Pharmacology, Biochemistry and Behavior 91 (2009) 566–572

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Activation of mu, delta or kappa opioid receptors by DAMGO, DPDPE, U-50488 or U-69593 respectively causes antinociception in the formalin test in the naked mole-rat (*Heterocephalus glaber*)

Philemon Kipkemoi Towett [a,*], Titus Ikusya Kanui [a,1], Geoffrey Moriaso Ole Maloiy [b,1], Francis Juma [c,2], Jacob Olongida Ole Miaron [a,1]

[a] Neurophysiology and Neuropharmacology Research Laboratory, Department of Veterinary Anatomy and Physiology, University of Nairobi, P.O. Box 00100-30197, Nairobi, Kenya
[b] Comparative and Integrative Physiology Research laboratory, University of Nairobi, P.O. Box 00100-30197, Nairobi, Kenya
[c] Department of Clinical Pharmacology, University of Nairobi, P.O. Box 00100-30197, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 31 July 2008
Received in revised form 18 September 2008
Accepted 23 September 2008
Available online 1 October 2008

Keywords:
Formalin test
Naked mole-rat
Nociception
Antinociception
DAMGO
DPDPE
U-50488
U-69593

ABSTRACT

Data available on the role of the opioid systems of the naked mole-rat in nociception is scanty and unique compared to that of other rodents. In the current study, the effect of DAMGO, DPDPE and U-50488 and U-69593 on formalin-induced (20 μl, 10%) nociception were investigated. Nociceptive-like behaviors were quantified by scoring in blocks of 5 min the total amount of time (s) the animal spent scratching/biting the injected paw in the early (0–5 min) and in the late (25–60 min) phase of the test. In both the early and late phases, administration of 1 or 5 mg/kg of DAMGO or DPDPE caused a naloxone-attenuated decrease in the mean scratching/biting time. U-50488 and U-69593 at all the doses tested did not significantly change the mean scratching/biting time in the early phase. However, in the late phase U-50488 or U-69593 at the highest doses tested (1 or 5 mg/kg or 0.025 or 0.05 mg/kg, respectively) caused a statistically significant and naloxone-attenuated decrease in the mean scratching/biting time. The data showed that mu, delta or kappa-selective opioids causes antinociception in the formalin test in this rodent, adding novel information on the role of opioid systems of the animal on pain regulation.

1. Introduction

Although nociception is fundamental to all animals, it has not been studied well in lower vertebrates such as the naked mole-rat. Naked mole-rats are primitive poikilothermic mammals found in semi-arid areas of Eastern Africa. They are subterranean rodents whose physiology is rather unique and interesting. Compared to other rodents of similar size, naked mole-rats have long life span (>28 years) (Buffenstein, 2005). They are virtually blind and on their skin are vibrissae-like hairs that are useful for underground locomotion. They have prominent incisors and tactile hairs, both of which have large representation in the somatosensory cortex (Catania and Remble, 2002; Crish et al., 2003; Park et al., 2003; Henry et al., 2006).

Research on the nervous system of the naked mole-rat has shown that its skin lacks substance P- and calcitonin gene-related peptide-(CGRP) immunoreactive fibers (Park et al., 2003). Substance P and CGRP are among other neuropeptides involved in pain transmission and are up-regulated in response to noxious peripheral stimulation in rats (Zhang et al., 1994). The absence of SP and CGRP in the skin of the naked mole-rat raises queries on how this primitive rodent responds to noxious stimuli. Detailed study of its skin innervations revealed numerous non-peptidergic C-fibers, A-δ fibers and lanceolate endings supplied by Aβ fibers containing SP and CGRP (Park et al., 2003). The abundance of these fibers may suggest that they are crucial in nociception in this fossorial rodent. Aβ fibers are low threshold mechanoreceptors (Gottschaldt et al., 1973) but may also act as nociceptive fibers (Djouhri et al., 1998; Djouhri and Lawson, 2004).

Investigations on the roles of opioid systems of the naked mole-rat have also revealed some peculiarities. In the hot plate test (60 °C) Kanui and Hole (1990) reported that morphine caused aggression instead of analgesia. In a later study, we reported increased pain sensitivity in the hot plate test (60 °C) after acute administration of pethidine (Towett and Kanui, 1993). These reports suggested that the opioid drugs used had no analgesic effects but instead were pronociceptive in the hot plate test in this animal. However, studies performed using the formalin test in the same species of rodent demonstrated analgesic effects of morphine (Kanui et al., 1993) and codeine (Karim et al., 1993). This suggests a difference in the roles of opioid systems of the naked mole-rat on different kinds of pain. Such differences have also been documented in other rodents (Abbott et al., 1986).

* Corresponding author. Tel.: +254 02 4446764.
E-mail addresses: towettp@yahoo.com (P.K. Towett), tkanui@uonbi.ac.ke (T.I. Kanui), geoffreymaloiy@yahoo.com (G.M.O. Maloiy).
[1] Tel.: +254 02 4446764.
[2] Tel.: +254 02 726300.

0091-3057/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2008.09.011

The unique anatomy, behavior and physiology of the naked mole-rat make it very interesting for comparative studies of pain regulation. It is therefore essential to increase the knowledge about the nociceptive and antinociceptive responses in this particular species. Data available indicate that only morphine and codeine (mu agonists) have been tested for analgesia using the formalin test in the naked mole-rat (Kanui et al., 1993; Karim et al., 1993). To the best of our understanding, no report on the role of highly selective mu, delta or kappa opioid agonists in the formalin-induced pain in this animal is available. In a recent study, we reported that stimulation of mu or delta opioid receptors caused hyperalgesia while activation of kappa receptors caused antinociception in the hot plate test (Towett et al., 2006). In the light of these recent data, it is important to find out how receptor-selective opioids modulate formalin-induced pain in the naked mole-rat and how the data will compare with those of other rodents.

