Amino Acids (2013) 45:171–178
DOI 10.1007/s00726-013-1484-2

ORIGINAL ARTICLE

Side-effects of analgesic kyotorphin derivatives: advantages over clinical opioid drugs

Marta M. B. Ribeiro · Sónia Sá Santos · David S. C. Sousa · Margarida Oliveira · Sara M. Santos · Montserrat Heras · Eduard Bardaji · Isaura Tavares · Miguel A. R. B. Castanho

Received: 28 November 2012 / Accepted: 23 February 2013 / Published online: 8 March 2013

Abstract The adverse side-effects associated with opioid administration restrain their use as analgesic drugs and call for new solutions to treat pain. Two kyotorphin derivatives, kyotorphin-amide (KTP–NH_2) and ibuprofen–KTP–NH_2 (IbKTP–NH_2) are promising alternatives to opioids: they trigger analgesia via an indirect opioid mechanism and are highly effective in several pain models following systemic delivery. In vivo side-effects of KTP–NH_2 and IbKTP–NH_2 are, however, unknown and were evaluated in the present study using male adult *Wistar* rats. For comparison purposes, morphine and tramadol, two clinically relevant opioids, were also studied. Results showed that KTP-derivatives do not cause constipation after systemic administration, in contrast to morphine. Also, no alterations were observed in blood pressure or in food and water intake, which were only affected by tramadol. A reduction in micturition was detected after KTP–NH_2 or tramadol administrations. A moderate locomotion decline was detected after IbKTP–NH_2-treatment. The side-effect profile of KTP–NH_2 and IbKTP–NH_2 support the existence of opioid-based mechanisms in their analgesic actions. The conjugation of a strong analgesic activity with the absence of the major side-effects associated to opioids highlights the potential of both KTP–NH_2 and IbKTP–NH_2 as advantageous alternatives over current opioids.

Keywords Kyotorphin · Analgesic peptides · Opioids · Side-effects · Morphine · Constipation

Abbreviations

BBB	Blood–brain barrier
BP	Blood pressure
CNS	Central nervous system
DMSO	Dimethyl sulfoxide
i.p.	Intraperitoneal
KTP	Kyotorphin (Tyr-Arg)
KTP–NH_2	Kyotorphin–amide
IbKTP–NH_2	KTP–NH_2 linked to ibuprofen
MCs	Metabolic cages
SBP	Systolic blood pressure
SEM	Standard error of the mean

Introduction

Opium and its alkaloids are powerful centrally acting compounds in the treatment of chronic pain. In parallel, they trigger several side-effects, such as constipation, nausea,

Marta M. B. Ribeiro, Sónia Sá Santos contributed equally to this work.

M. M. B. Ribeiro · S. S. Santos · D. S. C. Sousa · S. M. Santos · M. A. R. B. Castanho (✉)
Faculdade de Medicina de Lisboa, Instituto de Medicina Molecular, Av. Professor Egas Moniz, 1649-028 Lisbon, Portugal
e-mail: macastanho@fm.ul.pt
URL: http://www.imm.fm.ul.pt/web/imm/physicalbiochemistry

M. Oliveira · I. Tavares
Departamento de Biologia Experimental, Faculdade de Medicina do Porto, Rua Dr. Plácido da Costa, no 91, Edifício CIM_FMUP, 4200-450 Porto, Portugal

M. Oliveira · I. Tavares
Instituto de Biologia Molecular e Celular, Rua Campo Alegre, 4150-180 Porto, Portugal

M. Heras · E. Bardaji
Laboratori d'Innovació en Processos i Productes de Síntesi Orgànica (LIPPSO), Departament de Química, Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

urinary retention, motor impairment, respiratory depression, addiction, and tolerance. Of these, constipation and nausea occur the most frequently (Benyamin et al. 2008; Stratton Hill 1997). In addition, the development of tolerance after chronic administration of opioids leads to dose escalation, which increases the incidence and severity of all side-effects. These problems lead clinicians to restrain the prescription of opioids to relieve pain (Jaffe and Martin 1990; Stein et al. 2003). Therefore, the development of potent analgesic drugs with fewer side-effects than classic opioids is greatly needed and requires a large focus of research.

