Psychopharmacology (1999) 143:190–196

ORIGINAL INVESTIGATION

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Systemic effects of E-2078, a stabilized dynorphin A(1–8) analog, in rhesus monkeys

Received: 5 August 1998 / Final version: 6 November 1998

Abstract *Rationale*: E-2078 ([*N*-methyl-Tyr[1], *N*-methyl-Arg[7], D-Leu[8]] dynorphin A(1–8) ethylamide) is a dynorphin A(1–8) analog with a reduced tendency to be biotransformed, when compared to the unmodified opioid peptide. E-2078 has been found to produce κ-opioid agonist effects in vivo in rodents. *Objective*: In the present studies, we investigated whether systemically administered E-2078 could produce κ-agonist effects in rhesus monkeys, in tests of antinociception, diuresis and ethylketocyclazocine (EKC) discrimination. *Methods*: E-2078 (0.32–18 mg/kg, SC, IM or IV) was tested in the warm water (50°, 55°C) tail withdrawal assay of thermal antinociception. The diuretic effects of E-2078 (0.056–1.8 mg/kg, SC) were also compared to those of the κ-agonist, U69,593 (0.01–0.32 mg/kg, SC). Lastly, the effects of E-2078 (0.1–3.2 mg/kg, SC or IV) were studied in rhesus monkeys trained to discriminate EKC (0.0056 mg/kg SC) from vehicle, in a food-reinforced operant procedure. *Results*: E-2078 did not produce thermal antinociception in rhesus monkeys following SC or IM administration, up to the largest doses presently studied (i.e., 18 and 10 mg/kg, respectively). E-2078 caused thermal antinociception by the IV route, but this effect was not apparently mediated by κ- or μ-opioid receptors, as shown by its insensitivity to quadazocine (1 mg/kg) pretreatment. However, SC E-2078 caused diuresis, and this effect was blocked by quadazocine pretreatment, consistent with mediation by κ-opioid receptors. E-2078 generalized in EKC-discriminating monkeys, but only after the largest dose (3.2 mg/kg), and only following IV administration. *Conclusions*: The present studies suggest that systemically administered E-2078 can produce some κ-receptor mediated effects in rhesus monkeys, but its profile of action is not identical to non-peptidic κ-agonists following all routes of administration, or across all experimental situations.

Key words Antinociception · Diuresis · κ-Opioid receptors · *Macaca mulatta*

Introduction

The effects of parenterally administered dynorphins have received increased attention recently. In rodents, parenterally administered dynorphin A(1–13) produced antinociceptive effects, although these effects were not mediated by opioid receptors, as shown by a lack of sensitivity to opioid antagonists (Hooke et al. 1995a). Furthermore, the des-Tyr[1] dynorphins, which do not bind to any type of opioid receptor (Walker et al. 1982), also caused antinociception (e.g., Hooke et al. 1995a). Parenterally applied dynorphins share another behavioral effect, namely a decrease in naloxone-precipitated morphine withdrawal signs (e.g., Takemori et al. 1992, 1993; Hooke et al. 1995b). Intravenously administered dynorphin A(1–13) may also produce anti-withdrawal and antinociceptive effects in rhesus monkeys (Aceto et al. 1982; Butelman et al. 1995a) and humans (Wen and Ho 1982; Ingham et al. 1996; Greenwald et al. 1997; Specker et al. 1998). However, because natural sequence dynorphins are relatively unstable under physiological conditions (e.g., Chou et al. 1994; Nylander et al. 1995; Yu et al. 1996), it is unclear whether the intact dynorphin or one of its biotransformation fragment(s) mediates the various behavioral effects following systemic administration.