The aim of the present study was therefore to evaluate the effects of the mu [D-Ala^2-NMePhe^4-Gly-ol-enkephalin (DAMGO)], delta [D-Pen^2-D-Pen^5-enkephalin (DPDPE)], and kappa [trans(+)-3,4-Dichloro-N-methyl-N- [2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulfonate (U-50488) and (5α,7α,8β)-(+)-N-Methyl-N- [7-(1-pyrrolidinyl)-1-oxaspirol[4.5]dec-8-yl]-benzeneacetamide (U-69593) receptor agonists on the formalin-induced pain in the naked mole-rat. In addition, the study aimed to verify the opioid-receptor involvement by attenuating antinociceptive effects with naloxone. The data collected suggest that mu- and delta-selective opioid agonists have antinociceptive effects in the formalin test contrary to what was earlier observed in the hot plate test (Towett et al., 2006) and provide additional knowledge on pain modulation by the opioid systems of the naked mole-rat.

2. Materials and methods

2.1. Animals

In this study, 200 adult male naked mole-rats (*Heterocephalus glaber*), weighing 20–40 g, were used in the experiments. They were trapped from the field and transported to a laboratory where they were kept in rooms with conditions almost similar to those of their natural habitat (Towett et al., 2006). Feeding was also as previously described (Towett et al., 2006). The naked mole-rats were allowed at least one month to acclimate to the laboratory conditions before they were used for the experiments.

2.2. Drugs

All the opioid agents used in the experiments were bought from Research Biochemicals International (RBI, Natick, USA). These were D-Ala^2-NMePhe^4-Gly-ol-enkephalin (DAMGO), D-Pen^2-D-Pen^5-enkephalin (DPDPE), trans-(±)-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide methane sulfonate (U-50488), (5α,7α,8β)-(+)-N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspirol [4.5]dec-8-yl]-benzeneacetamide (U-69593), and naloxone hydrochloride. DAMGO, DPDPE, U-50488 and naloxone were dissolved in physiological saline (0.9% NaCl). U-69593 was dissolved in 0.1 N hydrochloric acid. All the precautions regarding handling and stability of the drugs as recommended by the manufacturer were followed strictly. The opioid peptides chosen have been shown to be receptor-selective in rats and mice and their analgesic effects have been widely demonstrated (VonVoigtlander et al., 1983; Corbett et al., 1984; Porreca et al., 1987; Calcagnetti et al., 1988; Suh and Tseng, 1990).

2.3. Antinociceptive testing

To investigate antinociceptive effects, DAMGO (0.1–5 mg/kg), DPDPE (0.1–5 mg/kg), U-50488 (1–5 mg/kg), and U-69593 (0.0125–0.05 mg/kg) were administered intraperitoneally 30 min before nociceptive testing. Naloxone (1 or 5 mg/kg) was co-administered with an opioid receptor agonist 30 min before injecting formalin. DAMGO (1 mg/kg), DPDPE (1 mg/kg), U-50488 (5 mg/kg) and U-69593 (0.05 mg/kg) were each administered with naloxone (1 or 5 mg/kg). The selection of the dose ranges of the agonists and antagonists was based on previous report (Towett et al., 2006) and on preliminary dose response curves. Drugs were administered in a constant total volume of 50 µl. In all experiments, we used fresh preparations of the drugs. There was one control group for each agonist and control animals received drug vehicle. Naked mole-rats were randomly selected from a colony of 50–100 members and assigned to treatment groups. Each animal was used only once and the experiments were blinded.

A transparent observation chamber measuring 30×30×30 cm was used for behavioral assessment of the animals. Naked mole-rats were adapted to the chamber for 1 h every day during the acclimation period of 30 days and 30 min prior to the start of the experiments. The naked mole-rat was gently restrained and using a 100 µl syringe and a 26-gauge needle, 20 µl of 10% formalin in 0.9% NaCl was carefully injected subcutaneously into its right dorsal hind paw. The naked mole-rat was put back into the observation chamber and the observation period immediately started. Pain-like behaviors were quantified by scoring in blocks of 5 min the total amount of time (s) the animal spent scratching/biting the injected paw in the initial acute phase (early phase: 0–5 min) and in the prolonged tonic phase (late phase: 25–60 min). The mean of each 5-minute data for a group of naked mole-rats was calculated to give mean time (s) spent in scratching/biting the injected paw. The volume and the concentration of formalin used were based on data reported (Kanui et al., 1993, Karim et al., 1993). The experiments were performed between 8 a.m. and 2 p.m., and in a room with minimal noise and vibrations, and a temperature range of 25–28 °C. In all the experiments performed, the

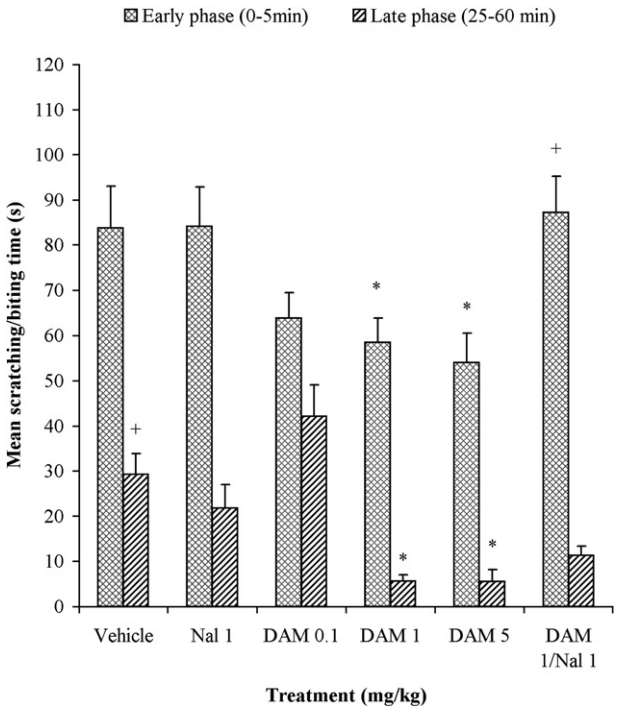

Fig. 1. Effects of i.p. administration of vehicle or DAMGO (0.1, 1 or 5 mg/kg), 1 mg/kg of naloxone (Nal 1) or a combination of 1 mg/kg of DAMGO and 1 mg/kg of naloxone (DAM 1/Nal 1) on the mean scratching/biting time. Data were plotted as mean ± S.E.M., and n = 9–11 animals per group. Each treated group (i.e. DAMGO and naloxone groups) was statistically compared with the vehicle group and * indicates a significant difference ($P<0.05$). For naloxone antagonism in each phase, $^+$ indicates significant difference ($P<0.05$, list significant difference) from DAM alone group or from DAM + Nal group.