Naturally occurring opioid peptides, such as endorphins, encephalins and endomorphins, are potential substitutes of exogenous opioids for pain relief (Gentilucci 2004; Janecka et al. 2010; Ribeiro et al. 2011c). Kyotorphin (KTP; L-Tyr–L-Arg), first isolated from bovine brain in 1979, is one example. This dipeptide plays an important role in pain regulation at the central nervous system (CNS) via an opioid-mediated mechanism (Takagi et al. 1979a, b). The remarkable analgesic activity of KTP in animal models was observed only when the molecule was directly injected into the brain (Shiomi et al. 1981), which is a consequence of its reduced ability to cross the blood–brain barrier (BBB). To improve KTP delivery to CNS, we have recently succeeded in designing two new KTP-derivatives: KTP–amide (KTP–NH_2) and KTP–NH_2 linked to ibuprofen (IbKTP–NH_2) (Ribeiro et al. 2011a, b). Following systemic administration in acute pain models, KTP–NH_2 and IbKTP–NH_2 exhibited analgesic activity similar to morphine, and lower tolerance (Ribeiro et al. 2011a, b). Here, side-effects typically associated to opioid administration were evaluated in vivo following a single administration of either KTP–NH_2, IbKTP–NH_2, morphine or tramadol. Morphine and tramadol are two commonly prescribed opioid drugs. Although several such drugs are nowadays available for prescription, morphine still remains the gold standard in analgesia (Benyhe 1994). Tramadol, a centrally acting analgesic drug mediated by both opioid and non-opioid mechanisms (Grond and Sablotzki 2004; Miranda and Pinardi 1998), has proven effective in different types of moderate to severe pain. This drug displays a safer side-effect profile than morphine, with lower incidence of constipation, sedation and tolerance (Dworkin et al. 2007).

In the present work, the metabolic profile of KTP–NH_2 and IbKTP–NH_2 was assessed in vivo, with particular attention to the gastrointestinal function. Drugs' effects on micturition, water and food intake, locomotion and cardiovascular function were also studied. Our data show that KTP-derivatives do not cause the major adverse side-effects of opioid drugs. These safer side-effect profiles, along with strong analgesic activity and ability to cross the BBB, enforce the potential of KTP–NH_2 and IbKTP–NH_2 as future drugs to treat pain.

Materials and methods

Compounds

The peptides KTP–NH_2 and IbKTP–NH_2 were synthesized as described elsewhere (Ribeiro et al. 2011a, b). Tramadol and morphine hydrochloride were obtained as pre-made solutions ready for injection (i.e. injectable ampoules; Labesfal Laboratórios, Portugal). All the compounds were dissolved or diluted in physiological saline solution (0.9 % NaCl containing 5 % of dimethyl sulfoxide, DMSO) prior to intraperitoneal (i.p.) injection (in a dosing volume of 1 mL/kg body weight). At the day of the experiment, animals were divided into groups according to the injected compound (single i.p. dose): KTP–NH_2 (32.3 mg/kg = 96 μmol/kg), IbKTP–NH_2 (24.2 mg/kg = 46 μmol/kg), morphine (5 mg/kg) and tramadol (10 mg/kg). The control group was i.p. injected with the vehicle: saline solution containing 5 % of DMSO. Selected doses of KTP-derivatives and morphine were previously optimized with antinociception studies (Ribeiro et al. 2011a, b), whereas the selected tramadol dose was chosen for its efficacy in rats when i.p. administered (Apaydin et al. 2000; Bianchi and Panerai 1998). These doses were chosen for inducing comparable levels of analgesia in rats after i.p. injection.

Animals

Experiments were performed on adult male *Wistar* rats (Harlan Ibérica, Spain) weighing between 253 and 340 g. Animals were housed together in groups with unrestricted access to water and food, and under controlled temperature and light conditions (22 ± 2 °C; lights on between 7 a.m. and 7 p.m.). All experiments were conducted during the light period of the 12:12 h cycle. In order to induce habituation to the researcher and to reduce stress, each animal was gently handled daily in the test room for at least 3 days prior to testing and was brought to the same room 2 h before the experiments. To induce habituation to the metabolic cage (MC) apparatus, animals were individually housed (1 rat per MC) for 24 h. All experiments were carried out in accordance with the guidelines of the European Community Council Directive (86/609/EEC), and were approved by the Portuguese Competent Authority for Animal Welfare (Direcção Geral de Veterinária) and the Ethical Committee of the Faculty of Medicine, University of Lisbon, for animal research.

Metabolic study

The metabolic study was performed using metabolic cages (Tecniplast, Italy), circular engineered cages whose design allows collecting and measuring faeces and urine

separately (funnelled into underside cylinders) as well as food and water intake. Animals were i.p. administered with each of the compounds or vehicle and immediately placed in the MC (1 rat/cage). Ad libitum access to a known amount of water (150 mL) and food (40 g) was available via a graduated drinking tube and a feeder chamber, respectively. The testing period was 6 h. This time period was chosen so as to cover the lifetimes of the compounds' analgesic activity and of their metabolization. At the end of the testing period, the remaining amount of water and food as well as the faecal pellets and urine expelled were quantified and registered. The urine pH was also measured using a pH universal indicator (Merck).

