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The cardiovascular and renal effects of a highly potent μ-opioid receptor agonist, cyclo[N^{ε},N^{β}-carbonyl-D-Lys^2,Dap^5]enkephalinamide

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Abstract

Investigation of the acute cardiovascular and renal effects of cyclo[N^{ε},N^{β}-carbonyl-D-Lys^2,Dap^5]enkephalinamide (cUENK6), the most potent μ-opioid receptor agonist, revealed dose-related effects, but most pronounced during the first hour post i.v. injections. During first hour, cUENK6 (3 μg/rat) stimulated ($P<0.001$) excretion of urine (1.1 ± 0.2 vs. 3.3 ± 0.3 ml/h), sodium (60 ± 10 vs. 124 ± 12 μeq/h), potassium and cGMP (1.76 ± 0.19 vs. 4.92 ± 0.80 nmol/h). These effects were inhibited by naloxone (4 mg/kg i.v.), but not by naloxonazine (35 mg/kg s.c.), or 4 mg/kg i.v. naloxone methiodide. cUENK6 stimulated urinary atrial natriuretic peptide (ANP)-like activity (113 ± 12 vs. 167 ± 20 pg/h, $P<0.02$) and the effect was totally abolished by naloxone. cUENK6 also suppressed the transient stress-induced elevation in blood pressures and heart rate that occurred over the first 30-min post-injection, an effect attenuated by naloxone. Plasma ANP increased 2-h post-injection (123 ± 11 vs. 192 ± 21 pg/ml, $P<0.005$), and was associated with augmented ANP mRNA levels in right atria and left ventricles. Thus, cUENK6 evokes renal effects by enhancing activity of the renal natriuretic peptide system.

Keywords: Opioids; Enkephalin analogue cUENK6; Naloxonazine; Diuresis; Natriuresis; Blood pressure; Natriuretic peptides; Urodilatin; Vasopressin

1. Introduction

Research in the last two decades has brought new scientific insights into the mechanisms of action of opioid peptides selectively targeting different types of opioid receptors (Dhawan et al., 1996). This research has been directed, on the one hand, toward the development of perfect analgesics devoid of physical dependence and deleterious side-effects, such as respiratory depression or inhibition of gastrointestinal transit common to opioid drugs; on the other hand, toward the generation of selective diuretics for hyponatremic disorders, such as congestive heart failure, liver cirrhosis or the syndrome of inappropriate antidiuretic hormone secretion, since certain opioids have been shown to inhibit vasopressin, the hormone involved in the primary control of water homeostasis (Robertson, 2001). Opioid peptides are widely distributed and, similarly to opioid alkaloids, synthetic opioid peptides are known to control cardiovascular and renal function (Kapusta, 1995), via mechanisms involving the central nervous system, peripheral activation of different hormonal systems or modulation of neurotransmitter release from neurons in the heart.

Since the first description in 1975 of the structure of two endogenous peptides, [met^5]- and [leu^5]enkephalins, several synthetic enkephalin analogues have been produced (Hansen and Morgan, 1984), with the goal of obtaining compounds possessing improved pharmacological properties. One promising direction expected to increase activity, metabolic and receptor stability has been cyclization via terminal and/or side-chain groups. We recently synthesized several potent enkephalin analogues cyclized via the ureido

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group and incorporating the side-chain amino groups of two α,ω-diamino acid residues. One of them, cyclo[N^{ε},N^{β}-carbonyl-D-Lys^2,Dap^5]enkephalinamide (cUENK6), appears to be the most potent μ-agonist reported so far, as determined by guinea pig ileum bioassay (Pawlak et al., 1997, 2001). In comparison with [leu^5]enkephalin, this new analogue showed 1160 times higher μ-agonist potency and about 270 times higher than that of morphine (Pawlak et al., 2001).

Several observations from our and other laboratories have revealed that μ-opioid agonists induce the release of the cardiac diuretic, natriuretic and vasorelaxant hormone, atrial natriuretic peptide (ANP) (Gutkowska and Schiller, 1996; Gutkowska et al., 1993; Vollmar et al., 1987). Indeed, we have shown that ANP is involved in the diuretic effect of morphine, H-Tyr-D-Ala-Phe-Phe-NH_2 (TAPP) (Gutkowska and Schiller, 1996), as well as clonidine, an α_2 adrenoceptor/imidazoline receptor agonist, all compounds acting via opioid receptors (Mukaddam-Daher and Gutkowska, 1999).