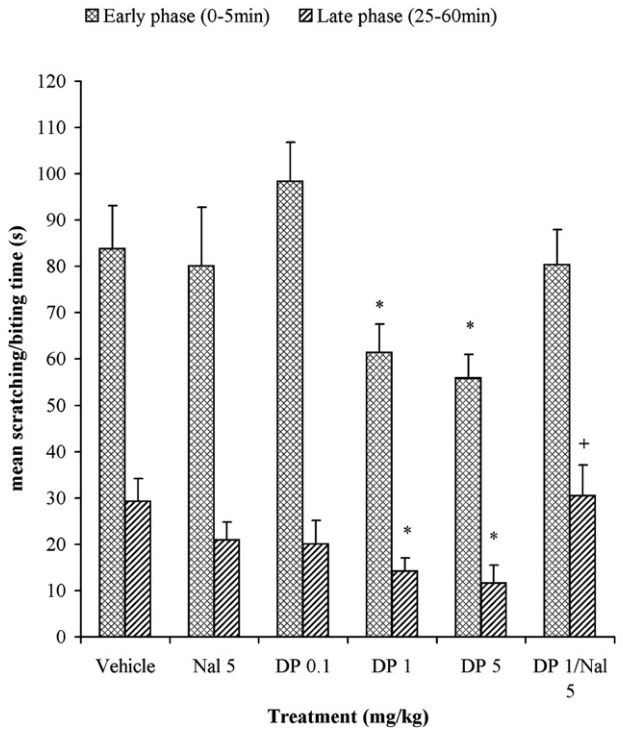

Fig. 2. Effects of i.p. administration of vehicle or DPDPE (0.1, 1 or 5 mg/kg), 5 mg/kg of naloxone (Nal 5), or a combination of 1 mg/kg of DPDPE and 5 mg/kg of naloxone (DP 1/Nal 5) on the mean scratching/biting time. Data were plotted as mean ± S.E.M., and $n = 10$ animals per group. Each treated group (i.e. DPDPE and naloxone groups) was compared with the vehicle group and * indicates a significant difference ($P<0.05$). For naloxone antagonism in the late phase, $^{+}$ indicates significant difference ($P<0.05$, list significant difference) from DP alone group.

principles of laboratory animal care (NIH Publication No. 85-23, revised 1996), as well as local guidelines regarding the use of animals in pain experiments were followed.

2.4. Data analysis

The response data from the early and the late phases were examined separately. The data were statistically assessed by an analysis of variance (ANOVA) and intergroup differences were analyzed by least significant difference. The results presented are means ± S.E. and the level of significance was set at 5% ($P<0.05$).

3. Results

3.1. Effects of mu agonist

The effects of DAMGO (0.1, 1 or 5 mg/kg) alone or in combination with naloxone (1 mg/kg) on the mean scratching/biting response are shown in Fig. 1. In either the early or the late phase, the effect of 1 or 5 mg/kg of DAMGO on the mean scratching/biting time was statistically significant ($P<0.05$) while that of 0.1 mg/kg dose was not ($P>0.05$, least significant difference, subsequent to ANOVA) when compared to the vehicle control group. Simultaneous administration of DAMGO (1 mg/kg) and naloxone (1 mg/kg) caused a statistically significant increase ($P<0.05$) in mean scratching/biting time in the early phase but not in the late phase ($P>0.5$) when each of the means was compared to the corresponding mean of 1 mg/kg of DAMGO alone. In both phases of the test, the means of naloxone- and vehicle-treated groups were insignificantly different. The mean of the group given the combined treatment (DAM+Nal) and that of the vehicle group was statistically different ($P<0.05$) in the late phase only.

3.2. Effects of delta agonist

The effects of DPDPE (0.1, 1 or 5 mg/kg) alone or in combination with naloxone (5 mg/kg) on the mean scratching/biting time are shown in Fig. 2. In both phases of the test, the effect of 1 or 5 mg/kg of the agonist on the mean scratching/biting time was statistically significant ($P<0.05$) while that of 0.1 mg/kg dose was not ($P>0.05$) when compared to the vehicle control group. In the early phase, co-administration of DPDPE (1 mg/kg) and naloxone (5 mg/kg) caused no change ($P>0.05$) in the mean scratching/biting time while in the late phase there was a significant ($P<0.05$) effect when each treatment was separately compared to the corresponding mean for the agonist-treated group (1 mg/kg of DPDPE). In both the early and late phases, the means of naloxone- and vehicle-treated groups were insignificantly different. Similarly, the mean of the group given the combined treatment (DP+Nal) and that of the vehicle group were not statistically different.

3.3. Effects of kappa agonists

The effects of U-50488 (1 or 5 mg/kg) alone or in combination with naloxone (5 mg/kg) on the mean scratching/biting time are shown in Fig. 3. In the early phase, no dose of U-50488 had statistically significant effect ($P>0.05$) on the mean scratching/biting time when compared to the vehicle control group. A higher dose (10 mg/kg) of U-50488 caused a severe motor impairment with no behavioral response following the injection of formalin. The standard errors of the means of the treatments in the early phase were large thus causing insignificant difference between the two groups (i.e. agonist-treated and agonist plus antagonist-treated groups) of animals. In the late phase, the effect of U-50488 (1 or 5 mg/kg) was statistically significant ($P<0.05$, compared to controls). Co-administration of U-50488 (5 mg/kg) and naloxone