Evaluation of locomotion

Evaluation of locomotor function was performed with an open field apparatus and carried out in a quiet and diffusely lit room with each group equally represented at the time of testing. The open field arena was an empty square box (67 × 67 × 51 cm high). Animals were i.p. administered with one of the compounds or with the vehicle. Fifteen minutes later, rats were placed individually in the centre of the arena, and their behaviour was video-recorded for 5 min using a camera mounted on the ceiling above the open field box. This 5-min time period is known to emphasize mice/rats exploratory behaviour (Gould et al. 2009), as well as to be appropriate to detect significant hyperactivity or behavioural sedation (Crawley 2007). Animal tracking along the different areas on the open field arena was analysed using the software Smart, version 2.5.10 (Panlab, S.L.U, Barcelona, Spain). For motor performance analysis, the arena was virtually divided into three concentric squares: borders (near the walls), periphery and centre. For the evaluation of the number of crossings, the open field arena was also virtually divided into 16 equal rectangles, and the number of times each animal crossed between two areas was measured. This analysis is independent of the squares being at the centre or at the periphery of the arena (Tuon et al. 2008). Results are shown as average velocity, % time spent resting, number of crossings and % time spent in the centre of the arena. Each animal was considered to be resting if the mean velocity was <3 cm/s. Average velocity is the mean velocity with the resting time excluded.

Blood pressure evaluation

The effects of KTP–NH_2 and IbKTP–NH_2 in blood pressure (BP) were evaluated according to previous protocols using the non-invasive tail-cuff method (Tavares et al. 1997). Briefly, after a training period of 7 days, the rats were inserted into a restrainer (LE 5610, Panlab S.I., Barcelona, Spain) and placed on a heating pad at 38 °C for 10–15 min. A tail-cuff detector (LE 5008 05PL Panlab S.I., Barcelona, Spain) was positioned on the animals' tail. Five BP measurements were collected as baseline values for each animal. Animals were unrestrained and allowed to recover for 10 min, after which they were injected i.p. with 32.3 mg/kg of KTP–NH_2 or 24.2 mg/kg of IbKTP–NH_2. Five BP measurements were obtained 15, 30 and 60 min after administration.

Statistical analysis

Data are represented as the groups' mean ± SEM (standard error of the mean). The significance of differences between groups was analysed with one way ANOVA followed by Tukey's multiple comparison test. The significance of differences in each group was analysed with Friedman's test followed by Dunn's multiple comparison test. All statistical analyses were calculated with Prism Software (GraphPad Software, version 5).

Results

Metabolic study

The metabolic response of *Wistar* male rats after KTP-derivatives or opioids administration was assessed using metabolic cages, which allowed accurate measurement of metabolic intake and output—the ingestion of food and water, and the production of faeces and urine. Morphine administration led to a blockade of bowel function with a total absence of faecal pellets for the whole 6-h period (Fig. 1a). On the other hand, KTP–NH_2-, IbKTP–NH_2- and tramadol-treated groups did not exhibit significant alterations of this behaviour.

All tested compounds appear to cause a reduction in urine output when compared to the control (Fig. 1b). Statistical significance was, however, only obtained for KTP–NH_2—and tramadol—treated animals. Urine pH measurement detected no statistical difference between the groups, with values ranging from 6.5 to 8.0 randomly. In addition to reducing voiding, tramadol was the only drug that changed eating and thirst patterns by causing a significant increase in the quantities of food and water intake (Fig. 2).

Evaluation of locomotion

The locomotion behaviour of animals after administration of KTP-derivatives, morphine and tramadol was recorded in an open field apparatus. Although no differences between groups were observed regarding the average velocity (Fig. 3a), IbKTP–NH_2 treated animals spent more

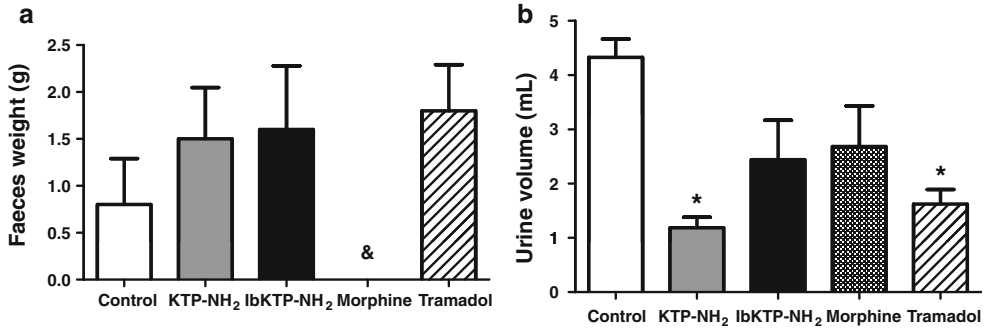

Fig. 1 Effect of KTP derivatives and opioids on faecal (**a**) and urine (**b**) output in rats, observed in animals i.p. administered with KTP–NH_2 (32.3 mg/kg), IbKTP–NH_2 (24.2 mg/kg), morphine (5 mg/kg), tramadol (10 mg/kg) or vehicle (saline with 5 % DMSO used as control) and individually placed in metabolic cages for 6 h. The quantity of faeces and urine was measured following this time period. In all experiments, $n = 5$ per group. $^{*}P < 0.05$ versus control, one way ANOVA ($P = 0.0124$) followed by Tukey's post test. $^{\&}$No faecal pellets were collected in the morphine group. Mean + SEM for all groups

