) were available. Data were obtained for the rate of responding (responses/s) during the response periods. Criterion levels of training were defined as a session in which the monkey obtained all the available food pellets and the response rate was above 1 response/s.

After training (one daily session, 5–6 days/week) and initial stabilization on the FR20 schedule, consecutive tests were separated by at least two consecutive training days of criterion performance. Tests were carried out either with a time course or cumulative dosing procedure. In time course testing, a control (no injection) cycle was followed by a single i.v. injection. Four response periods were then started at 5- to 60-min intervals after administration. Cumulative dose-effect curve tests were carried out with consecutive 15-min cycles (similar to training). Agonist dosing occurred in the first 1 to 2 min of the time-out period. Agonist doses increased by 0.5 log unit in consecutive cycles, and dosing continued until the monkeys did not earn a single pellet during the response period. In antagonist experiments, quazadocine (an opioid antagonist; 0.32 mg/kg) or cromolyn (a mast cell-stabilizing agent; 10 mg/kg) were administered s.c. 30 to 60 min before dynorphin A(1–17) or U69,593.

Design and Data Presentation. Dynorphins A(1–17) and A(2–17) were studied in time course experiments (0.1–3.2 mg/kg i.v.). The data for dynorphin A(1–17) were analyzed in a two-way (dose × time) repeated measures ANOVA. Active doses of dynorphin A(1–17) were also studied after quazadocine (0.32 mg/kg) and cromolyn (10 mg/kg) pretreatment. The U69,593 cumulative s.c. dose-effect curve was also studied alone and after pretreatment with quazadocine and cromolyn. Test data on rate of responding were converted to individual % control values. Control was defined as the mean response rate for the training day immediately preceding the test. Individual \( ED_{50} \) values were calculated by regression from the data points above and below 50% of control. Mean values (±95% CL) were calculated after log transformation of individual \( ED_{50} \) values. An in vivo affinity estimate for quazadocine (apparent \( K_i \)) was also calculated from the mean U69,593 \( ED_{50} \) value, according to a modified equation (\( pK_i = -\log[B/DR - 1] \)), where \( B \) was the dose of quazadocine in mol/kg and \( DR \) was the dose ratio (Negus et al., 1993). The same group of five subjects was used for the pharmacological comparisons in this assay; the 0.05 % level was adopted for all of the studies presented here.

Warm Water Tail Withdrawal Assay (Antinociception)

Apparatus and Procedure. The procedure used in the present study has been described in detail previously (Dykstra and Woods, 1986). Monkeys were seated in primate restraint chairs, and the lower portion of the shaved tail (approximately 10 cm) was immersed in a polycarbonate flask containing water at either 40°, 50°, or 55°C. Monkeys were tested at the three water temperatures in varying order, with tests in the same monkey separated from each other by approximately 2 min. Tail withdrawal latencies were timed manually on a stopwatch. To prevent tissue damage, tails were removed from the water if they remained immersed for 20 s (cutoff latency). Sessions began with control determinations at each water temperature, presented in a varied order among the monkeys.

After control determinations, the time course of the antinoceptive effects of dynorphins A(1–17) and A(2–17) (0.032–3.2 mg/kg i.v.) and U69,593 (0.032–0.18 mg/kg s.c.), were determined 5 to 60 min after administration. The effects of U69,593 (0.002–1 mg/kg s.c.) were also studied in a cumulative dose effect curve with a 30-min interinjection interval, with doses increasing by 0.5 log unit in each
cycle. Testing occurred at each temperature, starting 20 min after each injection. The cumulative U69,593 dose-effect curve was also re-determined 30 min after quadazocine (0.32 mg/kg s.c.) pretreatment. Sessions were carried out no more frequently than twice per week, at least 48 h apart (72 h apart, after quadazocine).

Data Analysis. Data for individual monkeys were converted to percent maximum possible effect (%MPE) by the following calculation: 

\[
%\text{MPE} = \left(\frac{\text{test latency} - \text{control latency}}{\text{cutoff latency} - \text{control latency}}\right) \times 100\%
\]

Individual ED_{50} values were calculated from individual %MPE values by linear regression, and a mean ED_{50} (±95% CL) was presented. Individual and mean quadazocine apparent \( p_K \) values for U69,593 were also calculated (see above). The same group of four subjects was used for all the pharmacological comparisons in this assay.