ANP is a member of the natriuretic peptide hormone family involved in the regulation of blood pressure and fluid homeostasis (De Bold et al., 1981), and inhibition of cardiac and vascular proliferation (Silberbach and Roberts, 2001). ANP is mainly synthesized in the mammalian atria, whereas other members such as brain natriuretic peptide (BNP) are produced by both atria and ventricles, and C-type natriuretic peptide (CNP) by the vasculature (Ruskoaho, 1992). To this family also belongs urodilatin, a 32-amino acid natriuretic peptide, synthesized in the kidney and directly excreted into the urine (Schulz-Knappe et al., 1988). Urodilatin has a similar structure to ANP and, through its natriuretic effects, contributes to body fluid regulation (Goetz et al., 1990).

The aim of this study was to evaluate the cardiovascular and renal effects of the new enkephalin analogue, cUENK6. The study was also designed to characterize the site and the receptor subtypes that mediate these actions.

2. Materials and methods

2.1. Preparation of cUENK6

cUENK6, the first side-chain to chain cyclized via an ureido group enkephalin analogue, was synthesized as described earlier (Pawlak et al., 2001). The crude product was purified to homogeneity by semi-preparative reverse phase-high performance liquid chromatography (RP-HPLC) on a Vidac C-18 column (250 × 10 mm), using the following system: A = 0.01% TFA/H_2O and B = 60% CH_3CN/A, with detection at 220 nm. Purity of the fractions was established by analytical HPLC on a Nucleosil column (250 × 7 mm), deploying a solvent system identical to that described above in the linear gradient mode (0–100% B in 30 min) at a flow rate of 1 cm^3/min. Homogeneous fractions containing enkephalin were combined and lyophilized. RP-HPLC-purified material (98% purity) was used in this study.

2.2. Animals

Experiments were performed in conscious female Sprague–Dawley rats (Charles River, Saint-Constant, Quebec) weighing 225–250 g. The animals were maintained at 22 °C under a 12-h light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.), and fed Purina Lab Rat Chow (Ralston-Purina, St. Louis, MO) and tap water ad libitum. Each rat received either saline vehicle or the opioid agonist and, within 1 week, the order of treatment was reversed. To avoid the effects of tolerance, no one rat received the opioid agonist two times. Experimental protocols were approved by the Animal Care Committee of the Centre Hospitalier de l'Université de Montréal following Canadian Council on Animal Care (CCAC) Guidelines.

2.3. Effects of cUENK6 on renal parameters

In an initial set of experiments, conscious and normally hydrated rats were studied to determine the dose–response relationship of systemically administered cUENK6 on urine volume, electrolytes and cGMP excretion. All experiments began at 8:00 a.m. The drug solutions were prepared freshly before the experiment. Each group of rats received one of various doses of cUENK6 ranging from 0.1 to 10 μg, dissolved in 300 μl of saline, injected into the tail vein. The whole injection procedure took about 60 seconds. Control rats received 300 μl of saline. The animals were then placed individually in Nalgene metabolic cages (Braintree Scientific, Braintree, MA), and spontaneously voided urine was collected every hour for four consecutive hours. At the end of the experiment, rats were returned to their home cages.

To determine whether opioid receptors are involved in the renal effects of cUENK6, other groups of rats were injected with an opioid receptor antagonist, naloxone hydrochloride (4 mg/kg) or saline–vehicle, 10 min before cUENK6 administration (Gutkowska and Schiller, 1996). Determination whether cUENK6 acts via activation of central or peripheral opioid receptors was achieved using the quaternary opioid receptor antagonist, naloxone methiodide that does not penetrate the blood brain barrier following systemic administration. The animals were injected with naloxone methiodide (4 mg/kg) or vehicle, 10 min prior to cUENK6 (3 μg). The selected dose of naloxone methiodide (4 mg/kg) is within the range of (0.3–10 mg/kg), which partially or completely blocked the cardioprotective effect of ischemic preconditioning mediated by a peripheral opioid receptors in the intact rat heart (Schultz et al., 1997), and similar to the commonly used 5 mg/kg injections that have been shown to antagonize several peripheral effects of morphine (Milanes et al., 2001).