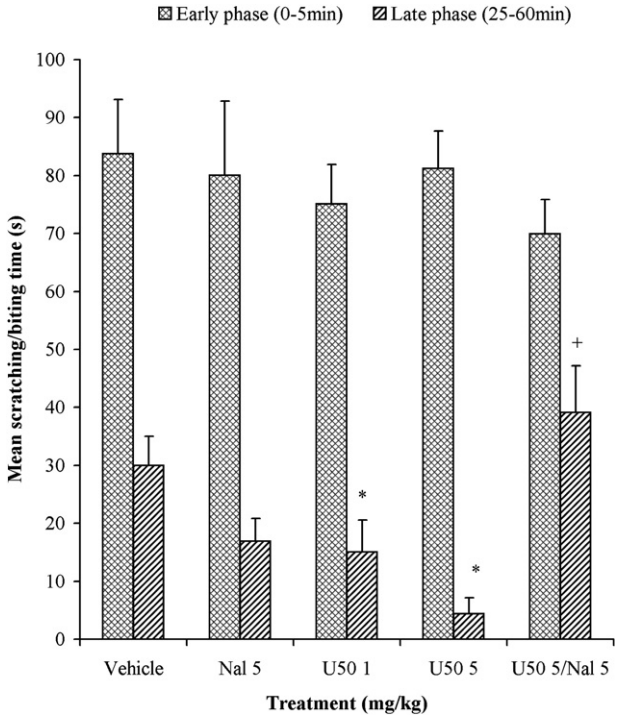

Fig. 3. Effects of i.p. administration of vehicle or U-50488 (1, or 5 mg/kg) or 5 mg/kg of naloxone (Nal 5), or a combination of 5 mg/kg of U-50488 and 5 mg/kg of naloxone (U50, 5/Nal 5) on the mean scratching/biting time. Data were plotted as mean ± S.E.M., and $n = 10$ animals per group. Each treated group (i.e. U-50488 and naloxone groups) was compared with the vehicle group and * indicates a significant difference ($P<0.05$). For naloxone antagonism in the late phase, $^{+}$ indicates significant difference ($P<0.05$, list significant difference) from U50 alone group.

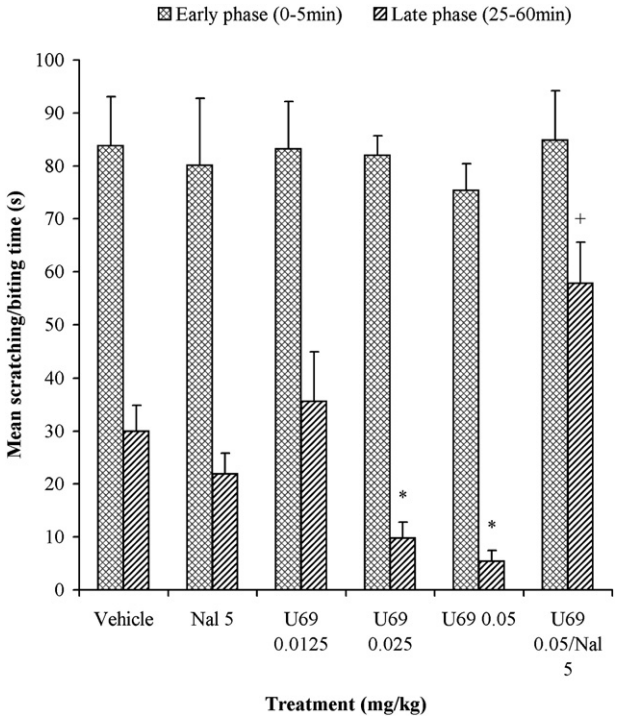

Fig. 4. Effects of i.p administration of vehicle or U-69593 (0.0125, 0.025 or 0.05 mg/kg), 5 mg/kg of naloxone (Nal 5), or a combination of 0.05 mg/kg of U69593 and 5 mg/kg of naloxone (U69, 0.05/Nal 5 on the mean scratching/biting time. Data were plotted as mean ± S.E.M., and $n = 10$ animals per group. Each treated group (i.e. U-69593 and naloxone groups) was compared with the vehicle group and * indicates a significant difference ($P < 0.05$). For naloxone antagonism in the late phase, $^{+}$ indicates significant difference ($P < 0.05$, list significant difference) from U69 alone group or the vehicle group.

(5 mg/kg) caused a statistically significant effect ($P < 0.05$, compared to agonist-treated group) on the mean scratching/biting time in the late phase.

The effects of U-69593 (0.0125, 0.025 or 0.05 mg/kg) alone or in combination with naloxone (5 mg/kg) are shown in Fig. 4. In the early phase, no dose of the agonist had statistically significant effect on the mean scratching/biting time. Doses higher than 0.05 mg/kg caused severe motor impairment. In the late phase, the effect of 0.025 or 0.05 mg/kg of the agonist on the mean scratching/biting time was statistically significant ($P > 0.05$, compared to controls) whereas that of the lower dose used was not. In the late phase only, co-administration of U-69593 (0.05 mg/kg) and naloxone (5 mg/kg) caused a mean scratching/biting time that was significantly different from that of the agonist- and vehicle-treated groups ($P < 0.05$).

4. Discussion

The formalin test is widely used in behavioral studies for studying antinociceptive agents. The test has two distinct phases of pain related behavior both of which have different mechanisms. The first phase is attributed to a direct peripheral stimulation of nociceptive afferents whilst the second phase results from peripheral inflammatory reaction involving the release of proinflammatory substances (Hunskaar and Hole, 1987; Shibata et al., 1989) and the spinal processes induced by the first phase (Dickenson and Sullivan, 1987). Formalin elicits pain-related behavior in rodents by activating Transient Receptor Potential cation channel, subfamily A, member 1 (TRPA1) on primary afferent nociceptors (McNamara et al., 2007). No study on TRPA channels is reported in naked mole-rat. However, the scratching and biting behavior induced by 20 μl of 10% formalin in this rodent suggests a similar mechanism of action as documented in other animals.

Although the formalin-induced behavior in the naked mole-rat is similar to that observed in other animals (Dubuisson and Dennis, 1977; Hunskaar et al., 1985; Hunskaar and Hole, 1987), in this animal the volume and the concentration of formalin required to induce the nocifencive behavior are higher (Tjolsen et al., 1992). This suggests that the reaction to formalin is weaker in the naked mole-rat. This could be due to the low body temperature and low metabolic rate of this animal (McNab, 1966, 1968). Park et al. (2008) also reported a reduced pain behavior after formalin injection and attributed it to the absence of neuropeptides in the skin, altered connectivity of C fibers in the spinal cord and the lack of proton sensitivity of single nociceptors in this rodent. Concerning formalin-induced behavior, naked mole-rats resemble TRPA1-knockout mice or mice injected with TRPA1 antagonists (Bautista et al., 2006; McNamara et al., 2007), suggesting that these channels may be fewer or absent in this animal. This may therefore explain the need to use high concentrations of formalin.