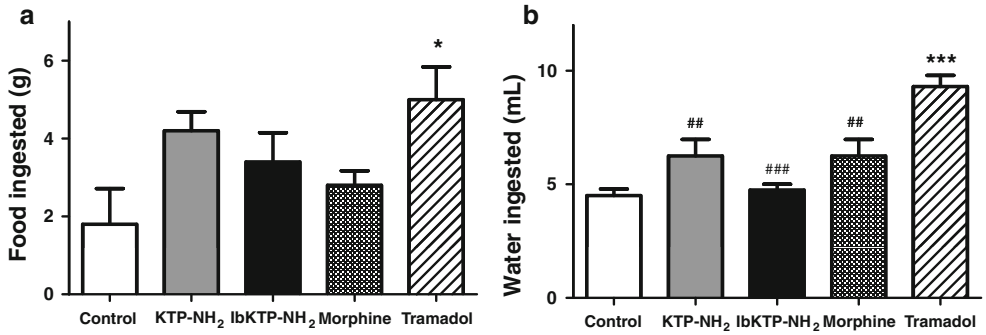

Fig. 2 Effect of KTP derivatives and opioids on food (**a**) and water intake in rats (**b**) observed in animals i.p. administered with KTP–NH_2 (32.3 mg/kg), IbKTP–NH_2 (24.2 mg/kg), morphine (5 mg/kg), tramadol (10 mg/kg) or vehicle (saline with 5 % DMSO used as control) and individually placed in metabolic cages for 6 h. The amount of food and water consumed was measured after this time period. In all experiments, $n \geq 4$ per group. $^{*}P < 0.05$, $^{***}P < 0.001$ versus control; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ versus tramadol, one way ANOVA [$P = 0.0396$ in (**a**), $P < 0.0001$ in (**b**)] followed by Tukey's post test. Mean + SEM for all groups

time resting (Fig. 3b) and displayed a lower number of crossings between areas (Fig. 3c), which suggests the occurrence of motor impairments. Also, these animals were the only ones with a reduced time spent in the centre of the arena (Fig. 3d). KTP–NH_2 and opioids morphine and tramadol did not affect the evaluated locomotion parameters.

Blood pressure evaluation

The effects of KTP–NH_2 and IbKTP–NH_2 in systolic BP are shown in Fig. 4. KTP–NH_2 induced a transient and non-statistically significant increase in BP 15 min after injection (83.33 ± 26.84 mmHg), after which BP values decreased to baseline values. IbKTP–NH_2 induced a transient and non-significant decrease in BP values 15 min following injection (35.00 ± 13.05 mmHg) after which BP values returned to baseline values. No differences from baseline values were detected 60 min after the injection of KTP–NH_2 or IbKTP–NH_2.

Discussion

Improving the safety of opioid-like drugs, currently of limited applicability due to major side-effects, is crucial for further pharmaceutical development. In this work, two new kyotorphin derivatives, KTP–NH_2 and IbKTP–NH_2, were studied regarding their side-effect profiles and compared with the widely used opioids morphine and tramadol.

The absence of constipation after KTP–NH_2 and IbKTP–NH_2 injection is an important advantage of these peptides over many opioids, namely morphine. The constipation observed as a result of morphine administration is mainly related to morphine's high affinity to μ-opioid receptors (Reimer et al. 2009). Tramadol, on the other hand and in spite of its μ-agonist activity, has about 6,000-fold lower affinity for μ receptors than morphine. This may explain the maintenance of gastrointestinal motility in tramadol-treated animals (Benyamin et al. 2008; Grond and Sablotzki 2004).