The opioid receptor subtype that mediates cUENK6 actions was determined by using a selective μ-1 opioid receptor antagonist, naloxonazine (10 mg/kg i.v.) or vehicle, injected 10 min prior to 3 μg cUENK6. Other groups were

pretreated with naloxonazine (35 mg/kg s.c.) 24 h before saline or cUENK6 injections.

Urinary sodium (Na^+) and potassium (K^+) concentrations were measured with a flame photometer (Perkin-Elmer 51, Norwalk, CT) and excretion rates per hour were calculated. Urinary cGMP excretion was measured by radioimmunoassay, according to a previously described method (Mukaddam-Daher et al., 1995).

2.4. Effect of cUENK6 on circulating hormones and ANP gene expression

A separate group of rats was injected with 3 μg cUENK6 or saline and sacrificed 20 or 120 min later. Blood was collected for hormone analysis, and hearts were quickly dissected and frozen in liquid nitrogen for ANP mRNA determination.

For ANP measurements, 2 ml of blood were collected in chilled test tubes containing protease inhibitors, 2 mg EDTA, 20 μl of 1 μM phenylmethylsulfonyl fluoride (Sigma P-7626, St. Louis, MI) and 20 μl of 0.5 μM pepstatin A (Sigma No. 4265). After centrifugation for 20 min at 4 °C at 4000 RPM, the separated plasma was stored at − 80 °C until assayed. Plasma ANP and urine ANP-like activity were measured by radioimmunoassay after prior extraction on C_{18} Sep Pak cartridges (Waters, Milford, MA), as described previously (Gutkowska, 1987). The ANP antibodies used cross-react (100%) with urodilatin. Plasma vasopressin was quantitated by radioimmunoassay after prior extraction with acetone/petroleum ether (Bichet et al., 1986).

ANP gene expression in the heart was measured by semi-quantitative reverse transcription–polymerase chain reaction (RT-PCR) as described previously (Gutkowska et al., 2000). Briefly, total RNA was isolated from rat hearts with Trizol (Life Technologies, Rockville, MD) according to the manufacturer's specifications. First-strand cDNA was synthesized in a final volume of 40 μl containing first-strand buffer, 2 μg rat cardiac RNA as a control, 2 μg hexanucleotide primer (Amersham Pharmacia, Pistacaway, NJ) and avian myeloblastosis virus reverse transcriptase (12 units/μg RNA; Life Technologies). Ten microliters of cDNA were added to a PCR mixture and amplified for 25–33 cycles by incubation at 95 °C for 1 min, 57 °C for 1 min and at 72 °C for 1.5 min, in the presence of ANP specific primers, in a Robocycler gradient 40 thermocycler (Stratagene, Cedar Creek, TX). Control RT-PCRs were conducted by omitting reverse transcriptase or RNA from the reaction mixture. The fluorescent bands were counted and analyzed with the Storm 840 Imaging System and ImageQuant software (Molecular Dynamics, Sunnyvale, CA). To validate the use of this RT-PCR as a tool for the semi-quantitative measurement of ANP mRNA, dose–response curves were charted for different amounts of total RNA extracted from heart tissue and the samples were quantitated in the curvilinear phase of PCR amplification. These data were normalized to corresponding values of GAPDH mRNA PCR products amplified from the same samples.

2.5. Hemodynamic measurements

Another group of rats was implanted with pressure transducers to track systolic, diastolic and mean arterial pressures and heart rates by telemetry (Data Science International, St. Paul, MN) as previously described (Gutkowska et al., 2000). Arterial pressures, heart rate and animal activity were measured every minute for 2 h before and 4 h after saline–vehicle or cUENK6 (3 μg) injection. In a separate group of rats, naloxone (4 mg/kg) was injected before treatment.

2.6. Statistical analysis

Telemetric BP and HR data in all groups, were averaged at 15-min intervals and expressed as differences from baseline data obtained over 2 h before treatment. Comparisons between groups were made by analysis of variance (ANOVA). Non-paired Student's *t*-test was used to compare values in cUENK6-treated rats vs. corresponding saline-treated controls. $P < 0.05$ was considered significant. All data are expressed as mean ± S.E.M.