Animals used in these studies were maintained in accordance with the Institutional Animal Care and Use Committees of the University of Michigan and 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). A preliminary report of portions of these findings was made at the Society for Neuroscience meeting in San Diego, Calif., USA, November 1995

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One strategy to reduce the aforementioned difficulties associated with peptides is to study the effects of compounds with a reduced tendency to be biotransformed in vivo. The dynorphin A(1–8) analog, E-2078 ([*N*-methyl-Tyr[1], *N*-methyl-Arg[7], D-Leu[8]] dynorphin A(1–8) ethylamide, e.g., Tachibana et al. 1988) is one such compound. E-2078 is able to cross the blood-brain barrier in rodents (e.g, Terasaki et al. 1989, 1991). It is also very stable when incubated in human and rhesus monkey blood, compared to unmodified dynorphin A(1–8), and no major E-2078 biotransformation fragments were detected following IV administration in rhesus monkeys (Yu et al. 1997a). E-2078 was detected in CSF following administration of a bolus IV dose (10 mg/kg) in rhesus monkeys (Yu et al. 1997b). E-2078 also exhibited binding selectivity for κ- over μ- and δ-receptors in monkey brain (Butelman et al. 1998). In particular, E-2078 exhibited a 27-fold binding selectivity for "κ_1" ([^{3}H]U69,593) sites over μ ([^{3}H]DAMGO) sites, and a 378-fold selectivity for κ_1 over δ ([^{3}H] p-Cl-DPDPE) sites. E-2078 also displayed 6-fold selectivity for κ_1 sites over all the κ-sites labeled by [^{3}H]bremazocine (Butelman et al. 1998). Therefore, E-2078 is an attractive compound for the study of dynorphin pharmacology following parenteral administration in human and non-human primates.

In rats, SC and IV administration of E-2078 produced antinociceptive and diuretic effects (Nakazawa et al. 1990; Salas et al. 1992); these effects have also been observed after non-peptide κ-opioid agonists in several species (Slizgi and Ludens 1982; Leander 1983; Dykstra et al. 1987a). Analgesic and diuretic effects of IM E-2078 were also observed in two studies conducted in humans (Ohnishi et al. 1994; Fujimoto and Momose 1995). In the present studies, we examined the pharmacology of parenterally administered E-2078 in rhesus monkeys, in assays of thermal antinociception, diuresis and EKC-discrimination. Studies in rhesus monkeys can be of particular value in understanding the in vivo profile of E-2078 in comparison to non peptide κ-agonists, because the opioid receptor binding of κ-ligands, as well as their behavioral profile (and sensitivity to antagonism), have been studied in several endpoints in this species (e.g., Dykstra et al. 1987a,b; Negus et al. 1993; Emmerson et al. 1994; France et al. 1994; Butelman et al. 1998). Direct comparisons of κ-agonists, including dose-effect curve and antagonism determinations, may not be practical in human studies, and therefore the present experiments can provide a more general perspective on the in vivo effects of E-2078. Non-peptide κ-agonists produce thermal antinociception, diuresis and EKC-like discriminative stimulus effects following systemic (e.g., SC) administration in rhesus monkeys, and as expected, their effects in these assays are blocked by the opioid antagonist, quadazocine (e.g., Dykstra et al. 1987a; France et al. 1994). Thus, if E-2078 shared this profile, it would be expected to produce quadazocine-sensitive agonist effects in these assays in the present experiments.

Materials and methods

Subjects

Seventeen adult rhesus monkeys (*Macaca mulatta*; nine males, eight females) were housed individually with free access to water; they were fed Purina Monkey Chow daily and fresh fruit weekly. They were kept in a room maintained at 20–22°C, on a 12-h light:dark cycle (lights on at 0700 hours).

Warm water tail withdrawal assay (antinociception)

Apparatus and procedure

The procedure used in the present study was similar to that described by 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 maintained at 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 microprocessor (IBM PCjr) via a push-button switch. In order to prevent tissue damage, tails were removed from the water if they remained immersed for 20 s (cut-off latency). Sessions began with one control determination at each water temperature, presented in a varied order among the monkeys.

Following control determinations, the time course of E-2078's antinociceptive effects was determined 15–150 min after IV administration. In two separate experiments, single large doses of E-2078 were studied by the IM (10 mg/kg) and SC (17.8 mg/kg) routes (n=3). Single pretreatment SC doses of E-2078 (0.56 and 1.8 mg/kg) were also administered 15 min before the smallest fully effective dose of the κ-opioid agonist U69,593 (0.1 mg/kg, SC), in order to investigate possible low-efficacy actions of the peptide by this route (i.e., to test whether under these conditions E-2078 would antagonize the effects of U69,593).