Serum Prolactin Levels

Procedure. Chair-trained monkeys were tested after extensive habituation to the experimental conditions. Monkeys were chaired (in the same chairs as in the antinociception experiments) and brought into the experimental room between 0930 and 1000 on each test day. A single indwelling catheter (24 gauge; Angiocath, Becton Dickinson, Sandy, UT) was acutely placed in a superficial leg vein and secured with elastic tape. A multisample injection port (Terumo, Elkton, MD) was attached to the hub of the catheter; the port and catheter were flushed (0.3 ml of 50 U/ml heparinized saline) before use and after each blood sampling or i.v. injection. Approximately 15 min after catheter placement, two baseline blood samples were collected, 5 min apart from each other (defined as −10 and −5 min relative to the onset of dosing). At each sampling point, a 1.5-ml blood aliquot was placed in an EDTA Vacutainer (Becton Dickinson, Franklin Lakes, NJ; these samples were not analyzed in the present studies). This was followed by a second 1.5-ml blood sample, which was placed in a plain Vacutainer and kept at room temperature until the time of spinning (3000 rpm at 4°C) and serum separation. The serum samples (approximately 400 \( \mu \)l) were then kept at −40°C until the time of analysis. These samples were analyzed in duplicate with a standard human prolactin radioimmunoassay kit (44 samples/kit; Nichols Diagnostics Institute, San Juan Capistrano, CA), following manufacturer’s instructions. The reported sensitivity limit of the assay was 0.14 ng/ml; the highest measurable concentration, using standard calibration curves, was 150 ng/ml; standard calibration curves were determined for each kit with human prolactin (3–150 ng/ml). The reported cross-reactivity of this assay was of largest magnitude for human growth hormone (0.07% cross-reactivity). The highest measurable concentration, using a standard human prolactin radioimmunoassay kit (44 samples/kit; Nichols Diagnostics Institute, San Juan Capistrano, CA), following manufacturer’s instructions. The reported sensitivity limit of the assay was 0.14 ng/ml; the highest measurable concentration, using standard calibration curves, was 150 ng/ml; standard calibration curves were determined for each kit with human prolactin (3–150 ng/ml). The reported cross-reactivity of this assay was of largest magnitude for human growth hormone (0.07% cross-reactivity). The intra-assay coefficient of variation for samples tested with the present kits was 4.7%, whereas the interassay coefficient of variation was 10.1%.

Monkeys were tested either in a time course or cumulative dosing design. Time course studies were carried out by administering a single i.v. injection (after baseline sample collection) through the catheter port over 15 s, followed by flushing. Blood samples were then obtained at intervals (5–90 min) after administration. Cumulative i.v. dose-effect curve studies were carried out with a 30-min interinjection interval, with doses increasing by 0.5 log unit in each cycle. Sample collection occurred approximately 20 min after each injection.

Design and Data Presentation. Each experiment was carried out in four to five females in the follicular phase (days 2–12 of each cycle of approximately 28 days, as defined by the onset of visible bleeding). Consecutive experiments in the same subject were separated by at least 48 h (or 72 h after quadazocine administration). Dynorphin A(1–17) was studied under a single-dose time course design (0.00032–0.32 mg/kg). Selected dynorphin A(1–17) doses were also studied after 30-min pretreatment with quadazocine (0.32 mg/kg s.c.). Dynorphin A(2–17) (0.32 mg/kg i.v.) was studied in a time course procedure. An i.v. saline (0.1 ml/kg) time course was also studied for control purposes. U69,593 was studied under both time course (0.0032–0.032 mg/kg i.v.) and cumulative dose-effect curve designs (0.001–0.032 mg/kg i.v.); the U69,593 cumulative dose-effect curve was also re-determined after 30-min quadazocine (0.32 mg/kg s.c.) pretreatment.