In the present study, i.p. administration of DAMGO, DPDPE, U-50488 or U-69593 caused a dose-dependent and naloxone-attenuated decrease in the mean scratching/biting time, suggesting antinociception. The significant difference between the vehicle control group and the DAMGO plus naloxone group or U-69593 plus naloxone in the late phase suggests that the dose of the antagonist was low (Fig. 1) or high (Fig. 4) respectively. The present results agree with the published ones where morphine (Kanui et al., 1993) or codeine (Karim et al., 1993) also caused antinociception in the same rodent. However, there is one main difference between the current study and the previous reports. In the current study, the effects of opioid peptides shown to be receptor-selective in other rodents (VonVoigtlander et al., 1983; Corbett et al., 1984; Porreca et al., 1987; Calcagnetti et al., 1988; Suh and Tseng, 1990) were investigated, whereas in the published reports only mu-selective agonists (morphine and codeine) were tested for antinociception. To the best of our knowledge, opioid receptor-selectivity in the naked mole-rat has not been studied. Naloxone, a general opioid antagonist, was used to demonstrate that the effects of the four drugs used in this study were mediated through opioid receptors. This is true because naloxone was able to attenuate their antinociceptive effects. Administration of opioid peptides (e.g. DPDPE) at high doses may interact with mu receptors to cause mu agonist effects in the animal (Millan, 1986; Murray and Cowan, 1991) hence the need to use highly selective receptor antagonists. However, the antinociceptive doses (1 or 5 mg/kg, i.p.) of DPDPE in this study are rather low and not likely to have mu agonist effects in this animal. The use of receptor-selective antagonists would shed some light on the specific roles of the four opioid peptides used in this study. However, the current study provided novel information on the effects of DAMGO, DPDPE, U-50488 or U-69593 on the formalin-induced nociception in the naked mole-rat.

The first phase of the formalin test was not significantly affected by U-50488 or U-69593 at all the doses used in this study. This finding contrasts with a number of studies performed in other rodents. These studies reported antinociception after i.c.v. (Calcagnetti et al., 1988), i.t. (Chapman and Dikenson, 1992; Haley et al., 1990; Pelissier et al., 1990), systemic (Murray and Cowan, 1991) or peripheral (Hong and Abbott, 1995; Nozaki-Taguchi and Yamamoto, 1998; Barr et al., 2003) administration of mu, delta and kappa agonists (e.g. U-50488) in both phases of the formalin test. Some researchers reported antinociceptive effects of i.t. U-50488 in the early phase only in rats (Machelska et al., 1997). The use of other chemogenic tests of nociception have also revealed the antinociceptive effects of opioid agonists including kappa ones in other rodents (Stein et al., 1988; 1989; Catheline et al., 1998, 1999). We cannot exactly explain why our results differ with those for other rodents. However, some of the possible reasons include the fact that the doses of the kappa agonists used compared to those used in the other rodents were relatively lower. Subsequently, the peritoneal fluids over-dilute the peptides resulting in little amounts reaching the CNS or the peripheral sites. An attempt to use higher doses of either U-50488 or U-69593 resulted in marked motor depression.

Species difference may also account for the lack of antinociceptive effects of the kappa agonists in the early phase of the test in this study (Murray and Cowan, 1991). Opioid transport systems determine distribution and elimination of peptides in the body. Distribution of exogenously administered drugs influences their physiological efficacy (Weber et al., 1991, 1992). Perhaps the low metabolism in the naked mole-rat influences the activity of the opioid transport systems causing a lack of kappa effect in the early phase of the formalin test. The difference between mu or delta and kappa effects in the early phase in this report may suggest different mechanisms of action of opioid peptides on nociceptive fibers (Nozaki-Taguchi and Yamamoto, 1998). An opioid receptor characterization may reveal interesting information in this unique rodent and this requires investigation.

Opioid effects are mediated by central and peripheral opioid receptors (Stein, 1995; Stein et al., 2001; Labuz et al., 2007) and the latter have been widely demonstrated in animals. In the current report, the opioid peptides were administered systemically (i.p.) and might have acted centrally as well as peripherally. Studies done in mice have shown that systemically injected opioids cause potent analgesia (Baamonde et al., 1991, 2005; Murray and Cowan, 1991; Weber et al., 1991, 1992) and this is in agreement with our present report. Opioid receptors are synthesized in the dorsal root ganglion (Schafer et al., 1995) and are then transported into the peripheral and central terminals via axonal transport.

Peripherally mediated opioid antinociception has been widely documented in rats and mice (Hong and Abbott, 1995; Catheline et al., 1998, 1999; Stein, 1995; Stein et al., 1988, 1989, 2001; Labuz et al., 2007). Peripheral antinociceptive effects of opioid peptides are mainly present in inflamed tissue (Stein et al., 2001). This is because inflammation enhances axonal transport of opioid receptors (Hassan et al., 1993) thus increasing their numbers in the periphery. Inflammation also increases contact between the opioid receptors and the peptides by disrupting the innermost layer of the perineurium (Olsson, 1990; Antonijevic et al., 1995). This evidence clearly indicates that inflammation potentiates the antinociceptive effects of peripherally or systemically administered opioid peptides. In the present study, it is possible that the four opioid peptides used acted peripherally considering that the formalin test is inflammatory in modality.

