Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 484 (2004) 217–223
www.elsevier.com/locate/ejphar

Decrease in serotonin concentration in raphe magnus nucleus and attenuation of morphine analgesia in two mice models of neuropathic pain

Sourisak Sounvoravong, Mihoko N. Nakashima, Mitsuhiro Wada, Kenichiro Nakashima*

Division of Analytical Research for Pharmacoinformatics, Department of Clinical Pharmacy, Graduate School of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan

Received 16 June 2003; received in revised form 4 November 2003; accepted 11 November 2003

Abstract

The alleviation of neuropathic pain cannot be satisfactorily achieved by treatment with opioids. There is much evidence to indicate that the active site of morphine for inducing effective analgesia is in the raphe magnus nucleus, where serotonin (5-HT, 5-hydroxytryptamine) acts as a primary transmitter. Therefore, we developed the hypothesis that 5-HT released in the raphe magnus nucleus could be related to the effectiveness of morphine in two mice models of neuropathic pain, diabetic (DM)-induced neuropathy and sciatic nerve ligation (SL). Two weeks after a single administration of streptozotocin, or 10 days after sciatic nerve ligation, mice were subcutaneously (s.c.) injected with morphine at 3, 5 and 10 mg/kg. The antinociceptive effect of morphine was estimated in the tail-pinch test; 5-HT content was measured after induction of neuropathic pain by microdialysis followed by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Morphine produced as insufficient antinociceptive effect in SL mice at all doses compared with that in sham-operated mice, while in DM mice, morphine given s.c. at 5 and 10 mg/kg produced antinociceptive effects compared with those in non-diabetic mice, but not at 3 mg/kg. The 5-HT content of dialysates, expressed as AUC for 75 min, in SL and DM mice was less than that in control mice. However, morphine given s.c. at 5 mg/kg did not significantly affect 5-HT levels in both mice models compared to their controls. These results suggest that the decrease in 5-HT levels in the raphe magnus nucleus may be related to attenuation of the analgesic effect of morphine caused by the abnormal pain state found in diabetes and partial peripheral nerve injury.

Keywords: Monoamine; 5-HT (5-hyroxytryptamine, serotonin); Microdialysis; Morphine; Neuropathic pain

1. Introduction

Neuropathic pain is a chronic or persistent pain. This pain is characterized by alterations in pain perception that includes an enhanced sensitivity to noxious stimuli (hyperalgesia) and abnormal pain sensitivity to previously non-painful stimuli (allodynia). Neuropathic pain is difficult to treat with conventional analgesics (Arner and Meyerson, 1988). Despite controversy over the effectiveness of opioids in the management of neuropathic pain, opioids are still used as the mainstay of treatment. However, their effects are curtailed by tolerance and dependence, in particular with chronic or neuropathic pain. Furthermore, much evidence suggests that morphine produces attenuated analgesia in some models of neuropathic pain (Kamei and Kasuya, 1995). Thus, research over the past few decades has been focused on identification of non-opioid drugs which affect the central mechanisms of pain relief. Antidepressant agents that block the re-uptake of monoamines provide the most effective treatment of neuropathic pain in clinical patients (MacFarlane et al., 1997) or in animal models of neuropathic pain (Jett et al., 1997; Abdi et al., 1998; Esser and Sawynok, 1999). Although serotonin (5-HT, 5-hydroxytryptamine) has been studied extensively, its precise role in the brain remains poorly understood, because the selective pharmacologic probes for each subtype of 5-HT receptor are not sufficient. Under normal conditions, 5-HT is implicated in numerous processes from mood to sleep, and alterations in its activity are hypothesized to contribute to many neuropsychiatric conditions, from migraine headache to depression (Nestler et al., 2001). Although central sites of action for tricyclic antidepressants and selective 5-HT re-uptake inhibitors have been

* Corresponding author. Tel./fax: +81-95-819-2450.
E-mail address: naka-ken@net.nagasaki-u.ac.jp (K. Nakashima).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.11.028

proposed (Bel and Artigas, 1993; Kreiss and Lucki, 1995), little is known about the pathophysiology of supraspinal monoaminergic systems in abnormal pain conditions that would be affected by these drugs.

The raphe magnus nucleus is suggested to be the descending pathway for pain mediation, and evidence reveals that it is a site of morphine action for producing effective analgesia (Jensen and Yaksh, 1986; Nestler et al., 2001). Furthermore, it has also been suggested that the raphe magnus nucleus is a central part of the serotonergic system. However, little is known about changes in 5-HT content in abnormal pain states. Therefore, it is interesting to know whether the levels of 5-HT in the raphe magnus nucleus are implicated in the abnormal pain processes and change in the analgesic potency of morphine in these animal models of neuropathic pain.