Serum prolactin values are presented as mean ± S.E.M. Individual dose-effect curves were analyzed with a four-parameter logistic equation to yield a sigmoidal dose-effect curve (variable slope) with the aid of a nonlinear regression program (GraphPad Prism, San Diego, CA). For the nonlinear regressions, the bottom of the dose-effect curve was kept at a fixed value of 0; all other parameters were determined by the program during the regression calculation. A quadazocine-apparent \( p_K \) value was calculated for U69,593 from individual dose ratios. The same group of subjects (\( n = 4–5 \) per experiment) was typically used for the pharmacological comparisons in this assay; the 0.05 \( \alpha \) level was adopted for all of the studies presented here.

Chemicals. Quadazocine methanesulfonate (Sanofi Winthrop, Malvern, PA) and cromolyn sodium (Sigma, St. Louis, MO) were dissolved in sterile water for s.c. injections in the midscapular region of the back (1–2 drops of lactic acid were added for quadazocine solutions). U69,593 (Pharmacia & Upjohn, Kalamazoo, MI) was dissolved in sterile water with one to two drops of lactic acid and injected s.c., as above, or i.v. (for i.v. injections through the catheter port, the U69,593 stock solution was further diluted with saline). Dynorphins A(1–17) and A(2–17) (National Institution on Drug Abuse/Research Triangle Institute, Research Triangle Park, NC) were dissolved in saline approximately 5 min before use and injected i.v. Compounds were typically injected in volumes of 0.1 ml/kg. All doses are expressed as the above forms of the compounds.

Results

Schedule-Controlled Food-Reinforced Responding. Monkeys were shaped to lever-press and were gradually trained on the FR20, multiple-cycle session. After at least 4 weeks of training, animals reached a stable level of responding. The mean (\( n = 5 \)) rate of responding for the 4 days before the onset of testing for each monkey was 1.89 responses/s (S.E.M. = 0.22). In a control experiment (\( n = 5 \)), a s.c. saline injection (0.5 ml) was administered at the beginning of four consecutive response cycles. These saline injections did not affect rates of responding (mean rates of responding ranged from 104 to 110% of control). Likewise, a single i.v. saline bolus did not affect responding measured in a time course session (5–60 min after administration; mean rates of responding ranged from 96 to 108% of control).

Dynorphin A(1–17) (0.1–3.2 mg/kg i.v.) was studied in time course experiments. Dynorphin A(1–17) caused a dose-dependent and time-dependent reduction in responding in all subjects. A two-way (dose \( \times \) time) repeated measures ANOVA for response rate revealed significant dose (\( F_{3,9} = 4.99; p < .026 \)) and time (\( F_{3,9} = 14.4; p < .001 \)) main effects, but no significant interaction. Peak reductions in response rates were observed 5 min after administration, and responding gradually returned to near-control levels by 60 min (see Fig. 1). Immediately after injection of the two largest doses (1 and 3.2 mg/kg), monkeys typically displayed transient facial flushing and scratching, and occasionally a brief (1–2 min) period of apparent sedation. A dose-effect curve for rate of responding was plotted at the time of peak effect (5 min after administration); the resulting ED_{50} value for dynorphin A(1–17) in this assay was 0.67 mg/kg (95% CL = 0.25–1.88 mg/kg; Fig. 2). Dynorphin A(2–17) (0.32 and 3.2 mg/kg i.v.), in contrast, did not produce consistent effects on rate of responding, between 5 and 60 min after i.v. bolus administration. No
overt signs (flushing or sedation) were observed after dynorphin A(2–17). The U69,593 cumulative s.c. dose-effect curve was also studied, and the ED$_{50}$ value for U69,593 under these conditions was 0.0060 mg/kg (95% CL 0.0038–0.0093; Fig. 2).