Supraspinal and spinal antinociceptive effects of the opioid peptides used in this study are also widely documented in other rodents. In most of these studies, the peptides were centrally administered (Dickenson and Sullivan, 1987; Calcagnetti et al., 1988; Murray and Cowan, 1991; Chapman and Dikenson, 1992; Machelska et al., 1997,). Central administration of peptides is preferred to systemic routes because in the latter peptides may not cross the blood brain barrier (BBB) intact. However, several studies have shown that peptides and their analogs can cross the BBB when administered i.p., i.v., S.C., or orally (Rapoport et al., 1980; Zlokovic et al., 1987; Weber et al., 1991, 1992; Chen and Polack, 1997). Behavioral studies employing the formalin test have also shown that systemically administered opioid peptides have antinociceptive effects in rodents (Baamonde et al., 1991, 2005; Murray and Cowan, 1991; Weber et al., 1991, 1992). However, some researchers have reported no analgesia of systemically administered opioid peptides in some animal models (Hong and Abbott, 1995). One of the reasons for lack of analgesia following systemic administration of an opioid peptide is its rapid elimination from the CNS (Weber et al., 1991, 1992; Chen and Polack, 1997). For instance, biliary excretion of DPDPE is responsible for its short duration of analgesia in mice (Weber et al., 1992).

Species or strain differences may also account, in part, for the poor opioid analgesia after systemic administration (Neilan et al., 2003; Schiller, 2005). Difference in pharmacokinetics of a peptide in different animal species may account for the poor opioid analgesia after systemic injection (Weber et al., 1992). Many aspects of the anatomy and the physiology of the naked mole-rat are unique. The present and the previous report (Towett et al., 2006) on systemic administration of opioid peptides tend to suggest that these compounds cross the BBB in this animal. However, this is not conclusive and there is a need to examine the ability of peptides to cross the BBB of this animal. There is also a need to investigate effects of centrally administered opioid peptides in the naked mole-rat and compare the results with the systemic ones.

The doses of the opioid peptides that caused antinociception in either the formalin or the hot plate tests in the naked mole-rat are lower compared to those reported to produce the same in other rodents. For instance, in the hot plate test DPDPE at doses, less than 40 mg/kg (i.v.) did not cause antinociception in mice (Chen and Polack, 1997) and U50488 at doses less than 3 mg/kg (s.c.) had no antinociceptive effects in the formalin test in mice (Murray and Cowan, 1991). The differences between these reports and the studies in the naked mole-rat are the routes of administration (i.p. versus i.v. or s.c.), the nociceptive test and the species of the animal used. In the i.v. route, the drug reaches the CNS faster than in either the i.p. or s.c. route (Weber et al., 1992) and therefore lower doses of drugs administered i.v. compared to those given i.p. are expected to cause antinociception in animals. In the naked mole-rat higher doses (>5 mg/kg) of DAMGO or DPDPE induced hyper-activity and hyper-excitability, whereas higher doses of U-50488 (>5 mg/kg) or U-69593 (>0.05 mg/kg) induced severe motor depression. This suggests a robust effect of opioid peptides in this animal. This could be due to a high density of opioid receptors in the CNS, low enzymatic degradation, or low biliary excretion of peptides in this animal versus other rodents. All these factors should be further investigated.

In this study, all the opioid peptides tested caused antinociception, whereas in our earlier study increased pain sensitivity was observed after DAMGO or DPDPE (Towett et al., 2006). Formalin test involves a continuous stimulus (Dennis and Melzack, 1979; Wheeler-Aceto et al., 1990), whereas the hot plate test is a brief, phasic nociceptive test (Dennis and Melzack, 1980). In the two tests, the motor response (elevation and licking/biting of the paw) are similar but not identical. Research has shown that the mechanisms of different types of pain are different in the CNS (Dennis and Melzack, 1980; Abbott et al., 1986). A number of studies on voltage-dependent calcium channels (VDCC) suggest their differential roles on types of pain in animals (Malberg and Yaksh, 1994; Diaz and Dickenson 1997). Naked mole-rats have unique physiology compared to other mammals and therefore the mechanisms of different types of pain may be unique.

Formalin nociception involves the release of pronociceptive and proinflammatory substances such as SP, calcitonin gene-related peptide, met-enkephalin, neurotensin, somatostatin and excitatory amino acids (Kantner et al., 1988; Skilling et al., 1988; McCarson and Goldstein, 1991; Zhang et al., 1994; Dickenson et al., 1997; Furst, 1999). Peripheral noxious stimulation causes a release of these chemicals from peripheral and spinal nociceptive afferents. Very little is known about the peptides involved in pain transmission in the naked mole-rat. It has been reported that this animal lacks C fibers immunoreactive to calcitonin gene-related peptide and SP but has abundant A-delta fibers (Park et al., 2003). Formalin induces activity in nociceptive C-fibers as well as in A-delta fibers (Puig and Sorkin, 1995). The absence of peptidergic C-fibers in the naked mole-rat suggests that these fibers are perhaps not involved in pain transmission in this rodent. Transmission of pain signals in the naked mole-rat may be a function of other types of nerve fibers. Studies have shown that the skin of the naked mole-rat has numerous peptidergic Aβ fibers (Park et al., 2003). Aβ fibers are nociceptive in normal, non-injured tissues (Djouhri et al., 1998) and are activated in the first phase of the formalin test (Puig and Sorkin, 1995). It is possible that Aβ fibers are very crucial in pain transmission in the naked mole-rat, and this needs to be further investigated.

In summary, the current data indicate that mu-, delta- and kappa-selective opioid peptides are antinociceptive in the formalin nociception in the naked mole-rat. The data presented here differ with that obtained using the hot plate test in the same rodent, thus adding more

information on the mechanisms of different types of nociception. Although the data is the first of its kind, it does not show how the opioid systems of the subterranean rodent modulate formalin nociception. However, it adds novel information on the effects of opioid systems on nociception in the animal.

Acknowledgments

We express our gratitude to German Academic exchange program (DAAD) for funding this work. We also gratefully thank all those who gave us technical and any other support during the course of the study.