The purpose of this study was to evaluate the analgesic effect of morphine in two neuropathic pain models in mice, produced by either streptozotocin-induced diabetic (DM) neuropathy or sciatic nerve ligation (SL), and also to measure changes in the raphe magnus nucleus 5-HT content in these neuropathy models.

2. Materials and methods

2.1. Animals

Male ddY strain mice (16–18 g weight, Otsubo Experimental Animals, Nagasaki, Japan) were purchased and housed in groups of six to eight animals. Mice were maintained under an ambient temperature (22 ± 1 °C) and relative humidity (55 ± 5%) in a control room with free access to laboratory diet (MF, Oriental Yeast, Tokyo) and tap water. After reaching a weight of 23 to 28 g, they were used for experiments. All procedures for using of animals in this study were approved by the Nagasaki University Animal Care and Use Committee.

2.2. Drugs and chemicals

Streptozotocin (Wako, Tokyo, Japan), and morphine HCl (Takeda Pharm., Osaka, Japan) were dissolved in 0.9% saline. Artificial CSF consisted of 125 mM NaCl, 2.5 mM KCl, 0.5 mM $NaH_2PO_4-H_2O$, 5 mM $Na_2HPO_4-2H_2O$, 1.0 mM $MgCl_2-6H_2O$, 1.2 mM $CaCl_2-2H_2O$ and 5 mM D-glucose. All other chemicals were analytical grade and purchased from Wako (Tokyo, Japan).

2.3. Induction of neuropathic pain

2.3.1. Sciatic nerve ligation

Neuropathic pain was induced by sciatic nerve ligation (Seltzer et al., 1990). Briefly, all animals were anesthetized by intravenous (i.v.) injection of pentobarbital sodium (Nembutal, 50 mg/kg). The common sciatic nerve was exposed unilaterally (right side) at the mid-thigh level, taking care to avoid interruption of the epineural circulation. Two ligatures with silk (No. 8) were placed around the nerve and tightened until they elicited a brief twitch in the high limb. The muscle layers and incision were closed and sutured with silk (No. 3). Control group or sham-operated mice were exposed to the same procedure but without ligature of sciatic nerve. Ten days after the operation, the development of neuropathic pain in mice was classified by application of the von Frey filament (a method described by Chaplan et al., 1994). Almost all of the mice with sciatic nerve ligation developed neuropathic pain, indicated by thresholds of von Frey filament of less than 1 g (0.07–0.6 g). In sham-operated mice, thresholds of von Frey filament were greater than 1 g (1.4–2 g). Mice (SL and sham-operated mice) were randomized and divided into three groups and treated subcutaneously (s.c.) with morphine at 3, 5, and 10 mg/kg.

2.3.2. Diabetic neuropathy

Diabetes was induced with a single intravenous injection of streptozotocin at 200 mg/kg of body weight. Streptozotocin was dissolved in saline adjusted to pH 4.5 with 1.5 mM citric acid as vehicle and injected immediately into a tail vein. The control group of age-matched non-diabetic mice received an injection of vehicle only. Diabetes was indicated by a serum glucose level greater than 400 mg/dl (by measurement of tail blood with Glucose test kit, Dex., Bayer). Two weeks after treatment with streptozotocin, approximately 98% of streptozotocin-treated mice developed diabetes and were used in the experiments. Mice (diabetic and control) were randomized and divided into three groups and treated s.c with morphine at 3, 5, and 10 mg/kg.

2.4. Measurement of mechanical allodynia

The method was as previously reported by Chaplan et al. (1994). The experiment was performed in a quiet room, as follows. Mice were individually placed in a clear plastic cage with a wire-mesh bottom (1-cm^2 perforations). The von Frey filament (North Coast Medical) was applied at a 90° angle for 5 s, at five random locations on the plantar surface of each hind paw from below the mesh floor at several levels of force (0.04, 0.07, 0.16, 0.4, 0.6, 1.0, 1.4 and 2.0 g). Paw withdrawal threshold was defined as the minimum force in grams required to elicit a withdrawal reflex of the paw, at least once in each of the five trials. Only robust and immediate withdrawal responses produced by the stimulus were counted. A mean force of 1.4 g was found to elicit a withdrawal response in normal mice, but after induction of neuropathy, this was less than 1 g. Mechanical allodynia was measured 10 days after sciatic nerve ligation

and 2 weeks after streptozotocin injection to classify development of neuropathic pain in mice.