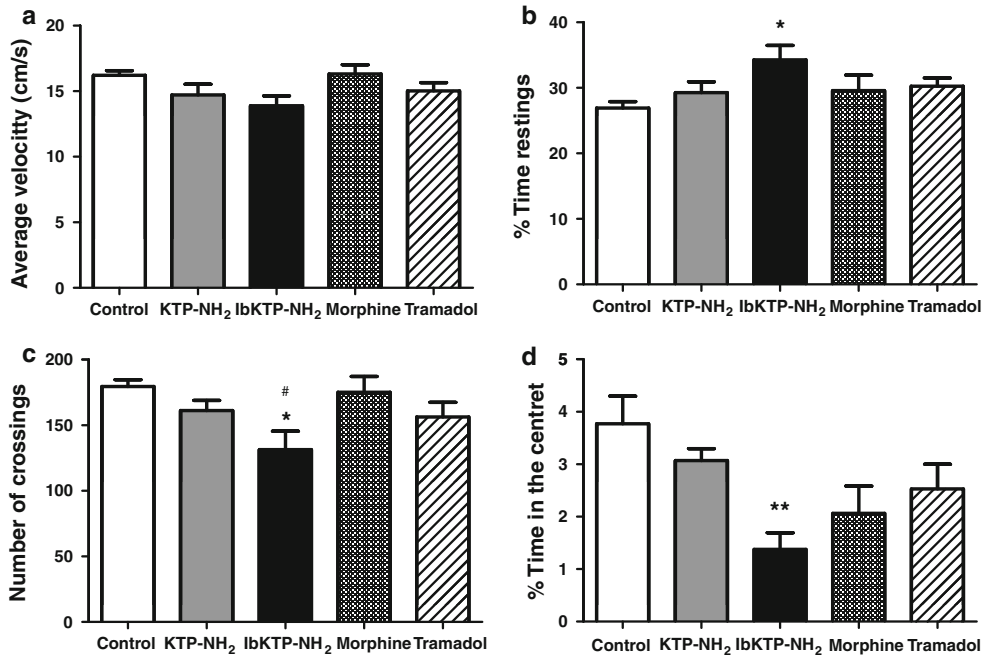

Fig. 3 Locomotion functions evaluation after administration of KTP derivatives and opioids. Animals were individually placed in an open field apparatus 15 min after being i.p. injected with KTP–NH_2 (31.3 mg/kg), IbKTP–NH_2 (24.2 mg/kg), morphine (5 mg/kg), tramadol (10 mg/kg), or vehicle (saline with 5 % DMSO used as a control). Behaviour was recorded for a 5-min time period and data are shown as average velocity (**a**), % time spent resting (**b**), number of crossings (**c**) and % time spent in the arena centre (**d**). In all experiments, $n \geq 6$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$ versus morphine, one way ANOVA [$P = 0.0811$ in (**b**), $P = 0.0225$ in (**c**), $P = 0.0065$ in (**d**)] followed by Tukey's post test. Mean + SEM for all groups

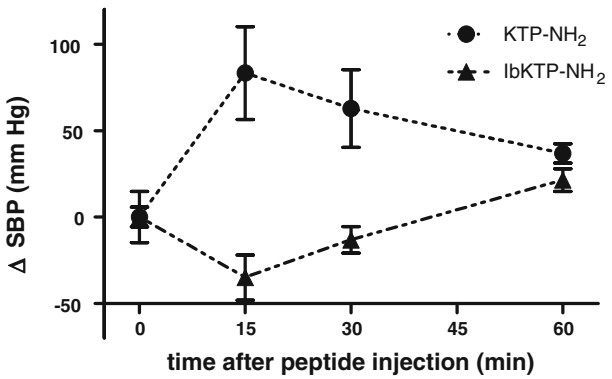

Fig. 4 Effect of Kyotorphin derivatives on animals' systolic blood pressure (SBP) after i.p. administration of KTP–NH_2 (31.3 mg/kg) and IbKTP–NH_2 (24.2 mg/kg). Results expressed as the variation of the SBP at each evaluation time point in relation to baseline values (time 0, obtained immediately before injection). In all experiments, $n \geq 4$ per group. Data expressed as mean ± SEM

Both KTP–NH_2 and tramadol lowered the micturition volume. Opioid-receptor mediated inhibition of micturition can occur mainly at the CNS, caused by stimulation of μ-opioid receptors but also δ receptors (Pandita et al. 2003). Therefore, KTP–NH_2 results agree with previous data regarding the central activity of this peptide through opioid pathways (Ribeiro et al. 2011a). It should be stressed that KTP–NH_2 has probably a minor physiological effect since urine output reduction did not result in major urinary retention because increases in blood pressure were not triggered. On the other hand, IbKTP–NH_2 did not cause a similar effect. However, the molar concentration dose of IbKTP–NH_2 used is about half of the KTP–NH_2. Also, non-steroidal anti-inflammatory drugs, like ibuprofen, do not induce urinary retention (Cole et al. 1988; Malinovsky et al. 1998). This may provide at least a partial explanation for the differences between the effect of KTP–NH_2 and IbKTP–NH_2 in urinary retention.

As to tramadol, doses up to 10 mg/kg were previously reported to increase bladder storage capacity without impairing bladder emptying (Pandita et al. 2003), which may explain the reduction in urine output observed. This effect is possibly due to supraspinal μ-opioid receptor activation and 5-hydroxytryptamine reuptake inhibition (Pandita et al. 2003). Such activity of tramadol is potentially interesting in the treatment of detrusor overactivity and nocturia. Similarly, the KTP–NH_2 effect may suggest interesting properties for the treatment of micturition disturbances.

Our study revealed that only tramadol changed eating and thirst patterns, causing an increase in both. Increase in water intake might be related to dry mouth, a frequent side-effect of tramadol (Grond and Sablotzki 2004). Further interpretation of data is hampered because the role of

opioids in ingesting and drinking behaviour is still unclear (Yeomans and Gray 2002). Nevertheless, the fact that none of the KTP-derivatives affected food and water intake is a clear benefit especially because humans subjected to opioid treatment are frequently bedridden or have reduced motility.