References

Abbott FV, Franklin KB, Libman R. A dose-ratio study of mu and kappa agonists in formalin and thermal pain. Life Sci 1986;39:2017–24.
Antonijevic I, Mousa SA, Schafer M, Stein C. Perineurial defect and peripheral opioid analgesia in inflammation. J Neurosci 1995;15:165–72.
Baamonde A, Dauge V, Gacel G, Roques BP. Systemic administration of (Tyr-D-Ser(O-tert-butyl)-Gly-Phe-leu-Thr(O-tert-butyl), a highly selective delta opioid agonist, induces mu receptor-mediated analgesia in mice. J Pharmacol Exp Ther 1991;257:767–73.
Baamonde A, Lastra A, Juarez L, Garcia V, Hidalgo A, Menedez L. Effects of the local administration of selective μ-, δ- and k-opioid receptor agonists on osteosarcoma-induced hyperalgesia. Naunyn-Schmiedeberg's Arch Pharmacol 2005;372:213–9.
Barr GA, Limon E, Luthman RA. Analgesia induced by local planter injections of opiates in the formalin test in infant rats. Dev Psychobiol 2003;42:111–22.
Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, et al. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. Cell 2006;124:1269–82.
Buffenstein R. The naked mole-rat: a new long-living model for human aging research. J Gerontol A Biol Sci Med Sci 2005;60:1369–77.
Calcagnetti DJ, Helmstetter FJ, Fanselow MS. Analgesia produced by centrally administered DAGO, DPDPE and U50488H in the formalin test. Eur J Pharmacol 1988;153:117–22.
Catania KC, Remble MS. Somatosensory cortex dominated by the representation of teeth in the naked mole-rat brain. Neurobiology 2002;99:5692–7.
Catheline G, Guilbaud G, Kayser V. Peripheral component in the enhanced antinociceptive effect of systemic U-69593, a kappa-opioid receptor agonist in mononeuropathic rats. Eur J Pharmacol 1998;357:171–8.
Catheline G, Le Guen S, Besson JM. Effects of U-69593, a kappa-opioid receptor agonist, on carrageenin-induced peripheral edema and Fos expression in the rat spinal cord. Eur J Pharmacol 1999;370:287–96.
Chapman V, Dikenson AH. The combination of NMDA antagonism and morphine produced profound antinociception in the rat dorsal horn. Brain Res 1992;573:321–3.
Chen C, Polack GM. Blood-brain disposition and antinociceptive effects of [D-Pencillamine^{2,5}]enkephalin in the mouse. J Pharmacol Exp Ther 1997;283:1151–9.
Corbett AD, Gillan MGC, Kosterlitz HW, McKnight AT, Paterson SJ, Robson LE. Selectivities of opioid peptide analogues as agonists and antagonists at the delta receptor. Br J Pharmacol 1984;83:271–9.
Crish SD, Rice FL, Park TJ, Comer CM. Somatosensory organization and behavior in naked mole-rat. I. Vibrissa-like body hairs comprise a sensory array that mediates orientation to tactile stimuli. Brain Behav Evol 2003;62:141–51.
Dennis SG, Melzack R. Comparison of phasic and tonic pain in animals. Adv Pain Res Ther 1979;3:747–60.
Dennis SG, Melzack R. Pain modulation by 5-hydroxytryptaminergic agents and morphine as measured by three pain tests. Exp Neurol 1980;69:260–70.
Diaz A, Dickenson AH. Blockade of N- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurons produced by subcutaneous formalin inflammation. Pain 1997;69:93-100.
Dickenson AH, Sullivan AF. Subcutaneous formalin-induced activity of dorsal horn neurons in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain 1987;30:349–60.
Dickenson AH, Chapman V, Green GM. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. Gen Pharmacol 1997;28:633–8.
Djouhri L, Lawson SN. A beta-fiber nociceptive primary afferent neurons: a review of incidence and properties in relation to other afferent A-fiber neurons in mammals. Brain Res Rev 2004;46:131–45.
Djouhri L, Bleazard L, Lawson SN. Association of somatic action potential shape with sensory receptive properties in guinea-pig dorsal root ganglion neurons. J Physiol 1998;513:857–72.
Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 1977;4:161–74.
Furst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull 1999;48:129–41.
Gottschaldt KM, Iggo A, Young DW. Functional characteristics of mechanoreptors in sinus hair follicles of the cat. J Physiol (Lond) 1973;235:287–315.
Haley JE, Ketchum S, Dickenson AH. Peripheral k-opioid modulation of the formalin response: an electrophysiological study in the rat. Eur J Pharmacol 1990;277:21–8.
Hassan AHS, Ableitner A, Stein C, Herz A. Inflammation of the rat paw enhances axonal transport of opioid receptors in the sciatic nerve and increases their density in the inflamed tissue. Neuroscience 1993;55:185–95.
Henry EC, Remple MS, O'rian MJ, Catania KC. Organization of somatosensory cortical areas in the naked mole-rat (*Heterocephalus glaber*). J Comp Neurol 2006;495:434–52.
Hong Y, Abbott FV. Peripheral opioid modulation of pain and inflammation in the formalin test. Eur J Pharmacol 1995;277:21–8.
Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.
Hunskaar S, Fasmer OB, Hole K. Formalin test in mice, a useful technique for evaluating mild analgesics. J Neurosci Methods 1985;14:69–76.
Kantner RM, Goldstein BD, Kirby ML. Regulatory mechanisms for substance P in the dorsal horn during a nociceptive stimulus: axoplasmic transport vs. electrical activity. Brain Res 1988;385:282–90.
Kanui TI, Hole K. Morphine induces aggression but not analgesia in the naked mole-rat (*Heterocephalus glaber*). Comp Biochem Physiol 1990;96C:131–2.
Kanui TI, Karim F, Towett PK. The formalin test in the naked mole-rat (*Heterocephalus glaber*): analgesic effects of morphine, nefopam and paracetamol. Brain Res 1993;600:123–6.
Karim F, Kanui TI, Mbugua S. Effects of codeine, naproxen and dexamethasone on formalin-induced pain in the naked mole-rat. NeuroReport 1993;4:25–8.
Labuz D, Mousa SA, Schafer M, Stein C, Machelska H. Relative contribution of peripheral verses central opioid receptors to antinociception. Brain Res 2007;1160:30–8.
Machelska H, Labuz D, Przewlocki R, Przewlocka B. Inhibition of nitric oxide synthase enhances antinociception mediated by mu, delta, and kappa opioid receptors in acute and prolonged pain in the rat spinal cord. J Pharmacol Exp Ther 1997;282:977–84.
Malberg AB, Yaksh TL. Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception. J Neurosci 1994;14:4882–90.
McCarson KE, Goldstein BD. Release of substance P into the superficial dorsal horn following nociceptive activation of the hind paw of the rat. Brain Res 1991;568:109–15.
McNab BK. The metabolism of fossorial rodents: a study of convergence. Ecology 1966;47:712–33.
McNab BK. The influence of fat deposits on the basal rate of metabolism in desert homeotherms. Comp Biochem Physiol 1968;26:337–43.
McNamara CR, Mandel-Brehm J, Bautista DM, Deranian JS, Zhao M, Hayward NJ, et al. TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci USA 2007;104:13525–30.
Millan MJ. Multiple opioid systems and pain. Pain 1986;27:303–47.
Murray CW, Cowan A. Tonic pain perception in the mouse: differential modulation by three receptor-selective opioid agonists. J Pharmacol Exp Ther 1991;257:335–41.
Neilan CL, King MA, Rossi G, Ansonoff M, Pintar JE, Schiller PW, et al. Differential sensitivities of mouse strains to morphine and [Dmt1] DALDA analgesia. Brain Res 2003;974:254–7.
Nozaki-Taguchi N, Yamamoto T. Involvement of nitric oxide in peripheral antinociception mediated by kappa- and delta-opioid receptors. Anesth Analg 1998;87:388–93.
Olsson Y. Microenvironment of the peripheral nervous system under normal and pathological conditions. Crit Rev Neurobiol 1990;5:265–311.
Park TJ, Comer C, Carol A, Ying Lu, Hong SH, Rice FR. Somatosensory organization and behavior in naked mole-rats: II. Peripheral structures, innervation, and selective lack of neuropeptides associated with thermoregulation and pain. J Comp Neurol 2003;465:104–20.
Park TJ, Ying Lu, Juttner R, Smith ESJ, Hu L, Brand A, et al. Selective inflammatory pain insensitivity in the African naked mole-rat (*Heterocephalus glaber*). PLoS Biol 2008;6(1):e13. doi:10.1371/journal.pbio.0060013.
Pelissier T, Paeile C, Soto-Moyano R, Saavedra H, Hernandez A. Analgesia produced by intrathecal administration of kappa opioid agonist, U-50,488H, on formalin evoked cutaneous pain in the rat. Eur J Pharmacol 1990;190:287–93.
Porreca F, Heyman JS, Mosberg JI, Omnass JR, Vaught JL. Role of mu and delta receptors in the supraspinal and spinal analgesic effects of [D-Pen^2-,D-Pen^5]enkephalin in the mouse. J Pharmacol Exp Ther 1987;241:393–400.
Puig S, Sorkin LS. Formalin-evoked activity in identified primary afferent fibers: systemic lidocaine suppresses phase-2 activity. Pain 1995;64:345–55.
Rapoport SI, Pettibrew KD, Ohno K. Entry of opioid peptides into the central nervous system. Science 1980;207:84–6.
Schafer M, Imai Y, Uhl GR, Stein C. Inflammation enhances peripheral μ-opioid receptor mediated analgesia, but not μ-opioid receptor transcription in dorsal root ganglia. Eur J Pharmacol 1995;279:165–9.
Schiller PW. Opioid peptide-derived analgesics. AAPSJ 2005;7:E560–5.
Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain 1989;38:347–52.
Skilling SR, Smullin DH, Beitz AJ, Larson AA. Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. J Neurochem 1988;51:127–32.
Stein C. The control of pain in peripheral tissues by opioids. New Engl J Med 1995;332:1685–90.
Stein C, Millan MJ, Yassouridis A, Herz A. Antinociceptive effects of mu- and kappa-agonists in inflammation are enhanced by a peripheral opioid receptor-specific mechanism. Eur J Pharmacol 1988;155:255–64.
Stein C, Millan MJ, Shippenberg TS, Peter K, Herz A. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J Pharmacol Exp Ther 1989;248:1269–75.
Stein C, Machelska H, Schafer M. Peripheral analgesic and antiinflammatory effects of opioids. Z Rheumatol 2001;60:416–24.
Suh HH, Tseng LF. Different types of opioid receptors mediating analgesia induced by morphine, DAMGO, DPDPE, DADLE and beta-endorphin in mice. Naunyn-Schmiedeberg's Arch Pharmacol 1990;342:67–71.
Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5-17.
Towett PK, Kanui TI. Effects of pethidine, acetylsalicylic acid, and indomethacin on pain and behavior in the naked mole-rat. Pharmacol Biochem Behav 1993;45:153–9.