In separate antagonist pretreatment studies (n=4), quadazocine (1 mg/kg) was administered SC, 30 min before E-2078 (5.6 mg/kg, IV) or U69,593 (0.1 mg/kg, SC). This dose of quadazocine has been shown to antagonize μ- and κ-receptor mediated effects in a variety of situations in rhesus monkeys (e.g., Dykstra et al. 1987b). Likewise, the above E-2078 and U69,593 doses were studied because they were the smallest doses to produce a maximal or near maximal antinociceptive effect in 50°C water in the same group of subjects. Sessions were carried out no more frequently than twice per week, at least 48 h apart.

Data analysis

Data from individual monkeys were converted to percent maximum possible effect (%MPE) by the following calculation: %MPE=[(test latency–control latency)/(cutoff latency–control latency)]×100%. Individual ED_{50} values (i.e., the doses that would cause 50%MPE) were calculated from individual %MPE values by linear regression and a mean ED_{50} (±95% confidence limits) was presented. Untransformed latency data from the agonist time course experiment were analyzed in a two-factor (drug dose, pretreatment time) repeated measures ANOVA. The smallest E-2078 dose (0.32 mg/kg) was not included in this analysis, as some of the subjects were not the same as in the larger three doses. This was followed by Newman-Keuls comparisons, when appropriate. The quadazocine antagonist pretreatment %MPE data were analyzed with a paired t-test; α was 0.05, two-tailed.

Diuresis

Procedure

Urine volumes were collected at hourly intervals over 3 h subsequent to the administration of E-2078 (0.056–1.8 mg/kg, SC) or U69,593 (0.01–0.32 mg/kg, SC). Tests were performed in the home cage of each monkey (n=3–4), with a clean cage pan placed under the grid floor for urine collection. Water was available via automatic spouts throughout the session. In agonist dose-effect curve studies, single doses of E-2078 or U69,593 were administered SC. In antagonist studies, quadazocine (1 mg/kg, SC) was administered 30 min prior to E-2078 (0.56 mg/kg, SC) or U69,593 (0.032 mg/kg, SC). These single doses were chosen as they were the smallest doses that produced maximal or near maximal diuretic effects in this group of subjects. Sessions were carried out twice per week.

Data analysis

Individual 3-h cumulative volumes for each session were determined and analyzed in a one-factor (drug dose) repeated measures ANOVA. When significant, Newman-Keuls post-hoc comparisons were performed. In antagonist experiments, individual urine volumes were analyzed with paired t-tests (α was 0.05, two-tailed).

EKC discrimination

Apparatus

All experiments utilized similar operant panels, consisting of two primate response levers (BRS-LVE model PRL-001; Laurel, Md., USA). The levers required a force of 0.25 N, over a distance of 2 mm, in order for a response to be recorded. A panel of 7.5 W stimulus lights was located above, and a food receptacle located between the levers, mounted on one wall of the testing chamber. Delivery of 300 mg banana flavored food pellets (Noyes formula G/T, Lancaster, N.H., USA) was controlled by an externally mounted food dispenser (Gerbrands model G5210, Arlington, Mass., USA). A PC compatible computer connected to an interface controlled the scheduling of events and recorded data. A lever press was counted as a response.

Procedure

Chair-trained monkeys (n=2–4) were trained to respond for pellets under a fixed ratio 30 (FR30) schedule of reinforcement. Subsequently, discrimination training was undertaken in which reinforcer delivery was made contingent upon the selection of the lever previously paired with a drug stimulus. For all subjects, EKC (0.0056 mg/kg SC) was paired with right, and saline with left lever selection. Training sessions consisted of two to five multiple cycles; each cycle comprising two periods: an initial 10-min time-out period in which light stimuli were extinguished and responding had no consequences, followed by a 5-min response period in which light stimuli were illuminated, and monkeys could obtain up to ten pellets (reinforcers) through completion of the FR30 schedule of reinforcement on the drug-appropriate lever. If ten reinforcers were obtained prior to the end of the response period, the stimulus lights were extinguished and responding had no consequences. Monkeys were trained 5–6 times a week and were not tested until greater than 80% drug appropriate responding was attained prior to the first reinforcer delivery (as well as across the session overall); this criterion was maintained across four out of five consecutive training sessions. In addition, no tests were permitted unless response rates were maintained within 20% across four of five consecutive sessions.