2.5. Measurement of nociceptive response by the tail-pinch test

The tail-pinch test was conducted according to modified Haffner's method as previously reported by Takagi et al. (1966). Briefly, mice were pre-tested by pinching their tail base with forceps, adjusted to elicit a nociceptive response within 2 s. The nociceptive response was indicated by vocalization or by biting of the forceps. After drug injection, the pressure stimuli were applied with a cut-off time of 10 s to prevent tissue damage. The tail-pinch test was conducted before drug administration and at 15-min intervals for a period of 90 min after drug injection. The data were converted to %maximum possible effect (MPE, details as mentioned in calculation and statistics).

2.6. In vivo brain microdialysis experiments

Two weeks after a single administration of streptozotocin, or 10 days after ligation of the sciatic nerve, mice were anesthetized with pentobarbital (50 mg/kg, i.v.). A microdialysis guide cannula (CUP 7, BAS) was implanted stereotaxically in the raphe magnus nucleus (coordinates: *A*, 0 mm; *L*, −5.8 mm from bregma; *V*, 4.5 mm from the skull surface, Keith and Franklin, 1997) and fixed with dental cement (GC, Tokyo, Japan). Animals were allowed to recover from surgery for at least 3 days before the microdialysis probe was inserted through the guide cannula. The microdialysis membrane probes (CMA/77/2 CUP) were 2 mm long, with an outer diameter of 0.24 mm and a 6000-Da cut-off. Artificial CSF was infused at a flow rate of 1 μl /min.

To establish baseline levels of 5-HT, five samples were taken at 15-min intervals, starting 90 min after implantation of the probe. Samples of 15 μl were collected into microvials containing 5 μl of 0.1 M HCl, resulting in a total sample volume of 20 μl. Following baseline sampling, each group of mice was divided into two sub-groups, one of which was injected s.c. with saline and the other with morphine at 5 mg/kg (since this dose of morphine produced antinociceptive effects up to 50% of MPE in each control mice). After injection, sample microdialysates were collected as before at 15-min intervals and these data were used to monitor the alteration of 5-HT levels after administration of morphine.

Dialysate samples were analyzed by reversed-phase high-performance liquid chromatography with electrochemical detection (HPLC-ECD, ED 623B). The conditioning cell was set at +400 mV. Samples were injected directly into a Rheodyne valve fitted with a 10 μl loop (Rheodyne, Cotati, USA) and separated on an ODS-5 column (Develosil, 250 × 1.5 mm i.d). The mobile phase was a mixture of 0.06 M acetic acid/0.13 M Na_2HPO_4/1 mM 1-octanesulfonic acid sodium salt/5% EDTA containing 12% methanol and 2% acetonitrile, at a flow rate of 0.17 ml/min.

2.7. Calculation and statistics

Antinociceptive effects of drug are presented as percentage of the maximum possible effect (%MPE) obtained from individual scores and calculated according to the formula: %MPE=(score after drug treatment-score before drug treatment) × 100/(peak score in control group treated with morphine at 10 mg/kg-score before drug treatment) and expressed as mean of %MPE ± S.E.M.

The 5-HT levels measured by HPCL-ED were calculated from a calibration curve using the formula: $Y=(X+1.021)/0.9796$ ($r=0.99$, limit of detection was 2.5 fmol). The 5-HT concentration is expressed as pmol of AUC for 5 microdialysate samples. The experimental data were statistically analyzed using Student's *t*-test. Probability values less than 0.05 were considered significant.

3. Results

3.1. Development of mechanical allodynia in SL and DM mice

Mechanical allodynia was detected in SL mice 10 days after surgery and in DM mice 2 weeks following i.v. administration of streptozotocin. In SL mice, the threshold to elicit the paw withdrawal response was reduced to 0.34 ± 0.03 and 0.106 ± 0.04 g in contralateral and ipsilateral paws, respectively, and was significantly lower than that in sham-operated mice ($P<0.01$) (Fig. 1(A)).

In DM mice, the threshold to elicit the paw withdrawal response was reduced to 0.24 ± 0.05 g, which was significantly lower than that in non-diabetic mice ($P<0.01$). (Fig. 2(A)).

3.2. Antinociceptive effects of morphine in SL mice

Fig. 1(B), morphine injected s.c. into groups of sham-operated mice at 3, 5 and 10 mg/kg produced an antinociceptive effect in a dose-dependent manner, 35.08 ± 5.6%, 57.87 ± 10% and 100 ± 0% of MPE for 3, 5 and 10 mg/kg, respectively. However, in groups of SL mice, %MPE was lower, 23.99 ± 4.5%, 26.55 ± 3.2% and 37.48 ± 7.2%, after s.c. administration of morphine at 3, 5 and 10 mg/kg, respectively.