Quadazocine (0.32 mg/kg s.c.) was administered 30 to 60 min before the two largest dynorphin A(1–17) doses (1 and 3.2 mg/kg) and before the U69,593 cumulative dose-effect curve. Quadazocine alone did not affect responding when tested 10 min before the onset of dosing with dynorphin or U69,593. Quadazocine did not block the rate-decreasing effects of dynorphin A(1–17) (1 or 3.2 mg/kg), or its overt signs such as facial flushing. By comparison, quadazocine (0.32 mg/kg) caused a 6-fold rightward shift in the U69,593 dose-effect curve (Fig. 2) (ED$_{50}$ 0.033; 95% CL 0.0076–0.14). The large confidence limits on this ED$_{50}$ value were probably due to the fact that one of the five animals tested was not antagonized by this quadazocine dose. A paired $t$ test was calculated on individual log ED$_{50}$ values for the U69,593 dose-effect curve in the presence or the absence of quadazocine. This $t$ test was significant ($t[4]=2.91; p < .05$), consistent with a quadazocine-induced rightward shift in the U69,593 dose-effect curve. The quadazocine apparent $pK_B$ value against the mean U69,593 dose-effect curve was 6.83 (see Materials and Methods for details of apparent $pK_B$ calculation).

Cromolyn (10 mg/kg s.c.) was administered 30 min before the three largest dynorphin A(1–17) doses and the U69,593 cumulative dose-effect curve. Cromolyn alone did not affect rates of responding when measured 20 min after administration (data not shown). Cromolyn caused a 2.5-fold shift in the rate-suppressant effects of dynorphin A(1–17) (0.32–3.2 mg/kg) at the time of peak effect (5 min after administration) but did not affect the U69,593 dose-effect curve (Fig. 2).

**Antinociception.** After initial training, monkeys displayed a consistent pattern of tail withdrawal latencies in the different water temperatures. Monkeys typically left their tails in 40°C water until the cutoff (20 s), whereas they removed their tails from 50°C or 55°C water rapidly (within 1–2 s). Saline (0.1 ml/kg, administered as an i.v. bolus, did not affect tail withdrawal latencies relative to control ($n = 4$; data not shown). Individual values in 50°C water after a saline i.v. bolus were all less than 5%MPE (tested 5–60 min after injection).

Dynorphin A(1–17) (0.032–3.2 mg/kg i.v., $n = 4$) only produced a partial effect (peak mean 25%MPE) in 50°C water at the peak time (15 min after bolus administration; see Fig. 3), and was ineffective in 55°C water (Fig. 3). Under the same conditions, dynorphin A(2–17) also produced partial antinociception (peak 45%MPE, at 15 min after administration) in 50°C water, and no effect in 55°C water. Larger dynorphin A(2–17) doses were not studied due to an apparent plateau at the two largest doses studied in this paper (0.32 and 3.2 mg/kg).

The $\kappa$-selective agonist U69,593 (0.032–0.18 mg/kg s.c. bolus doses) produced dose-dependent antinociception in 50°C water in all subjects ($n = 4$). At the largest U69,593 bolus dose studied (0.18 mg/kg) the animals had maximal or
near-maximal effects in 50°C water, and partial effects in 55°C water (see Fig. 3). Larger U69,593 doses were not studied due to the appearance of tremors in some of the subjects. The ED$_{50}$ value for bolus U69,593 administration in 50°C water at the time of peak effect (15 min after administration) was 0.055 mg/kg (95% CL = 0.041–0.074). U69,593 was also studied under a cumulative dosing procedure (0.0032–0.1 mg/kg s.c.); the ED$_{50}$ value obtained under these conditions (0.062 mg/kg; 95% CL = 0.042–0.032) was similar to that obtained from the bolus dosing experiment (above). Quada-zocine (0.32 mg/kg) pretreatment alone did not affect tail withdrawal latencies when measured 20 min after administration (data not shown). This quada-zocine pretreatment caused a 7-fold shift in the U69,593 cumulative dose-effect curve (ED$_{50}$ = 0.43 mg/kg; 95% CL = 0.22–0.89). The quada-zocine apparent p$_{K_B}$ value for the antinociceptive effects of U69,593 was 6.96 (95% CL = 6.61–7.31).