Towett PK, Kanui TI, Juma FD. Stimulation of mu and delta opioid receptors induces hyperalgesia, while stimulation of kappa receptors induces antinociception in the hot plate test in the naked mole-rat (*Heterocephalus glaber*). Brain Res Bull 2006;71:60–8.

VonVoigtlander PF, Lahti RA, Ludens JH. U-50,488: a selective and structurally novel non-mu (kappa) opioid agonist. J Pharmacol Exp Ther 1983;224:7-12.

Weber SJ, Greene DL, Sharma SD, Yamamura HI, Kramer TH, Burks TF, et al. Distribution and analgesia of [3H][D-Pen^2,D-Pen^5] enkephalin and two halogenated analogs after intravenous administration. J Pharmacol Exp Ther 1991;259:1109–17.

Weber SJ, Greene DL, Hruby VJ, Yamamura HI, Porreca F, Davis TP. Whole body and brain distribution of [^{3}H]cyclic [D-Pen^2,D-Pen^5]enkephalin after intraperitoneal, intravenous, oral and subcutaneous administration. J Pharmacol Exp Ther 1992;263:1308–16.

Wheeler-Aceto H, Porreca F, Cowan A. The rat paw formalin test: comparison of noxious agents. Pain 1990;40:229–38.

Zhang RX, Mi ZP, Qiao JT. Changes of spinal substance P, calcitonin gene-related peptide, somatostatin, met-enkephalin and neurotensin in rats in response of formalin-induced pain. Regul Pept 1994;51:25–32.

Zlokovic BV, Lipovac MN, Beglay DJ, Davson H, Rakic L. Transport of leucin-enkephalin across the blood-brain barrier in the perfused guinea pig brain. J Neurochem 1987;49:310–5.