Testing sessions were performed as described above, with a 15-min cycle time (10-min time-out, 5-min response periods), and reinforcer delivery was made contingent upon completion of the fixed ratio schedule regardless of the lever selected. Vehicle and drugs were administered at the beginning of each cycle using a cumulative dosing procedure.

Data analysis

For discrimination data, lever selection was expressed as percent EKC responding by dividing the number of responses on the EKC lever by the total number of responses. The distribution of responding below 20% and above 80% on the EKC lever was designated as saline and EKC lever selection, respectively. The percent of control response rates for individual monkeys were calculated by dividing the response rates for each cycle by the saline control response rates for that session, and are presented graphically.

Drugs

E-2078 (Eisai, Tsukuba, Japan) was dissolved in saline approximately 15 min prior to use. Ethylketocyclazocine (EKC) methanesulfonate and quadazocine methanesulfonate (Sanofi-Winthrop, Malvern, Pa., USA) and U69,593 (Upjohn Co., Kalamazoo, Mich., USA) were dissolved in sterile water. All drugs were administered in volumes of 0.05–0.1 ml/kg body weight. All drug doses are expressed as the drug forms mentioned above. All injections were typically made in a volume of 0.1 ml/kg; bolus IV injections were made over a period of approximately 5–10 s.

Results

Antinociception

The monkeys reliably left their tails in 40°C water until the cut-off (20 s), but rapidly removed their tails from 50 or 55° water (typically within 2 s). Intravenous E-2078 doses (0.32–10 mg/kg) dose-dependently produced antinociception in 50° water (Fig. 1).

The duration of action and peak antinociceptive effects of E-2078 IV doses were generally smaller in 55° than in 50° water. In 50° water, there were significant dose [$F(2,4)=16.43$; $P<0.05$] and time [$F(6,12)=18.19$; $P<0.001$] main effects, and a significant interaction [$F(12,24)=4.79$; $P<0.001$]. Likewise, in 55° water, there were significant dose [$F(2,4)=24.51$; $P<0.01$] and time [$F(6,12)=25.48$; $P<0.001$] main effects, and a significant interaction [$F(12,24)=7.12$; $P<0.001$]. After administration of the larger doses (i.e., 3.2–10 mg/kg, IV), some monkeys appeared sedated (as defined by unresponsiveness to non-noxious visual, auditory or tactile stimuli) for approximately 5–10 min after administration.

An ED_{50} value for antinociception in 50° water was calculated from %(MPE) data obtained 15 min after administration. The ED_{50} value was 2.0 mg/kg (95% CI=1.6–2.6 mg/kg), therefore smaller than the doses which caused unresponsiveness to environmental stimuli. Bolus doses of E-2078 administered via IM (10 mg/kg) and SC (17.8 mg/kg) routes did not produce antinociception (data not shown). Larger E-2078 doses were not administered by the IM and SC routes due to supply limitations. Single E-2078 SC doses [0.56 (n=4), 1.8 mg/kg (n=4)] were also administered 15 min before a