3.3. Antinociceptive effects of morphine in DM mice

Fig. 2(B), in non-diabetic mice, morphine injected s.c. at 3, 5 and 10 mg/kg also produced antinociceptive effects in a dose-dependent manner, as indicated by %MPE of 34.99 ± 7.3%, 54 ± 7.3% and 100 ± 0% for 3, 5 and 10

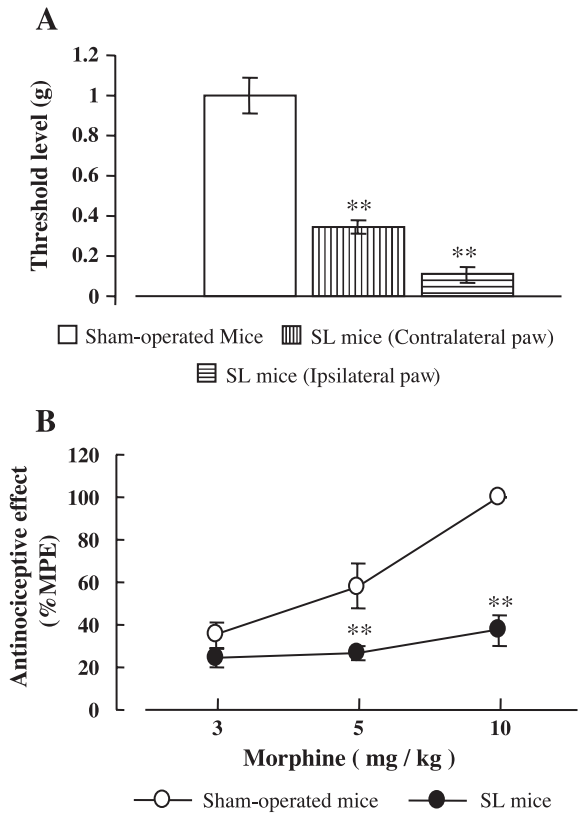

Fig. 1. Development of mechanical allodynia and antinociceptive effects of s.c. administration of morphine in NM and SLM. (A) Mechanical allodynia was estimated by application of von Frey filament to both hind paws 10 days after surgery. The results are expressed as means ± S.E.M., $^{**}P<0.01$ compared with Sham-operated mice ($n=9$). (B) Animals were treated with morphine at 3, 5 and 10 mg/kg, s.c., and antinociceptive effects were estimated by tail-pinch test. The results are expressed as the mean of %MPE ± S.E.M. $^{**}P<0.01$ compared with sham-operated mice ($n=6-7$).

mg/kg, respectively. However, in DM mice, the %MPE of morphine at 3 mg/kg was significantly lower than in non-diabetic mice (17.74 ± 3.19%), while the %MPE for morphine at 5 and 10 mg/kg was 51.26 ± 11% and 107 ± 0.07%, respectively.

3.4. 5-HT content in raphe magnus nucleus of SL and DM mice

After a recovery period of 3 days after insertion of the guide cannula, the stylet was replaced with a microdialysis probe, which was connected to a swivel to allow free movement of the mouse. The probe was perfused with CSF at a rate of 1 μl/min. Microdialysate samples were collected every 15 min for 75 min and immediately injected into the HPLC-ECD system. Data were used to calculate the AUC for microdialysate collected over 75 min. The 5-HT concentration in the raphe magnus nucleus was 1730 ± 132 pmol in sham-operated mice and 215 ± 33.2 pmol in SL mice ($^{**}P<0.01$). Meanwhile, the 5-HT concentration in DM mice was 496 ± 72.9 pmol, significantly less than the concentration of 1752 ± 166 pmol found in non-diabetic mice.

3.5. Effect of morphine on 5-HT content in raphe magnus nucleus of SL and DM mice

After the five baseline samples were collected, animals were injected s.c. with either morphine at 5 mg/kg or with saline. Neither morphine nor saline induced an alteration of 5-HT levels in control or neuropathy model mice. The AUCs for the samples collected over 75 min are shown in Fig. 3(B): (1-S) after saline injection in sham-operated mice, the AUC was 1980 ± 186 pmol, (1-M) after morphine injection in sham-operated mice, the AUC was 2495 ± 194 pmol; (2-S) after saline injection in SL mice, the AUC was 67 + 19.2 pmol; (2-M) after morphine injection in SL mice, the AUC was 135 ± 38.6 pmol; (3-S) after saline injection in non-diabetic mice, the AUC was 1580 ± 147 pmol; (3-M) after morphine injection in non-diabetic mice, the AUC was 1940 ± 82.2 pmol; (4-S) after saline injection in DM mice,