3. Results

3.1. Effect of cUENK6 on renal parameters

Intravenous administration of cUENK6 evoked a dose-dependent increase in renal parameters, measured during

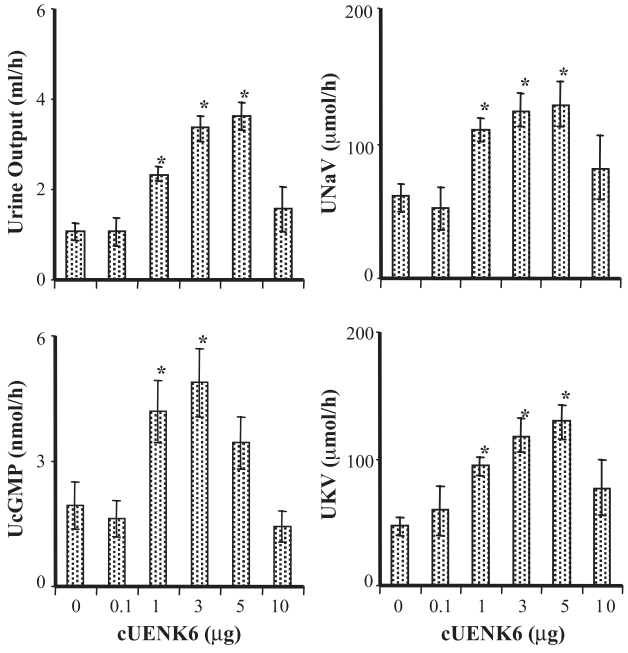

Fig. 1. Effect of increasing doses of cUENK6 (0–10 μg/rat) on urine output and the urinary excretion of sodium (UNaV), potassium (UKV), and cGMP, during the first hour post-injection. Each dose was injected in at least 10 animals. *P<0.001 vs. saline vehicle-treated controls.

the first hour after injection (Fig. 1). The diuretic impact was significant with 1-, 3- and 5-μg doses. The effect of the highest dose of 10 μg was less effective on the first hour, but was also observed on the second hour. The intermediate dose of 3 μg induced a significant increase ($P<0.001$) in the excretion of urine (1.1 ± 0.2 vs. 3.3 ± 0.3 ml/h), Na^+ (60 ± 10 vs. 124 ± 12 μmol/h), K^+ (46 ± 8 vs. 118 ± 13 μmol/h) and cGMP (1.76 ± 0.19 vs. 4.92 ± 0.80 nmol/h).

Cumulative urine output over a 4-h period was also increased in a dose-related manner. The dose of 3 μg augmented cumulative urine output from 2.1 ± 0.2 ml/4 h in the saline control group to 4.9 ± 0.3 ml/4 h. In addition, the 4-h urine volume excreted with 10 μg of cUENK6 was also significantly higher (3.1 ± 0.3 ml/4 h, $P<0.05$) than in the control group. This was likely due to heightened urine output during the second hour. However, cumulative Na^+, K^+ and cGMP excretions were not different with increasing doses of cUENK6.

Naloxone (4 mg/kg) pretreatment totally abolished the renal responses to 3 μg cUENK6, resulting in urine volume, Na^+ and K^+ excretions not different from vehicle-treated controls. Similarly, naloxone pretreatment abolished the increase in cGMP excretion during the first hour (3.47 ± 0.72 vs. 1.13 ± 0.42 nmol/h, $n=14$, $P<0.03$) (Fig. 2).

In another group, the peripherally acting naloxone methiodide had no significant effect on renal parameters evoked by cUENK6, indicating lack of peripheral opioid receptor effects. However, cGMP excretion was partially inhibited by naloxone methiodide pretreatment (1.35 ± 0.15 vs. 0.85 ± 0.07 nmol/h, $P<0.03$, $n=9$–18), but remained significantly higher than saline treated controls (0.30 ± 0.13 nmol/h, $P<0.01$).