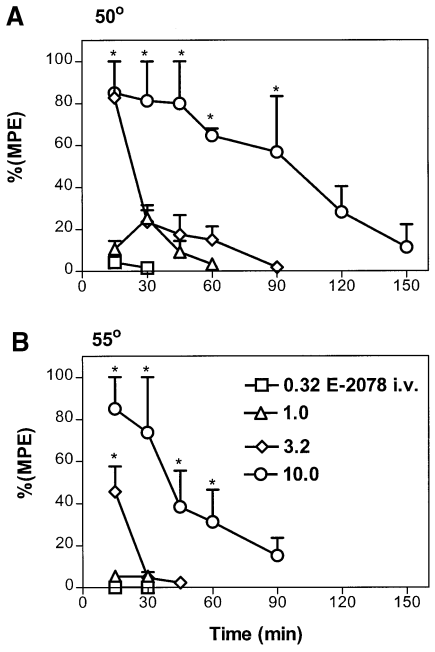

Fig. 1A, B Time course of the antinociceptive effects of E-2078 (0.32–10 mg/kg IV; *n*=3–4) in 50° (**A**) and 55°C water (**B**). *Abscissae*: time from injection; *ordinates*: percent maximum possible effect. There were significant dose, time and interaction effects in both water temperatures. *Significantly different from the respective control (pre-drug) value (Newman-Keuls post-hoc comparison; $P<0.05$). □ 0.32, △ 1.0, ◇ 3.2 and ○ 10.0 E-2078

single dose of U69,593 (0.1 mg/kg, SC). This dose of U69,593 was the smallest fully effective dose in 50° water, measured at the peak time, i.e., 15 min after administration. Neither of the two E-2078 pretreatment doses caused a decrease in the peak antinociceptive effects of U69,593 (0.1 mg/kg; data not shown).

In preparation for quadazocine antagonism experiments, a single E-2078 bolus IV dose (5.6 mg/kg; *n*=4) was determined to be the lowest dose that produced maximal antinociceptive effects 5 min after administration, in a second group of subjects. Testing at this early time point did not occur in the time course experiments reported above, which had been carried out previously. This endpoint (i.e., antinociception 5 min after 5.6 mg/kg E-2078, IV) was then studied 30 min following pretreatment with quadazocine (1 mg/kg, SC; Fig. 2).

Quadazocine (1 mg/kg) did not block the antinociceptive effects of E-2078 under these conditions, either in 50 or 55° water. For control purposes, the same quadazocine pretreatment was administered 30 min before a single maximally effective dose of the κ-agonist U69,593 (0.1 mg/kg, SC). This quadazocine pretreatment completely blocked the peak U69,593 effect (which occurred 15 min after administration) in both 50 and 55° water [50° water: $t(3)=140$; $P<0.001$; 55° water: $t(3)=3.35$; $P<0.05$]. In several previous published studies, this quadazocine pretreatment dose clearly antagonized the effects of both μ- and κ-agonists in this assay (e.g., Dysktra et al. 1987b).

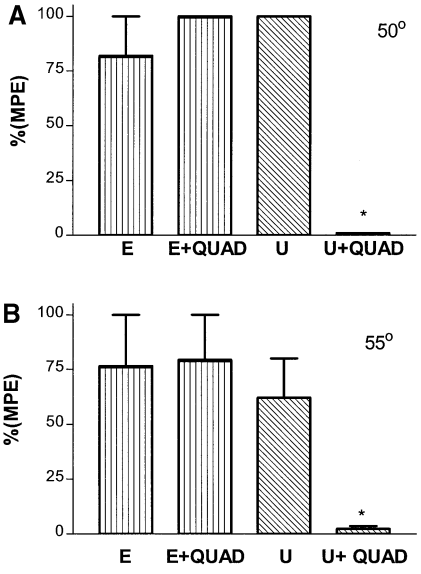

Fig. 2A, B Quadazocine antagonism of the antinociceptive effects of E-2078 (*E*; 5.6 mg/kg, IV) and U69,593 (*U*; 0.1 mg/kg, SC); *n*=4. Quadazocine (*QUAD*; 1.0 mg/kg, SC) was administered 30 min before administration of E-2078 and U69,593. Data are shown at the time of peak antinociceptive effect (5 min after E-2078 administration and 15 min after U69,593 administration). Other details as in Fig. 1. *Significantly different from baseline [paired *t*-test; $P<0.001$ (**A**); $P<0.05$ (**B**)]