**Serum Prolactin Levels.** Preinjection prolactin values were reliably low in the subjects tested; the mean preinjection values in the experiments reported here were typically 5.5 ng/ml or less. The preinjection samples, obtained at −10 and −5 min relative to the onset of drug administration, did not differ from each other (see Fig. 4). Administration of a bolus saline i.v. injection (0.5 ml) did not elevate prolactin levels above preinjection values between 5 and 90 min after administration (see Fig. 4).

A two-way (dose × time) repeated measures ANOVA was calculated for dynorphin A(1–17); significant dose ($F_{4,8}$ = 40.41; $p < .0001$) and time ($F_{4,8}$ = 91.53; $p < .0001$) main effects were found, as well as a dose by time interaction ($F_{16,32}$ = 15.8; $p < .0001$). Peak prolactin levels were observed 5 min after dynorphin A(1–17) administration, and gradually declined by 90 min. The three largest dynorphin A(1–17) doses (0.0032–0.32 mg/kg) produced a similar peak effect and time course. Prolactin levels returned to baseline by 90 min after administration of all the active doses of dynorphin A(1–17) (0.001–0.032 mg/kg), as shown by nonsignificant Dunnett’s tests compared with the baseline value (i.e., the mean of the −10- and −5-min samples).

Quada-zocine (0.32 mg/kg s.c.) was administered 30 min before the lowest maximally effective dynorphin A(1–17) dose (0.0032 mg/kg). Quadracizine (0.32 mg/kg) alone did not affect serum prolactin levels relative to baseline when measured 20 min after administration (data not shown). However, this quada-zocine pretreatment fully antagonized the effects of dynorphin A(1–17) for the 90 min after administration (Fig. 5). This quada-zocine antagonism was surmounted by a larger dose of dynorphin A(1–17) (0.32 mg/kg; Fig. 5).

A dynorphin A(1–17) dose-effect curve was constructed from the time of peak effect (i.e., 5 min after administration;
see Fig. 6). The data were fit to a sigmoidal function, which allowed calculation of ED$_{50}$ and maximum effect values (see Table 1). A mean dose-effect curve for the peak effects of dynorphin A(1–17) (0.32 mg/kg) was also plotted in the presence of quadazocine. This curve did not appear to be sigmoidal or parallel to the baseline dynorphin A(1–17) dose-effect curve (see Fig. 6). This feature was likely due to variation in the magnitude of quadazocine-induced shifts among the different subjects. For this reason, a nonlinear (sigmoidal) regression of the dynorphin A(1–17) dose-effect curve in the presence of quadazocine was not calculated. However, individual dynorphin ED$_{50}$ values were calculated in the presence of quadazocine by linear regression (using the half-maximal prolactin value from the dynorphin baseline, i.e., 36.1 ng/ml); the resulting mean ED$_{50}$ value is presented in Table 1. Dynorphin A(1–17) baseline dose-effect curve data were also reanalyzed with linear regression, to achieve a direct comparison (Table 1). The ED$_{50}$ and 95% CL obtained for dynorphin A(1–17) by this method were very similar to those determined by nonlinear regression (Table 1). An apparent quadazocine pK$_B$ value against dynorphin A(1–17) could not be calculated due to the lack of a parallel shift in the mean dose-effect curve.

Dynorphin A(2–17) (0.32 mg/kg i.v., n = 4) did not cause changes in serum prolactin levels from baseline when tested 5 to 90 min after administration (Fig. 4). Larger doses of dynorphin A(2–17) were not probed because this dose (0.32 mg/kg) was already approximately 300-fold larger than the obtained ED$_{50}$ dose for the intact opioid peptide, dynorphin A(1–17) (see above and Table 1).

The selective κ-agonist U69,593 was studied in both time course (0.0032–0.032 mg/kg i.v.) and cumulative dosing designs (0.001–0.032 mg/kg i.v.). In time course studies, the peak effects of U69,593 were observed 5 min after administration and gradually declined through the course of the experiment. Serum prolactin values after the two largest U69,593 doses (0.01 and 0.032 mg/kg) had not returned to preinjection levels by the end of the experiment (90 min) as shown by significant Dunnett’s tests for this time point (compared to baseline, i.e., the mean of −10- and −5-min samples). A U69,593 dose-effect curve was obtained in a cumulative dosing procedure (30-min interinjection interval; samples were collected 20 min after each injection). The U69,593 dose-effect curve was fit to a sigmoidal function, for calculation of ED$_{50}$ and maximum effect (see Table 1). The cumulative U69,593 dose-effect curve was also redetermined 30 min after pretreatment with quadazocine (0.32 mg/kg s.c.). This quadazocine pretreatment caused a 10-fold rightward surmountable shift of the mean U69,593 dose-effect curve for serum prolactin levels. The apparent quadazocine pK$_B$ against the effects of U69,593 on serum prolactin was 7.2 (95% CL = 6.7–7.6).