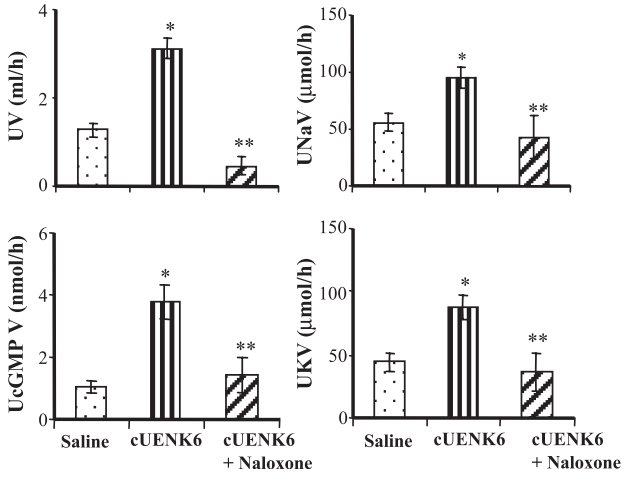

Fig. 2. Effect of naloxone (4 mg/kg) pretreatment on urine output and the urinary excretion of sodium (UNaV), potassium (UKV), and cGMP, during the first h post-treatment with 3 μg of cUENK6. At least 10 animals in each group. *$P<0.01$ saline vehicle-treated controls; **$P<0.01$ vs. cUENK6.

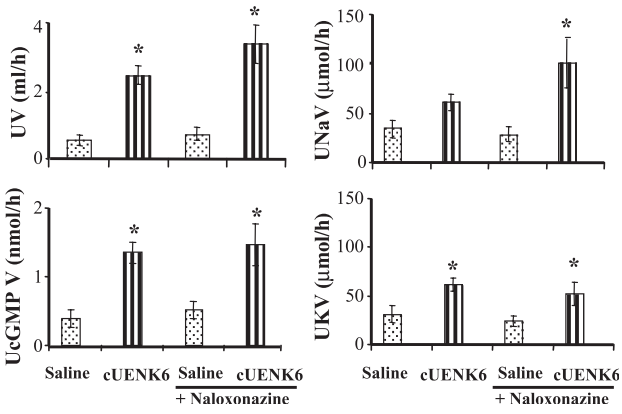

Fig. 3. Effect of 3 μg cUENK6 with and without 24 h pretreatment with naloxonazine (35 mg/kg, s.c.) on urine output and the urinary excretion of sodium (UNaV), potassium (UKV), and cGMP, during the first h post-treatment. *$P<0.01$ vs. corresponding saline vehicle-treated controls.

Pretreatment with naloxonazine (10 mg/kg) injected i.v., 10 min prior to treatment, totally antagonized the renal effects of cUENK6. However, the effects were not antagonized by a more specific dose of 35 mg/kg, injected s.c., 24 h before treatment. (Fig. 3), implying the contribution of opioid receptors, but not the μ-1 subtype. All antagonists injected alone did not alter basal renal parameters.

3.2. Effect of cUENK6 on hormonal parameters

Measurement of plasma ANP and plasma vasopressin concentrations, 20 and 120 min after drug administration, revealed that cUENK6 (3 μg) increased plasma ANP only 120 min after drug injection (123 ± 11 vs. 192 ± 21 pg/ml, $P<0.005$, $n=22$) (Fig. 4), and that there was no effect on plasma vasopressin levels at either time point. In the control group, plasma vasopressin was 2.2 ± 0.2 pg/ml and remained at 2.9 ± 0.7 pg/ml, 20 min after treatment with 3 μg cUENK6 (n = 16). On the other hand, cUENK6 (3 μg) significantly stimulated urinary excretion of ANP-like product from 113 ± 12 to 167 ± 20 pg/h ($P<0.02$, $n=20$) during the first hour post-injection. The effect was totally abolished (91 ± 15 pg/h) by pre-

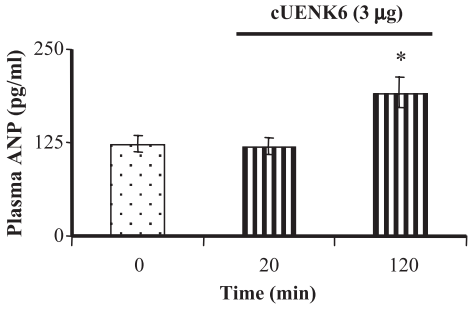

Fig. 4. Plasma ANP measured 20 and 120 min after 3 μg cUENK6 administration. *$P<0.005$ vs. corresponding saline vehicle-treated controls (n=22 rats).