Locomotion studies revealed that IbKTP–NH_2 impairs motor capacities and may eventually induce an anxiogenic effect because IbKTP–NH_2 treated animals spent less time in the centre of arena (Belzung 1999; Gould et al. 2009; Stohr et al. 1998). KTP–NH_2, on the other hand, did not change the parameters evaluated, which supports previously published locomotion data using the Rotarod-test (Ribeiro et al. 2011a). Therefore, one can conclude that KTP amidation brings no adverse effects regarding locomotor capacities. Original kyotorphin itself was shown not to induce ataxia, catalepsy or hypermobility in rats and goldfish (Kolaeva et al. 2000). Structurally, the only difference between KTP–NH_2 and IbKTP–NH_2 resides on the ibuprofen residue. Simultaneous administration of ibuprofen and opioids, mainly hydrocodone, is commonly used in clinical settings as this improves analgesic performance (Kolesnikov et al. 2003). However, some adverse effects are also associated with this treatment: anxiety, nausea and sleepiness, which are probably a synergistic combination of the side-effects of the two drugs. The results reported here for IbKTP–NH_2 treated animals in the open field test may be due to a similar synergistic effect of an opioid-like analgesic, kyotorphin, and ibuprofen. Nevertheless, these motor alterations do not interfere with the conclusion that IbKTP–NH_2 is an analgesic substance, since the nociceptive activity of neurons at the spinal cord is lower in IbKTP–NH_2 treated animals (Ribeiro et al. 2011b).

As to possible cardiovascular effects of KTP–NH_2 and IbKTP–NH_2, the present data did not show any alterations in blood pressure. This agrees with our preliminary data for KTP–NH_2 obtained 30 min after administration (Ribeiro et al. 2011a) and with results obtained with unmodified KTP: a transient and dose-dependent BP increase was detected after intracerebroventricular injection, but systemic administration did not cause any effects on BP (Summy-Long et al. 1998). These data show that amidation and conjugation of KTP with ibuprofen do not lead to cardiovascular effects of the peptide. Due to ethical constraints on the use of laboratory animals, the effects of morphine and tramadol in cardiovascular parameters were not evaluated since the literature provides extensive and clear data on their effects on BP. As to morphine, all studies performed with systemic administration reported effects on BP with the main difference depending on whether the animal is anaesthetized or not. For example, i.v. administration of morphine (0.1–2.5 mg/kg) in rats lightly anesthetized with pentobarbital sodium (Randich et al. 1991) induced dose-dependent hypotension. Gomes and colleagues stated that i.v. morphine (10 mg/kg) transiently increased BP in conscious rats whereas in anaesthetized animals hypotensive responses were obtained even with low doses (1 mg/kg) (Gomes et al. 1976). In the dose used in the present study (5 mg/kg), morphine was shown to produce a short lasting hypotension in non-anaesthetized animals (Gomes et al. 1976). As to tramadol, it is considered haemodynamically stable (Mildh et al. 1999) but at high doses (5 and 10 mg/kg) cardiac depression and hypotension are induced (Raimundo et al. 2006). The present experiments allow us, therefore, to conclude that KTP–NH_2 and IbKTP–NH_2 do not appear to induce effects in BP, suggesting that they do not stimulate cardiovascular effects.

In this work, we characterized the side-effect profile of new KTP-derivatives and showed their advantages over opioids morphine and tramadol. Data reported here clearly demonstrate that KTP-derivatives display a safer side-effect profile and give further evidence on the mechanisms of action of these peptides, in agreement with previously obtained results (Ribeiro et al. 2011a, b): (1) opioid receptors are involved in KTP-derivatives mechanism of action, (2) their activity is mainly at the CNS-level, (3) KTP–NH_2 does not cause motor impairment or blood pressure changes, and (4) KTP-derivatives mechanisms of action are distinct from those of classic opioids.

The only side-effect of KTP–NH_2 is a reduction in micturition, which may be exploited as a positive effect in cases of detrusor overactivity. IbKTP–NH_2 causes a mild motor impairment that is, however, less harmful than the collection of severe side-effects resulting from the administration of morphine and tramadol. In conclusion, KTP–NH_2 and IbKTP–NH_2 associate strong analgesic activities with very important side-effect reduction when compared to morphine and tramadol (Table 1), which make them promising drugs for the future treatment of pain.