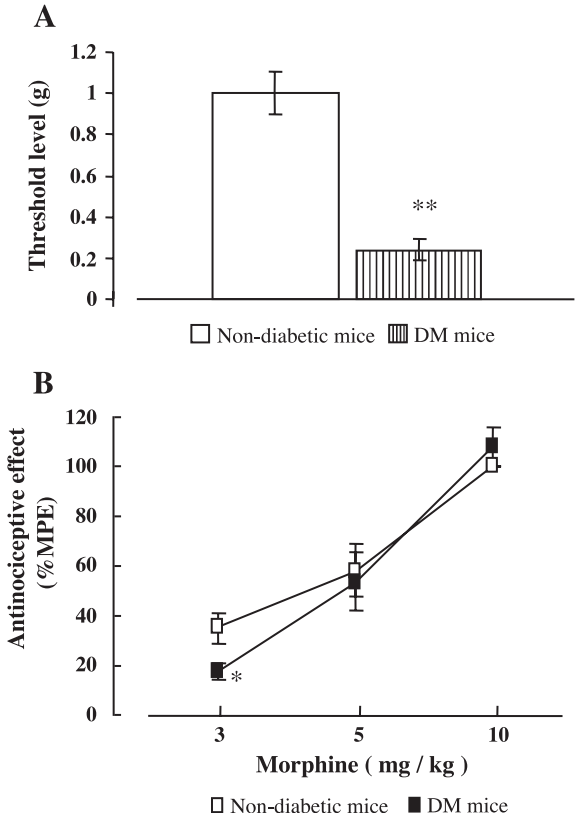

Fig. 2. Development of mechanical allodynia and antinociceptive effects of s.c. administration of morphine in Non-diabetic and DM mice. (A) Mechanical allodynia was estimated by application of von Frey filament to hind paw 2 weeks after i.v. injection of streptozotocin or vehicle. The results are expressed as means ± S.E.M., $^{**}P<0.01$ compared with non-diabetic mice ($n=12$). (B) Animals were s.c. treated with morphine at 3, 5 and 10 mg/kg, and antinociceptive effects were estimated by tail-pinch test. The results are expressed as the mean of %MPE ± S.E.M. $^{*}P<0.01$ compared with non-diabetic mice ($n=6-7$).