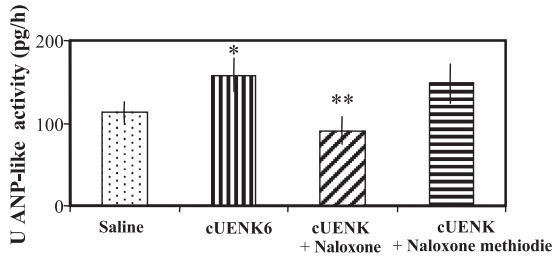

Fig. 5. Urinary excretion of immunoreactive ANP-like product during the first h after administration of 3 μg cUENK6 with and without pretreatment with naloxone (4 mg/kg), or naloxone methiodide (4 mg/kg). *P<0.02 vs. saline vehicle-treated controls. **P<0.05 vs. cUENK6 (n=10-20 rats, each).

treatment with naloxone, but not by naloxone methiodide (Fig. 5).

3.3. Effect of cUENK6 on cardiac ANP transcripts

The influence of cUENK6 on ANP mRNA was analyzed in all heart chambers by RT-PCR. Compared to values obtained in saline-treated controls (presented as 100%), a significant increase in ANP mRNA was detected in right atria (135%, $n=7$, $P<0.05$) and left ventricles (170%, $n=7$, $P<0.004$) 2 h after cUENK6 (3 μg) injection (Fig. 6).

3.4. Effect of cUENK6 on blood pressure and the heart rate

Arterial pressures, heart rate and animal activity were monitored by telemetry (Fig. 7) before and after treatment with saline vehicle or 3 μg cUENK6, with and without naloxone pretreatment. Saline injection resulted in a rapid increment of animal activity as well as increase in arterial pressures and heart rates, effects that reached maximum

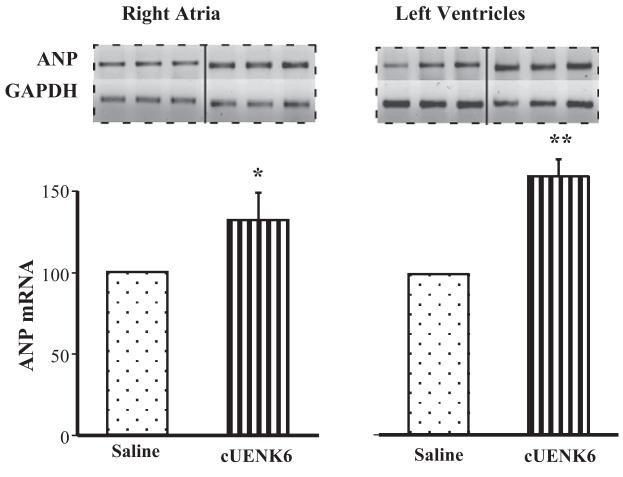

Fig. 6. RT-PCR analysis of ANP gene transcripts detected in the right atrium and left ventricle 2 h after treatment with either saline or 3μg cUENK6. ANP mRNA levels are normalized to corresponding GAPDH mRNA and presented as a percentage of vehicle-treated cotrols. *P<0.05 and **P<0.004 vs. saline vehicle-treated controls (n=7 rats each).

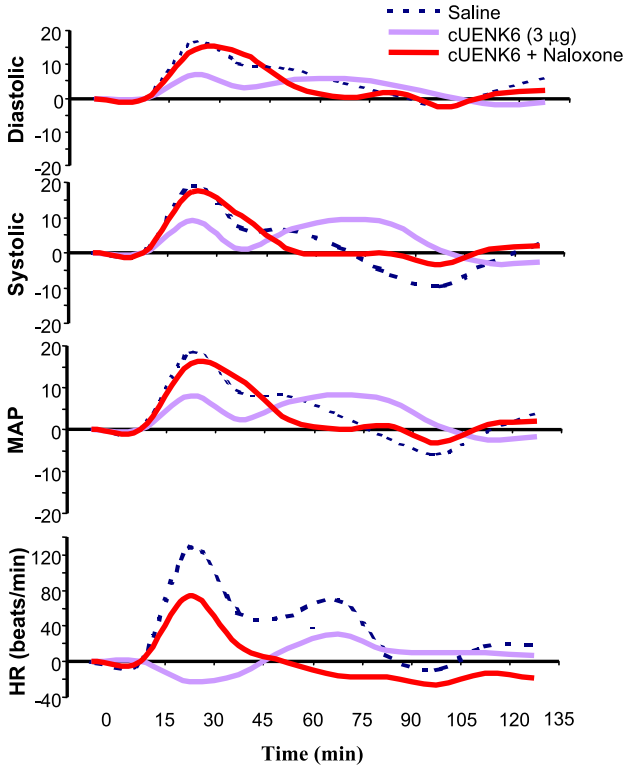

Fig. 7. Continuous telemetry recording of systolic, diastolic and mean arterial pressures and heart rate (HR) over 2 h after cUENK6 administration with and without naloxone pretreatment (4 mg/kg). Data are presented as mean values above basal recordings obtained over 2 h before drug injection (n=6 rats each). Error bars are omitted for clarity.