Discussion

Food-Reinforced Responding. Dynorphin A(1–17) (0.1–3.2 mg/kg) decreased food-reinforced responding, but its effects were not mediated by κ- or μ-opioid receptors, as shown by a lack of sensitivity to the opioid antagonist quadazocine (0.32 mg/kg). This quadazocine dose antagonized the effects of both κ- and μ-agonists in this procedure (Negus et al., 1993; present studies). A δ-receptor-mediated effect of dynorphin A(1–17) could have been insensitive to this quadazocine dose (see Negus et al., 1993), but δ-opioid receptor mediation is unlikely, given the low affinity of dynorphin A(1–17) for δ-receptors in rhesus monkey brain (K$_D$ = 129 nM; Butelman et al., 1998). However, future antagonism studies with the
suggest that this peptide has high efficacy at cloned

opioid biotransformation fragment, dynorphin A(2–17), did

causes mast cell degranulation in vivo in rhesus

monkeys (Johnson and VanHout, 1976). Under these condi-
tions, cromolyn also caused an apparent decrease in the
prominence of behavioral signs (e.g., flushing) after adminis-
tration of the larger dynorphin A(1–17) doses, but these effects were not rigorously quantified within these

studies. Overall, this suggests that the operant effects of i.v.
dynorphin A(1–17) are not mediated by \( \kappa \)- or \( \mu \)-receptors, but
may be partially a consequence of mast cell degranulation.

larger cromolyn doses could not be tested in the present
study due to solubility limitations; it may be feasible to
circumvent this problem by using larger cromolyn infusion
volumes (e.g., by the i.v. route) in future studies. The non-

opioid biotransformation fragment, dynorphin A(2–17), did
not cause prominent effects on operant behavior in these
studies, up to 3.2 mg/kg. This suggests that the putative
nonopioid receptor-mediated effect of dynorphin A(1–17) in
this procedure (see above) may be due to the complete peptide or to a biotransformation fragment including the Tyr
residue (e.g., Yu et al., 1996).

Antinociceptive Effects. Both dynorphin A(1–17) and
A(2–17) produced partial antinociceptive effects in 50°C wa-
ter, and no effect in 55°C water. By comparison, the selective
\( \kappa \)-agonist (U69,593) was maximally effective in 50°C water
and partially effective in 55°C water, up to the highest doses
previously studied. Previous studies have demonstrated that
some opioids [including partial agonists and dynorphin A(1–
13)] may be effective in lower water temperatures (e.g., 48° or
50°C water), but not in higher water temperatures (55°C) in
this assay (e.g., Walker et al., 1993; Butelman et al., 1995).

Possible reasons for the lack of more robust effects with
dynorphin A(1–17) in this assay therefore include limited
efficacy or affinity at \( \kappa \)-receptors. However, recent findings
suggest that this peptide has high efficacy at cloned \( \kappa \)-opioid
receptors in vitro, as measured by the accumulation of
[35S]GTP\( \gamma \)S (Zhu et al., 1997; Remmers et al., 1999), and has
high affinity for \(^{3}H\)U69,593 ("\( \kappa _{1} \)"") sites in rhesus monkey
brain (Butelman et al., 1998). Furthermore, dynorphin A(1–
17) was approximately equipotent and equieffective with
U69,593 in causing an opioid receptor-mediated increase in
serum prolactin levels after i.v. administration (see below).