Table 1 Comparative display of the side-effects following administration of either KTP–NH_2 (32.3 mg/kg), IbKTP–NH_2 (24.2 mg/kg), morphine (5 mg/kg), or tramadol (10 mg/kg)

Side-effect	Tested compounds			
	KTP–NH_2	IbKTP–NH_2	Morphine	Tramadol
Obstipation	−	−	+	−
Micturition reduction	+	−	−	+
Food intake increase	−	−	−	+
Water intake increase	−	−	−	+
Motor impairment	−	+	−	−
Blood pressure alterations	−	−	+[a]	+[b]

[a] From Gomes et al. (1976)

[b] From Raimundo et al. (2006)

Acknowledgments We thank Autonomic Nervous System Unit and Neurosciences Unit from Instituto de Medicina Molecular (Portugal), especially Prof. I. Rocha, Prof. A. Sebastião and Dr. L. Lopes for the support, helpful discussions and facilities provided. Manuel N. Melo is thanked for critical revision of the manuscript. Fundação para a Ciência e Tecnologia (Portugal) is acknowledged for funding: SFRH/BD/42158/2007 fellowship to M. Ribeiro and SFRH/BI/51213/2010 fellowship (for doctorate) to S. Sá Santos associated to Marie Curie IAPP. Marie Curie Industry-Academia Partnerships and Pathways (European Commission) is also acknowledged for funding (FP7-PEOPLE-2007-3-1-IAPP. Project 230654). The authors acknowledge and appreciate the financial support received from Faculdade de Medicina da Universidade de Lisboa and Fundação Amadeu Dias, Portugal (Project No. 2010029).

References

Apaydin S, Uyar M, Karabay NU, Erhan E, Yegul I, Tuglular I (2000) The antinociceptive effect of tramadol on a model of neuropathic pain in rats. Life Sci 66(17):1627–1637 [pii]:S0024320500004823

Belzung C (1999) Measuring exploratory behavior. In: Crusio WE, Gerlai RT (eds) Handbook of molecular genetic techniques for brain and behavior research (techniques in the behavioral and neural sciences), vol 13. Elsevier, Amsterdam, pp 739–749

Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, Glaser SE, Vallejo R (2008) Opioid complications and side effects. Pain Phys 11(2 Suppl):S105–S120

Benyhe S (1994) Morphine: new aspects in the study of an ancient compound. Life Sci 55(13):969–979

Bianchi M, Panerai AE (1998) Anti-hyperalgesic effects of tramadol in the rat. Brain Res 797(1):163–166 [pii]:S0006-8993(98)00388-6

Cole RS, Fry CH, Shuttleworth KE (1988) The action of the prostaglandins on isolated human ureteric smooth muscle. Br J Urol 61(1):19–26

Crawley JN (2007) What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice. 2nd edn. Wiley, Hoboken

Dworkin RH, O'Connor AB, Backonja M, Farrar JT, Finnerup NB, Jensen TS, Kalso EA, Loeser JD, Miaskowski C, Nurmikko TJ, Portenoy RK, Rice AS, Stacey BR, Treede RD, Turk DC, Wallace MS (2007) Pharmacologic management of neuropathic pain: evidence-based recommendations. Pain 132(3):237–251. doi:10.1016/j.pain.2007.08.033

Gentilucci L (2004) New trends in the development of opioid peptide analogues as advanced remedies for pain relief. Curr Top Med Chem 4(1):19–38

Gomes C, Svensson TH, Trolin G (1976) Effects of morphine on central catecholamine turnover, blood pressure and heart rate in the rat. Naunyn Schmiedebergs Arch Pharmacol 294(2):141–147

Gould TD, Dao DT, Kovacsics CE (2009) The open field test In: Gould TD (ed) Mood and anxiety related phenotypes in mice: characterization using behavioral tests. Neuromethods, vol 42, 1st edn. Humana Press, Totowa, pp 1–20. doi:10.1007/978-1-60761-303-9_1

Grond S, Sablotzki A (2004) Clinical pharmacology of tramadol. Clin Pharmacokinet 43(13):879–923. doi:43134

Jaffe JH, Martin WR (1990) Opioid analgesics and antagonists. In: Gilman AG, Rall TW, Nies AS, Taylor P (eds) Goodman and Gilman's the pharmacological basis of therapeutics. 8th edn. Pergamon Press, New York, pp 485–521

Janecka A, Perlikowska R, Gach K, Wyrebska A, Fichna J (2010) Development of opioid peptide analogs for pain relief. Curr Pharm Des 16(9):1126–1135 [pii]:BSP/CPD/E-Pub/00001

Kolaeva SG, Semenova TP, Santalova IM, Moshkov DA, Anoshkina IA, Golozubova V (2000) Effects of L-thyrosyl–L-arginine (kyotorphin) on the behavior of rats and goldfish. Peptides 21(9):1331–1336 [pii]:S0196-9781(00)00275-8

Kolesnikov YA, Wilson RS, Pasternak GW (2003) The synergistic analgesic interactions between hydrocodone and ibuprofen. Anesth Analg 97(6):1721–1723

Malinovsky JM, Le Normand L, Lepage JY, Malinge M, Cozian A, Pinaud M, Buzelin JM (1998) The urodynamic effects of intravenous opioids and ketoprofen in humans. Anesth Analg 87(2):456–461

Mildh LH, Leino KA, Kirvela OA (1999) Effects of tramadol and meperidine on respiration, plasma catecholamine concentrations, and hemodynamics. J Clin Anesth 11(4):310–316 [pii]:S0952-8180(99)00047-1