within 15 min, then declined. Treatment with cUENK6 (3 μg) attenuated these elevations, resulting in a mild sedative action observed as lower locomotor activity (6.9 ± 2.2 vs. 1.7 ± 0.7 pulses, $P<0.04$). Basal mean arterial pressure (average 88 ± 1 mm Hg) was augmented after saline injection by 18 ± 1 mm Hg, while the increase by cUENK6 (3 μg) was 8 ± 2 mm Hg. This effect was influenced by decreases in both systolic and diastolic blood pressures. Saline injection also resulted in increased heart rate that averaged, at 15-min post-injection, 128 ± 7 beats/min above baseline, while cUENK6 (3 μg) reduced heart rate to 22 ± 12 beats/min below baseline. The effects of cUENK6 were partially or totally inhibited by pretreatment with naloxone.

4. Discussion

This is the first in vivo study that investigates the renal and cardiovascular effects of cUENK6, the most potent known μ-opioid receptor agonist. Intravenous injection of cUENK6 in conscious and normally hydrated normotensive rats increased urine output as well as sodium, potassium and cGMP excretions in a dose-dependent manner; an action that was observed primarily during the first hour post-injection. The renal effects of cUENK6 were inhibited by

pretreatment with naloxone, but not by naloxone methiodide or naloxonazine, indicating that cUENK6 activates central μ-opioid receptors but not of μ-1 subtype. In addition, cUENK6 injection resulted in increased urinary ANP-like immunoactivity, suggesting new mechanisms for opioid renal actions. Another important finding was that cUENK6 decreased the injection-induced stress observed as an increase in animal activity, arterial pressures and heart rate, and that these effects were attenuated by pretreatment with naloxone, indicating opioid receptor mediation.

Three main opioid receptor types, μ, κ and δ, and several subtypes have been characterized pharmacologically (Dhawan et al., 1996). Opioid receptors are G protein-coupled, with seven transmembrane domains, and share high protein sequence identity (Befort et al., 1996). μ-opioid receptors share 58% sequence homology with δ opioid receptors and 67% sequence homology with κ-opioid receptors (Reisine and Bell, 1993). Activation of all opioid receptors induces, to different extents, cardiovascular and renal effects. Early studies have shown that opioids acting at κ receptors elicit profound dose-dependent diuresis without increased sodium and potassium excretion in humans and animals. This diuretic effect was originally believed to be mainly related to inhibition of vasopressin, because diuresis did not occur in Brattleboro rats that genetically lack vasopressin, and because synthetic vasopressin receptor antagonists (Leander et al., 1985) abolished the diuretic action of κ-opioids. On the other hand, Ashton et al. (1989) have shown that the κ-opioid, U50,488, induces potent diuretic effects without altering plasma vasopressin. Others suggested that κ receptor agonists may interact directly with renal vasopressin receptors, thus attenuating the action of endogenous vasopressin in the kidney (Slizgi and Ludens, 1982). These observations are consistent with our present finding that the diuretic effect of cUENK6 over the first hour was not associated with lower circulating vasopressin levels and are consistent with the hypothesis that opioids may alter renal excretory function by reducing tubular water reabsorption (Kapusta, 1995).

In our previous studies, we have shown that, at low doses, the diuretic actions of morphine, the most studied μ-opioid receptor agonist, are associated with stimulated release of a potent diuretic and natriuretic hormone, ANP, synthesized by the heart and other loci (Horky et al., 1985; Gutkowska et al., 1986, 1993). This finding was subsequently confirmed in humans (Ogutman et al., 1990). Similarly, another highly potent and selective μ-opioid agonist, TAPP, given i.v., induced a 10-fold increment of urine output, sodium, potassium and cGMP excretions, and these effects were associated with a 25-fold increase in plasma ANP. The actions of TAPP are peripherally mediated, because TAPP, in contrast to morphine, does not cross the blood–brain barrier, and specifically because the effects of ANP are inhibited by pretreatment with peripheral administration of anti-ANP (Gutkowska and Schiller, 1996).