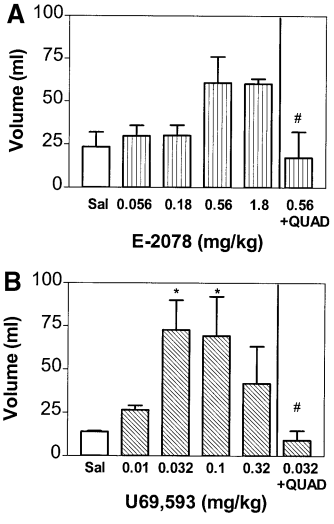

Fig. 3A, B Diuretic effect of E-2078 (**A**; *n*=3; three replications per dose) or U69,593 (**B**; *n*=4; three replications) and quadazocine (1 mg/kg; *QUAD*) antagonism. *Abscissae*: dose (mg/kg, SC); *ordinates*: cumulative urine output (ml) over 3-h test period. *Significantly different from the respective saline condition (*Sal*; $P<0.05$); #Significantly different from respective baseline condition [$P<0.009$ (**A**); $P<0.005$ (**B**)]. See Results for further details

Diuresis

Saline-injected monkeys produced approximately 10–25 ml of urine during the 3-h observation period. E-2078 (0.056–1.8 mg/kg, SC) increased urine output [$F(4,8)=4.05$, $P=0.04$] (Fig. 3), but post-hoc Newman-Keuls tests

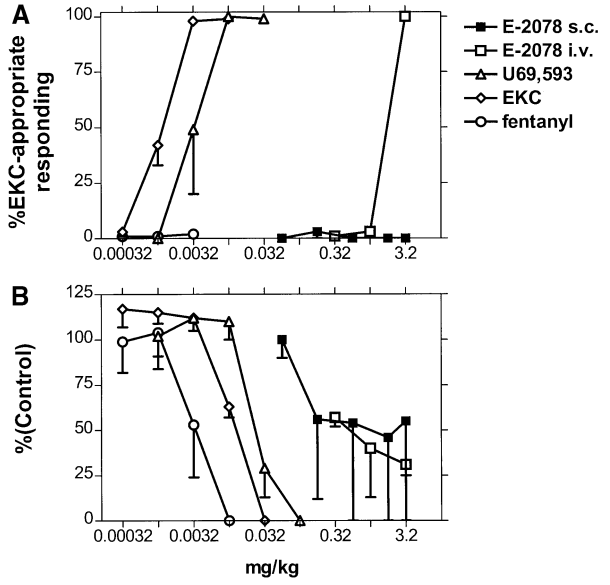

Fig. 4A, B Effects of SC and IV E-2078 (n=2–3) in monkeys trained to discriminate EKC (0.0056 mg/kg, SC) from vehicle. *Abscissae*: dose (mg/kg); *ordinate* **A**: percent EKC-appropriate responding, **B** percentage control rate of responding. ■ E-2078 SC, □ E-2078 IV, △ U69,593, ◇ EKC, ○ fentanyl

did not achieve significance. The largest diuretic effect of E-2078 (approximately 260% of control) was obtained after the 0.56 mg/kg dose. After the highest E-2078 doses (0.56 and 1.8 mg/kg), salivation, apparent sedation and vomiting were observed in several subjects.

U69,593 (0.001–0.32 mg/kg, SC) also increased urine output [$F(5,20)=7.35$, $P=0.0005$], reaching significance at the two intermediate doses (0.032, 0.1 mg/kg). Apparent sedation was also observed after the higher U69,593 doses. In antagonist experiments, quadazocine (1 mg/kg) prevented the diuretic effects of both E-2078 [0.56 mg/kg; $t(2)=10.3$, $P=0.009$] and U69,593 [0.032 mg/kg; $t(3)=7.67$, $P=0.005$], and returned urine output to control (i.e., saline-treated) levels).

EKC discrimination

When administered intravenously, E-2078 (0.32–3.2 mg/kg), engendered EKC-lever responding at the largest dose in the two subjects tested initially (Fig. 4). The mean E-2078 ED_{50} value for percent drug-appropriate responding in these two subjects was 1.8 mg/kg.