Thus, the low potency and effectiveness of dynorphin A(1–17)
relative to U69,593 in the antinociception assay may be due
to factors in addition to the above pharmacodynamic vari-
able. For example, it is currently unknown whether the
partial antinociceptive effect of dynorphin A(1–17) was due
to a nonopioid action of this peptide, such as that observed in
the operant assay above.

The partial effects of dynorphin A(2–17) in this procedure
are not likely mediated by opioid receptors, given that this
peptide lacks affinity for cloned \( \kappa \)-opioid receptors (e.g., Zhu
et al., 1995). Both intact and des-Tyr\(^{1}\) dynorphins caused
antinociceptive effects in rodents after systemic administra-
tion, and these effects were not sensitive to opioid antago-
nism (Hooke et al., 1995). Dynorphins A(1–17) and A(2–17)
also both decreased adenylate cyclase function in the rat
caudate-putamen (Claye et al., 1996). The receptors involved
in the nonopioid effects of the dynorphins have not been
identified at present, but these peptides have moderate af-
finity for several sites, including orphanin FQ, melanocortin,
and \( \alpha \)-methyl-D-aspartate receptors (Quillan and Sadee,
1997; Shukla et al., 1997; Zhang et al., 1998).

**Serum Prolactin Levels.** In contrast to the low potency
and/or low effectiveness of dynorphin A(1–17) in the above
assays, dynorphin A(1–17) was equipotent and equieffective
with U69,593 in increasing serum prolactin levels. Both com-
pounds also produced sigmoidal dose-effect curves in this
procedure. The dynorphin A(1–17) dose-effect curve was
shifted to the right by quazadocine (0.32 mg/kg) pretreat-
ment, but this shift was not parallel, probably due to varia-
tions in the magnitude of the shifts among the individual
subjects. By contrast, quazadocine shifted the U69,593 dose-
effect curve to the right in a parallel and surmountable
manner; a quazadocine apparent \( pK_{B} \) value was therefore
calculated for this effect. This value (7.2) was slightly higher
than values observed for quazadocine against \( \kappa \)-opioid-medi-
ated effects (e.g., Negus et al., 1993) but did not differ signif-
ificantly from the apparent \( pK_{B} \) value obtained with U69,593
under identical dosing conditions in the antinociception assay.
This finding is therefore consistent with a \( \kappa \)-opioid medi-
ation of the prolactin-increasing effects of U69,593 in these
studies. The nonparallel shift caused by quazadocine against
dynorphin A(1–17) precludes the calculation of an apparent

<table>
<thead>
<tr>
<th>Linear regression</th>
<th>ED(_{50}) (95% CL)</th>
<th>Maximum (95% CL)</th>
<th>Dose Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>dyn A(1–17)</td>
<td>0.0012 (0.00079–0.0018)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>quad/dyn</td>
<td>0.022 (0.0034–0.14)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Nonlinear regression</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dyn A(1–17)</td>
<td>0.0011 (0.00074–0.0016)</td>
<td>72.1 (60.3–83.9)</td>
<td></td>
</tr>
<tr>
<td>U69,593</td>
<td>0.0030 (0.0016–0.0057)</td>
<td>81.2 (59.4–102.9)</td>
<td></td>
</tr>
<tr>
<td>quad/U69(^{9})</td>
<td>0.032 (0.018–0.059)</td>
<td>82.2 (59.3–105.1)</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^{a}\) ED\(_{50}\) values in (mg/kg); the quazadocine/dynorphin A(1–17) data did not fit a sigmoidal dose-effect curve, and were therefore analyzed by linear regression. The dynorphin ED\(_{50}\) value was also recalculated by linear regression for direct comparison.

\(^{b}\) Maximum effect (ng/ml), determined from non-linear regression (sigmoidal dose-effect curve). Mean preinjection baselines for dynorphin A(1–17) and U69,593 were less than 3 ng/ml.