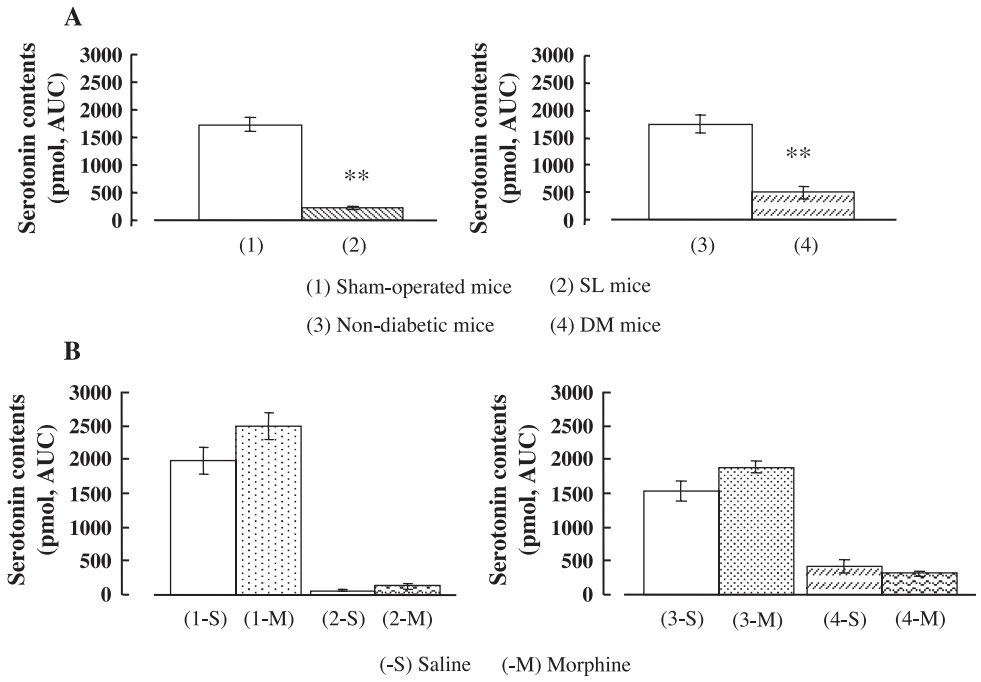

Fig. 3. 5-HT content in raphe magnus nucleus of SL and DM mice. (A) After induction of neuropathy, the 5-HT content was measured. Data are expressed as the AUC (in pmol) for 75 min and indicate means ± S.E.M. **$P < 0.01$, compared with 5-HT content in raphe magnus nucleus of control mice ($n = 7–8$). (B) 5-HT content after s.c. administration of saline or morphine in each group of mice, sham-operated mice treated with saline (1-S), with morphine (1-M); SL mice were treated with saline (2-S) or morphine (2-M); non-diabetic mice were treated with saline (3-S) or morphine (3-M); DM mice were treated with saline (4-S) or morphine (4-M). The values are expressed as the AUC (in pmol) for 75 min and indicate means ± S.E.M. ($n = 3–4$).

the AUC was 427 ± 96 pmol; and (4-M) after morphine injection in DM mice, the AUC was 327 ± 38.8 pmol.

4. Discussion

The results showed that morphine at all doses used in SL mice and at 3 mg/kg in DM mice produced weaker antinociceptive effects than in corresponding control mice, an effect which was related to reduction of raphe magnus nucleus 5-HT levels in these neuropathy models. It has been suggested that local stimulation and locally applied morphine produce effective analgesia in the rostral ventral medulla, including the raphe magnus nucleus (Nestler et al., 2001). Numerous evidence indicates that 5-HT is produced by several discrete brain stem nuclei, which appear in rostral and caudal clusters. The caudal nuclei comprise the raphe magnus and raphe pallidus (Nestler et al., 2001). Furthermore, it has been suggested that the 5-HT is the primary transmitter in the raphe magnus nucleus. Although many other transmitters are present in this area, such as γ-aminobutyric acid (GABA), glutamate, enkephalin, galanin, cholecystokinin and substance P, they are not necessarily co-localized with a primary transmitter in neurons involved in pronociception and antinociception (Mark, 2002).

Morphine given s.c. at 5 and 10 mg/kg produced antinociceptive effects in control and DM mice, but not in SL mice, showing that the antinociceptive effect of morphine is weaker in SL mice than in DM mice, suggesting that the systemic administration of morphine induced analgesia via different mechanisms in these mice models. Other studies suggested that the attenuated analgesic effect of morphine in nerve injury may be caused by a reduction in μ-opioid receptor density in the superficial laminae (I and II) of spinal dorsal horn neurons (DeGroot et al., 1997). These are the regions where nociceptive C- and Aδ-fibers of peripheral afferent nerves terminate (Light and Perl, 1977). Furthermore, Aβ-fibers of peripheral nerves do not normally contribute to nociceptive mediation, and there is weak expression of μ-opioid receptors mRNA in laminae III and IV, where Aβ-fibers terminate (Minami and Satoh, 1995). However, peripheral nerve injury could lead to contribution of Aβ-fibers to pain mediation, which may be associated with the attenuated analgesia induced by morphine in nerve injury. Another study has also revealed that after axotomy of peripheral nerves, the density of μ-opioid receptors was found to be decreased (DeGroot et al., 1997). The attenuated antinociceptive effect of morphine in DM may due to different mechanisms, e.g. the enhancement of intracellular calcium level in the spinal cord (Ohsawa and Kamei, 1999; Kamei et al., 2000). However, our finding shows that the decreased 5-HT content in the raphe magnus nucleus in DM and SL mice may also

support the explanation of attenuated analgesic effect of morphine in these mice. Further, another suggestion is that the diminished antinociceptive effect of morphine in streptozotocin-induced diabetic animals might be due, at least partly, to a decrease in 5-HT release from the spinal cord (Suh et al., 1996) and a reduced basal 5-HT release from the ventrobasal thalamus in animal models of neuropathic pain (Virginia et al., 2002).

Studies of the sites of opioid actions show that opioid analgesia can be produced from the periaqueductal gray and rostroventromedial medulla not only after electrical stimulation (Reynolds, 1969; Mayer et al., 1971; Gebhart, 1986), but also following local instillation of opioids (Jensen and Yaksh, 1986). In contrast, lesion of the raphe magnus nucleus prevents morphine-induced antinociception (Proudfit and Anderson, 1975; Yaksh et al., 1977; Chance et al., 1978; Proudfit, 1980). In addition, naloxone administered in the raphe magnus nucleus attenuates the antinociception produced by systemic administration of morphine or by electrical stimulation of the raphe magnus nucleus (Oliveras et al., 1977; Rivot et al., 1979).