In two subsequently tested subjects, IV E-2078 (0.32 mg/kg; three determinations in each subject) completely suppressed food-reinforced responding for at least 1 h after administration, and caused observable salivation (data not shown). In the first two subjects mentioned above, E-2078 generalized when administered as a bolus dose, as well as during a cumulative dosing procedure. As a bolus, the EKC-like discriminative stimulus effects of E-2078 were maximal 15 min after administration and were observed up to 30 min after administration (time course data not shown). When administered SC, E-2078 (0.01–3.2 mg/kg) failed to generalize to EKC up to doses which suppressed responding in two of three subjects tested. EKC (0.0001–0.01 mg/kg SC) and U69,593 (0.0001–0.032 mg/kg SC), but not the μ-agonist fentanyl (0.0001–0.01 mg/kg SC) engendered EKC lever responding. These effects of EKC and U69,593 were prevented by a 30-min pretreament with quadazocine (1 mg/kg; data not shown). The effects of E-2078 in the presence of quadazocine (1 mg/kg) could not be evaluated in later experiments, due to rate-decreasing effects of quadazocine alone in these subjects.

Discussion

E-2078 has been shown previously to be a stable dynorphin A(1–8) analog in primate plasma in vivo (Yu et al. 1997a) and was detected in the CSF following IV administration of a large dose (10 mg/kg; Yu et al. 1997b). Previous pharmacokinetic studies in rats have also shown that E-2078, administered by either the SC or IM routes, has substantial bioavailability (i.e., approximately 65% relative to the IV route; see Murahashi et al. 1989). In the present studies, the parenteral effects of E-2078 have been studied in rhesus monkeys, in assays sensitive to the effects of κ-agonists.

This dynorphin analog was dose-dependently effective in the warm water tail withdrawal procedure, both in 50 and 55° water. As expected, the duration of action and maximum effect for individual doses were temperature-dependent (e.g., Walker et al. 1993). Only the largest E-2078 dose (10 mg/kg) had a substantial effect in 55° water, and this effect was accompanied by prominent behavioral effects (i.e., unresponsiveness to environmental stimuli) which began 1 min after administration and persisted for approximately 5–10 min. This profile is not unlike that of several κ-opioid agonists, which produce prominent antinociception in 50 and 55°C water, as well as sedation, following systemic administration (Dykstra et al. 1987a). However, the antinociceptive effects of the smallest effective dose of E-2078 (5.6 mg/kg, IV) were not mediated by μ- or κ-opioid receptors, as shown by their lack of sensitivity to pretreatment by the opioid antagonist quadazocine (1 mg/kg). In contrast, this dose of quadazocine completely blocked the antinociceptive effects of a maximally effective dose of the arylacetamide κ-agonist, U69,593 (0.1 mg/kg, SC). In previous studies, the antinociceptive effect of IV dynorphin A(1–13), administered in a cumulative dosing procedure, was sensitive to antagonism by nor-BNI, consistent with mediation by κ-opioid receptors (Butelman et al. 1995a). Dynorphin A(1–13) is rapidly biotransformed in rhesus monkey and human blood in vitro (Butelman et al. 1995b; Chou et al. 1996), therefore part of the aforementioned antinociceptive effect may have been mediated by biotransformation fragment(s) of dynorphin A(1–13). Given the minimal biotransformation of E-2078 (Yu et al. 1997a), it would appear that the presently reported antinociceptive effect of IV E-2078 was caused by the

intact peptide. The receptor mediation of this effect of E-2078 is unclear at present, although some relevant issues should be considered. It has been shown recently that several non-opioid dynorphin fragments [e.g., dynorphin A(2–17)] produced antinociceptive effects in rodents following systemic administration (Hooke et al. 1995a). Furthermore, even intact dynorphins had similar effects, and these were also insensitive to opioid antagonism in these reports (Hooke et al. 1995a). Some effects of centrally administered dynorphins also follow the same profile (Walker et al. 1982). The receptor(s) mediating these effects have not been identified at present. The dynorphins are also known to cause mast cell degranulation by a non-opioid mechanism (e.g., Sydbom and Terenius 1985). It is therefore also possible that some of the non-opioid effects of systemically administered dynorphins (including E-2078) may be caused by some of the mediators released by these cells.