Therefore, it was unexpected to find, in the present study, that the renal and cardiovascular effects of cUENK6 cannot be explained by increased plasma ANP, especially that the renal responses were observed during the first hour post-treatment when plasma ANP levels were not altered. Our results, however, suggest that the effects of cUENK6 may result from activation of renal natriuretic peptide system, evidenced by a significant increase in urinary ANP-like activity. The antibody used in urinary ANP measurement cross-reacts with urodilatin, a renal member of the natriuretic peptide family. Urodilatin is the product of the same gene as ANP propeptide, but is processed differentially in the kidney and excreted in urine. Urodilatin acts in a paracrine fashion, released from distal tubular kidney cells into the tubular lumen and binds to luminal guanylyl cyclase natriuretic peptide receptors (GC-A) in the inner medullary collecting ducts, inducing the generation of cGMP with subsequent diuresis and natriuresis (Valentin et al., 1993). In support, recent evidence has been provided from the study of postprandial natriuresis in humans, showing that urinary sodium excretion correlated with urodilatin, but not with plasma ANP (Drummer et al., 1996). Therefore, we propose an additional mechanism for opioid-mediated renal effects, namely activation of renal natriuretic peptide system, which may in consequence induce urinary cGMP excretion, diuresis and natriuresis. Indeed, this is the first demonstration that a μ-opioid receptor agonist, cUENK6, activates renal natriuretic peptide system, which may explain, at least in part, its renal effect (Jin et al., 2001). In addition, several studies of endogenous opioid receptor blockade in vivo indicate inhibitory responses of sympathetic activity by endogenous opioids. Therefore, inhibition of renal nerve activity by cUENK6 may also lead to the observed renal effects.

Studies have identified three subtypes of μ receptors: μ-1, μ-2 and μ-3 (Randich et al., 1993). In the present study, we characterized the μ-opioid receptor subtype that mediates the effects of cUENK6 and found that μ-1 receptors are not involved, because of lack of inhibition of renal effects by naloxonazine, a selective μ-1 opioid receptor antagonist (Pasternak et al., 1980).

It has been known for a long time that opioids acting within the central nervous system (CNS) modulate arterial pressure. It is likely that the decrease in blood pressures and heart rate observed in the present study may be due to an analgesic effect of cUENK6 and subsequent decrease in pain sensation or mild sedative effect in response to the injection. It may also be consequent to inhibition of sympathetic nervous activity. Opioids suppress the release of catecholamines, both in vitro and in vivo, resulting in diminished effector responses to nerve stimulation (Wong et al., 1990). Opioid receptor activation evokes inhibitory effects on autonomic nerve traffic, presynaptic release of neurotransmitters and post-synaptic neurotransmitter function. These effects may involve activation of presynaptic α2-adrenoceptors and/or inhibition of beta-adrenoceptors (Kienbaum et al., 2001; Xiao et al., 1997).

It is noteworthy that although circulating ANP appears not to be involved in the renal and cardiovascular effects of cUENK6 observed in the first hour posttreatment, heart ANP gene expression was enhanced in right atria and left ventricles 2 h after opioid administration, and this increase was associated with elevated plasma levels. Opioid-stimulation of cardiac ANP gene in specific heart compartments has already been described in rat left ventricles 4 h after acute or chronic cocaine treatment under conditions of unaltered blood pressure (Besse et al., 1997), and in right atria 4 h after morphine administration (Fukui et al., 1991). The opioid-dependent enhancement of ANP gene expression may have important physiological benefits, such as explaining, at least in part, the cardioprotective effects of opioids in ischemic preconditioning, a phenomenon in which brief exposure of the myocardium to ischemia protects the heart against subsequent severe ischemia.

In summary, the acute cardiovascular and renal effects of the newly developed most potent μ-opioid receptor agonist, cUENK6, are mediated by μ receptors, but not the μ-1 subtype. In addition to other neurohormonal mechanisms, the immediate diuretic and saliuretic actions of cUENK6 may be routed through a renal natriuretic peptide. The delayed effect on the heart involving stimulation of atrial natriuretic peptide, could, at least in part, explain the cardioprotective outcomes of opioids.

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