Serotonergic cell bodies in the raphe magnus nucleus provide dense projections to the dorsal horn of the spinal cord, and this descending pathway has been shown to mediate the antinociceptive action of morphine (for review, see Fields et al., 1991; Gilbey and Stein, 1991). It has been reported that paracetamol significantly increases the 5-HT level in rat brain by 70%, and that pretreatment with *p*-chlorophenylalanine, a 5-HT receptor antagonist, reduces the 5-HT content in rat brain and this effect can be reversed by paracetamol (Luigi et al., 1996). Furthermore, another study has revealed that a reduced 5-HT level may also be implicated in patients affected by chronic daily headache (Paola et al., 2002). However, our data illustrate that s.c. injection of morphine at 5 mg/kg did not significantly alter 5-HT levels in control or mice with neuropathic. This suggests that an increase or a decrease in 5-HT release introduced by opioids would depend on the initial balance between GABAergic and glutamatergic input. Therefore, it is possible that the decreased 5-HT levels in the raphe magnus nucleus of the neuropathic pain mice may be due to a disturbance in this balance between GABAergic and glutamatergic input (Rui and Sidney, 2003). Furthermore, another study has also suggested that opiate enhances serotonergic activity in the brain, by increasing the turnover rate of 5-HT, without altering the overall concentration of neurotransmitter (Gorlitz and Frey, 1972).

In conclusion, this study shows that the analgesic effects of morphine are reduced in mice with neuropathic pain, increasing nociception to stimuli in association with a reduction of 5-HT levels in the raphe magnus nucleus. Therefore, 5-HT levels in the raphe magnus nucleus play a key role in inducing the analgesic effects of opioid receptor agonists. However, these agonists may not alter raphe magnus nucleus 5-HT levels in mice model of neuropathic pain.

Acknowledgements

The authors would like to thank Dr. Gwyn A Lord, University of London, for revising the manuscript and helpful suggestions.

References

Abdi, S., Lee, D.H., Chung, J.M., 1998. The anti-allodynic effects of amitriptyline, gabapentin, and lidocaine in a rat model of neuropathic pain. Anesth. Analg. 87, 1360–1366.

Arner, S., Meyerson, B.A., 1988. Lack of analgesic effect of opioids on neuropathic and idiopathic forms of pain. Pain 33, 11–24.

Bel, N., Artigas, F., 1993. Chronic treatment with fluvoxamine increases extracellular serotonin in frontal cortex but not in raphe nuclei. Synapse 15, 243–245.

Chance, W.T., Krynok, G.M., Rosecrans, J.A., 1978. Effects of medial raphe and raphe magnus lesions on the analgesic activity of morphine and methadone. Psychopharmacologia (Berlin) 56, 133–137.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53, 55–63.

DeGroot, J.F., Coggeshall, R.E., Carlton, S.M., 1997. The reorganization of μ opioid receptors in the rat dorsal horn following peripheral axotomy. Neurosci. Lett. 233, 113–116.

Esser, M.J., Sawynok, J., 1999. Acute amitriptyline in a rat model of neuropathic pain: differential symptom and route effects. Pain 80, 643–653.

Fields, H.L., Heinricher, M.M., Mason, P., 1991. Neurotransmitters in nociceptive modulatory circuits. Annu. Rev. Neurosci. 14, 219–245.

Gebhart, G.F., 1986. Modulatory effects of descending systems on spinal dorsal horn neurons. In: Yaksh, T.L. (Ed.), Spinal Afferent Processing. Plenum, New York, pp. 391–416.

Gilbey, M.P., Stein, R.D., 1991. Characteristics of sympathetic preganglionic neurones in the lumbar spinal cord of the cat. J. Physiol. 432, 427–443.

Gorlitz, B.D., Frey, H.H., 1972. Central monoamines and antinociceptive drug action. Eur. J. Pharmacol. 20, 171–180.

Jensen, T.S., Yaksh, T.L., 1986. III. Comparison of the antinociceptive action of mu and delta opioid receptor ligands in the periaqueductal gray matter, medial and paramedial ventral medulla in the rat as studied by the microinjection technique. Brain Res. 372, 301–312.

Jett, M.F., McGuirk, J., Waligora, D., Hunter, J.C., 1997. The effects of mexiletine, desipramine and fluoxetine in rat models involving central sensitization. Pain 69, 161–169.

Kamei, J., Kasuya, Y., 1995. The effects of diabetes on opioid-induced antinociception. In: Tseng, D. (Ed.), The Pharmacology of Opioid Peptides, vol. I. Harwood Academic Publishers, New York, NY, pp. 217–286.