E-2078 (10–15 mg) was an effective analgesic in humans after IM administration (Fujimoto and Momose 1995), whereas it was inactive both after SC and IM administration in the present assay in rhesus monkeys, up to high doses (10–17.8 mg/kg). In order to test whether this lack of effectiveness by the SC route was due to low or intermediate efficacy of E-2078 at κ-receptors (see France et al. 1994), E-2078 was administered as a pretreatment to the selective κ-agonist U69,593 (0.1 mg/kg). Under these conditions, E-2078 did not decrease the antinociceptive effect of U69,593. Thus, it did not appear that the lack of antinociceptive effectiveness of E-2078 by the SC route was due to low efficacy at κ-receptors, or at least the κ-receptor population that mediated the effects of U69,593 under these conditions. Consistent with this finding, E-2078 was highly efficacious in its stimulation of [^{35}S]GTPγS binding in cloned human κ-receptors, in vitro (Remmers et al. 1999). It should also be noted that these SC pretreatment E-2078 doses were the two doses that caused diuresis in the present studies.

The effectiveness of E-2078 as a diuretic by the SC route (see below) is in contrast with its inactivity by this route in the test of thermal antinociception. The reason for this is unclear at present; however, it is unlikely that an insufficient SC dose was studied in the antinociceptive assay. For example, for U69,593, the peak diuretic dose (0.032 mg/kg, SC) is approximately 0.5 log units smaller than a dose producing maximal antinociception (i.e., 0.1 mg/kg SC; see Results). However, for E-2078, a subcutaneous dose 1.5 log units larger than the peak diuretic dose (i.e., 17.8 versus 0.56 mg/kg) was still not effective in the antinociceptive assay. The diuretic effect of SC E-2078 was mediated by opioid (probably κ) receptors, as shown by the full blockade observed after quadazocine pretreatment. An E-2078-induced diuretic effect had also been observed in humans (Ohnishi et al. 1994) and rats (Salas et al. 1992). In the latter study, it was found that large naltrexone doses (10 mg/kg) were required to antagonize the diuretic effects of E-2078.

E-2078 generalized in monkeys trained to discriminate EKC from vehicle, but only after IV and not SC administration, when responding was not suppressed. Monkeys trained to discriminate EKC under these conditions typically generalize κ-agonists (e.g., France et al. 1994) but not compounds from other classes (e.g., fentanyl in the present study). Thus it could be assumed that a relatively large IV dose of E-2078 (i.e., 3.2 mg/kg) produced κ-receptor mediated discriminative stimulus effects, whereas the same doses administered by the SC route, did not. This apparent effect selectivity of SC E-2078 is in contrast with the profile observed with SC U69,593, since the latter compound was generalized in EKC-discriminating monkeys at doses lower than those required to produce diuresis in the present experiments. Intraperitoneally administered E-2078 generalized in rats trained to discriminate the κ-agonist spiradoline (Ohno et al. 1992). This suggests that E-2078 can produce interoceptive stimulus effects similar to those of non-peptide κ-agonists under several conditions. Ohno et al. (1992) also reported that the D_2-receptor preferring dopamine antagonists, haloperidol and sulpiride, generalized in the spiradoline-discriminating rats, indicating a possible commonality between the interoceptive effects of κ-agonists and dopamine antagonists (see also Spanagel et al. 1990).

Overall, the stable dynorphin A(1–8) analog, E-2078, produced a mixed pattern of opioid and non-opioid effects, following IV and SC or IM administration in rhesus monkeys. Future studies would be needed to characterize the nature of the non-κ-opioid effects of intravenously administered E-2078, and to more fully characterize the actions of parenterally administered E-2078 in rhesus monkeys.

Acknowledgements The authors would like to gratefully acknowledge the technical assistance of Dr. Y. Ai, Ms. Lisa MacMahan and Mr. Todd Harris. Support for this research was provided by USPHS Grants DA 00254, DA 01113, DA05130 and DA00049.

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