Miranda HF, Pinardi G (1998) Antinociception, tolerance, and physical dependence comparison between morphine and tramadol. Pharmacol Biochem Behav 61(4):357–360 [pii]:S0091-3057(98)00123-3

Pandita RK, Pehrson R, Christoph T, Friderichs E, Andersson KE (2003) Actions of tramadol on micturition in awake, freely moving rats. Br J Pharmacol 139(4):741–748. doi:10.1038/sj.bjp.0705297

Raimundo JM, Sudo RT, Pontes LB, Antunes F, Trachez MM, Zapata-Sudo G (2006) In vitro and in vivo vasodilator activity of racemic tramadol and its enantiomers in Wistar rats. Eur J Pharmacol 530(1–2):117–123. doi:10.1016/j.ejphar.2005.11.028

Randich A, Thurston CL, Ludwig PS, Timmerman MR, Gebhart GF (1991) Antinociception and cardiovascular responses produced by intravenous morphine: the role of vagal afferents. Brain Res 543(2):256–270 [pii]:0006-8993(91)90036-U

Reimer K, Hopp M, Zenz M, Maier C, Holzer P, Mikus G, Bosse B, Smith K, Buschmann-Kramm C, Leyendecker P (2009) Meeting the challenges of opioid-induced constipation in chronic pain management—a novel approach. Pharmacology 83(1):10–17. doi:10.1159/000165778

Ribeiro MM, Pinto A, Pinto M, Heras M, Martins I, Correia A, Bardaji E, Tavares I, Castanho M (2011a) Inhibition of nociceptive responses after systemic administration of amidated kyotorphin. Br J Pharmacol 163(5):964–973. doi:10.1111/j.1476-5381.2011.01290.x

Ribeiro MM, Pinto AR, Domingues MM, Serrano I, Heras M, Bardaji ER, Tavares I, Castanho MA (2011b) Chemical conjugation of the neuropeptide kyotorphin and ibuprofen enhances brain targeting and analgesia. Mol Pharm 8(5):1929–1940. doi:10.1021/mp2003016

Ribeiro MMB, Serrano ID, Sá Santos S (2011c) Turning endogenous peptides into new analgesics: the example of Kyotorphin derivatives. In: Castanho M, Santos N (eds) Peptide drug discovery and development: translational research in academia and industry, 1st edn. Wiley, Weinheim, pp 171–188

Shiomi H, Ueda H, Takagi H (1981) Isolation and identification of an analgesic opioid dipeptide kyotorphin (Tyr–Arg) from bovine brain. Neuropharmacology 20(7):633–638

Stein C, Schafer M, Machelska H (2003) Attacking pain at its source: new perspectives on opioids. Nat Med 9(8):1003–1008. doi:10.1038/nm908nm908

Stohr T, Schulte Wermeling D, Weiner I, Feldon J (1998) Rat strain differences in open-field behavior and the locomotor stimulating and rewarding effects of amphetamine. Pharmacol Biochem Behav 59(4):813–818 [pii]:S0091-3057(97)00542-X

Stratton Hill C (1997) Guidelines for treatment of cancer patients. The Revised Pocket Edition of the Final Report of the Texas Cancer Council's Workgroup on Pain Control in Cancer Patients, 2nd edn. Cancer Texas Council, Austin

Summy-Long JY, Bui V, Gestl S, Koehler-Stec E, Liu H, Terrell ML, Kadekaro M (1998) Effects of central injection of kyotorphin and L-arginine on oxytocin and vasopressin release and blood pressure in conscious rats. Brain Res Bull 45(4):395–403 [pii]:S0361-9230(97)00341-9

Takagi H, Shiomi H, Ueda H, Amano H (1979a) Morphine-like analgesia by a new dipeptide, L-tyrosyl–L-arginine (Kyotorphin) and its analogue. Eur J Pharmacol 55(1):109–111

Takagi H, Shiomi H, Ueda H, Amano H (1979b) A novel analgesic dipeptide from bovine brain is a possible Met-enkephalin releaser. Nature 282(5737):410–412

Tavares I, Almeida A, Albino-Teixeira A, Lima D (1997) Lesions of the caudal ventrolateral medulla block the hypertension-induced inhibition of noxious-evoked c-fos expression in the rat spinal cord. Eur J Pain 1(2):149–160 [pii]:S1090-3801(97)90073-2

Tuon L, Comim CM, Petronilho F, Barichello T, Izquierdo I, Quevedo J, Dal-Pizzol F (2008) Time-dependent behavioral recovery after sepsis in rats. Intensive Care Med 34(9): 1724–1731. doi:10.1007/s00134-008-1129-1

Yeomans MR, Gray RW (2002) Opioid peptides and the control of human ingestive behaviour. Neurosci Biobehav Rev 26(6): 713–728 [pii]:S0149-7634(02)00041-6