Kamei, J., Kentaro, T., Masahiro, O., Hideki, H., 2000. Modulation of the formalin-induced nociceptive response by diabetes: possible involvement of intracellular calcium. Brain Res. 862, 257–261.

Keith, B.J., Franklin, G.P., 1997. The Mouse Brain in Stereotaxic Coordinates. Academic Press, San Diego, CA, USA, pp. 92101–94495.

Kreiss, D.S., Lucki, I., 1995. Effects of acute and repeated administration of antidepressant drugs on extracellular levels of 5-hydroxytryptamine measured in vivo. J. Pharmacol. Exp. Ther. 274, 866–876.

Light, A.R., Perl, E.R., 1977. Differential termination of large-diameter and small-diameter primary afferent fibres in the spinal dorsal grey matter as indicated by labelling with horseradish peroxidase. Neurosci. Lett. 6, 59–63.

Luigi, A.P., Maurizio, S., Giovanni, V., 1996. The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. Eur. J. Pharmacol. 308, 31–40.

MacFarlane, B.V., Wright, A., O'Callaghan, J., Benson, H., 1997.

Chronic neuropathic pain and its control by drugs. Pharmacol. Ther. 75, 1–19.
Mark, J.M., 2002. Descending control of pain. Prog. Neurobiol. 66, 355–474.
Mayer, D.J., Wolfle, T.L., Akil, H., Carder, B., Liebeskind, J.C., 1971. Analgesia from electrical stimulation in the brainstem of the rat. Science 174, 1351–1354.
Minami, M., Satoh, M., 1995. Molecular biology of the opioid receptors: structures, functions and distributions. Neurosci. Res. 23, 121–145.
Nestler, E.J., Hyman, S.E., Malenka, R.C., 2001. Serotonin, acetylcholine and histamine. Molecular Neuropharmacology, a Foundation for Clinical Neuroscience. McGraw-Hill, USA, pp. 191–200.
Ohsawa, M., Kamei, J., 1999. Role of intracellular calcium in thermal allodynia and hyperalgesia in diabetic mice. Brain Res. 833, 278–281.
Oliveras, J.L., Hosobuchi, Y., Redjemi, F., Guilbaud, G., Besson, J.M., 1977. Opiate antagonist, naloxone, strongly reduces analgesia induced by stimulation of a raphe nucleus (centralis inferior). Brain Res. 120, 221–229.
Paola, S., Andrea, A., Ardesio, F., Virgilio, G., 2002. L-Arginine/nitric oxide pathway in chronic tension-type headache: relation with serotonin content and secretion and glutamate content. J. Neurol. Sci. 98, 9–15.
Proudfit, H.K., 1980. Effects of raphe magnus and raphe pallidus lesions on morphine-induced analgesia and spinal cord monoamines. Pharmacol. Biochem. Behav. 13, 705–714.
Proudfit, H.K., Anderson, E.G., 1975. Morphine analgesia: blockade by raphe magnus lesions. Brain Res. 98, 612–618.
Reynolds, D.V., 1969. Surgery in the rat during electrical analgesia induced by focal brain stimulation. Science 164, 444–445.
Rivot, J.P., Chaouch, A., Besson, J.M., 1979. The influence of naloxone on the C fiber response of dorsal horn neurons and their inhibitory control by raphe magnus stimulation. Brain Res. 176, 355–364.
Rui, T., Sidney, B.A., 2003. Influence of inhibitory and excitatory inputs on serotonin efflux differs in the dorsal and median raphe nuclei. Brain Res. 961, 109–120.
Seltzer, Z., Dubner, R., Shir, Y., 1990. A novel behavioral model of neuropathic pain disorders produced in rats by partial sciatic nerve injury. Pain 43, 205–218.
Suh, H.-W., Song, D.-K., Wie, M.-B., Jung, J.-S., Hong, H.-E., Choi, S.-R., Kim, Y.-H., 1996. The reduction of antinociceptive effect of morphine administered intraventricularly is correlated with the decrease of serotonin release from the spinal cord in streptozotocin-induced diabetic rats. Gen. Pharmacol. 27, 445–450.
Takagi, H., Inukai, T., Nakama, M., 1966. A modification of Haffner's method for testing analgesics. Jpn. J. Pharmacol. 16, 287–294.
Virginia, M.G., Yan, H., Kevin, V.H., Robert Jr, L.S., 2002. Reduced basal release of serotonin from the ventrobasal thalamus of the rat in a model of neuropathic pain. Pain 99, 336–359.
Yaksh, T.L., Plant, R.L., Rudy, T.A., 1977. Studies on the antagonism by raphe lesion of the antinociceptive action of systemic morphine. Eur. J. Pharmacol. 41, 399–408.