\(^{c}\) quazadocine (0.32 mg/kg s.c.) pretreatment.
pKₐ value, but the observed shift is consistent with an opioid mediation of this effect of dynorphin A(1–17) (see for comparison the lack of antagonism of the effects of dynorphin in the operant assay). One possible reason underlying the nonparallel shift in the dynorphin A(1–17) dose–effect curve could be a mediation of this effect by more than one opioid receptor type, for which quadazocine may have differential affinity (see Kenakin, 1993). Quadazocine has greater affinity for μ- than for κ-receptors in rhesus monkey brain (Negus et al., 1993), and both μ- and κ-opioid agonists can increase serum prolactin levels in mammals (see Moore and Lookingland, 1995; Ur et al., 1997). Dynorphin A(1–17) can also cause agonist effects at both κ- and μ-receptors in vitro, although its affinity for κ-receptors is higher than for μ-receptors in both human and nonhuman primates (Alt et al., 1998; Butelman et al., 1998; Zhang et al., 1998). Kappa- and μ-selective opioid antagonists may be used in the future to test whether both receptor types are involved in the prolactin-releasing effects of dynorphin A(1–17).

A shorter dynorphin fragment, dynorphin A(1–13), increased serum prolactin levels in female rhesus monkeys (Gilbeau et al., 1986). In the present experiment, i.v. dynorphin A(1–17) was more potent than i.v. dynorphin A(1–13) (Gilbeau et al., 1986; maximal effects were observed at 0.0032 and 0.06 mg/kg, or 1 and 37 nmol/kg, respectively). Interestingly, dynorphin A(1–17) was 32-fold more potent than dynorphin A(1–13) in stimulating the binding of [35S]GTPγS in cloned human κ-receptors in vitro (Remmers et al., 1999). Dynorphin A(1–17) also causes a maximum enhancement of serum prolactin levels than dynorphin A(1–13) (i.e., 28- versus 3.6-fold, respectively, as shown by this study and Gilbeau et al., 1986). These differences in potency and effectiveness could be due to experimental conditions (Gilbeau et al., 1986, used ovariectomized rhesus monkeys, rather than the intact female monkeys in the present studies) or to a difference in the in vivo profile of dynorphin A(1–17) versus A(1–13). For example, the i.v. potency of the two peptides could be differentially affected by biotransformation in blood (Chou et al., 1994; Muller and Hochhaus, 1995; Yu et al., 1996).

The nonopioid fragment dynorphin A(2–17) did not increase serum prolactin levels at a dose of 0.32 mg/kg, which was approximately 300-fold greater than the ED₅₀ value for the complete peptide under identical conditions (see above). This suggests that the effects of dynorphin A(1–17) in this assay are mediated by opioid receptors and not by a nonopioid biotransformation fragment. Serum prolactin increases may therefore provide a selective in vivo endpoint to differentiate the effects of opioid dynorphin peptides from those of their nonopioid biotransformation fragments.

General Summary. The present studies are, to our knowledge, the first to compare the behavioral and neuroendocrine effects of i.v. dynorphin A(1–17) in primates. Dynorphin A(1–17) decreased food-reinforced responding, but its effect in this assay was of low potency (compared to U69,593) and was not mediated by κ- or μ-opioid receptors. This peptide was partially effective in the warm water (50°C) tail withdrawal assay of thermal antinociception, and its nonopioid biotransformation fragment, dynorphin A(2–17), was also partially effective, whereas U69,593 was maximally effective. By contrast, i.v. dynorphin A(1–17) was equieffective and equipotent to U69,593 in increasing serum prolactin levels, and the effects of both compounds were sensitive to opioid antagonist pretreatment, whereas dynorphin A(2–17) was inactive. The selectivity of i.v. dynorphin A(1–17) for this neuroendocrine effect versus operant or antinociceptive effects suggests that this peptide is more potent in stimulating the hypothalamic κ-opioid receptors thought to mediate prolactin release, relative to κ-opioid receptors that mediate these behavioral effects. One possible reason for this selectivity is that i.v. dynorphin A(1–17) may have greater access to the hypothalamic opioid receptors that mediate prolactin release, as these receptors may be functionally outside the blood–brain barrier (e.g., Merchenthaler, 1991). By contrast, the κ-receptors which presumably mediate the antinociceptive effect of U69,593 may be located in areas of the central nervous system that are less accessible to systemically administered dynorphin A(1–17).

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References


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