Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 511 (2005) 199–206

www.elsevier.com/locate/ejphar

Differential respiratory effects of [Dmt[1]]DALDA and morphine in mice

Megumi Shimoyama[a,*], Tomoyuki Kuwaki[a,b], Akira Nakamura[a], Yasuichiro Fukuda[a], Naohito Shimoyama[c], Peter W. Schiller[d], Hazel H. Szeto[e]

[a]Department of Autonomic Physiology, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
[b]Department of Molecular and Integrative Physiology, Chiba University Graduate School of Medicine, Chiba, Japan
[c]Division of Pain Medicine and Palliative Care, National Cancer Center Hospital, Tokyo, Japan
[d]Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Canada
[e]Department of Pharmacology, Joan and Sanford I, Weill Medical College of Cornell University, New York, NY, USA

Received in revised form 7 December 2004; accepted 10 February 2005
Available online 17 March 2005

Abstract

H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]DALDA, dDAL), a highly selective mu-opioid peptide, produces potent analgesia without respiratory depression after intrathecal administration. Despite carrying 3+ net charge, dDAL is also a potent analgesic after systemic administration. We compared the respiratory effects of dDAL and morphine after subcutaneous administration in mice using whole body plethysmography. Analgesic doses of 3 and 10 times ED_{50} were examined. Both drugs dose-dependently decreased respiratory frequency and minute volume in room air. Tidal volume was increased by the lower dose of morphine, while it was decreased by the higher dose of dDAL. The decrease in minute volume by dDAL and morphine was completely reversed by naloxone. No difference in ventilatory response to CO_2 was observed between dDAL and morphine at three times ED_{50}. Ventilatory response to hypoxia was significantly diminished by dDAL compared to morphine and saline, and this effect of dDAL was naloxone-irreversible. Thus dDAL likely reduces the sensitivity of the peripheral chemoreflex loop through a non-opioid action.

Keywords: [Dmt[1]]DALDA; Morphine; Respiratory depression; Hypoxic respiratory response

1. Introduction

Opioids are potent analgesics widely used clinically but present side effects, the most serious being respiratory depression (O'Mahony et al., 2001; McNicol et al., 2003). Respiratory effects of opioids have been documented clinically and have also been demonstrated in basic studies (Morin-Surun et al., 1984; Stott and Pleuvry, 1991). Opioids are known to depress the respiratory center in the brain via the activation of mu and delta receptors (Santiago and Edelman, 1985; Kozaki et al., 2000). It has been reported that opioid receptors exist in rhythmogenic neurons in the pre-Botzinger complex of the ventrolateral medulla, which has been proposed to be the respiratory center (Gray et al., 1999). Morphine, although the most commonly used opioid, can produce early respiratory depression after systemic administration and late respiratory depression after intrathecal administration (O'Mahony et al., 2001; McNicol et al., 2003; Etches et al., 1989; Bailey et al., 1993). H-Dmt-D-Arg-Phe-Lys-NH_2 ([Dmt[1]]DALDA, dDAL), a dermorphin analog, is an opioid peptide highly selective to the mu receptor (Schiller et al., 2000). We showed that dDAL given spinally produces markedly less late respiratory depression compared to morphine in the rat at equipotent doses (Shimoyama et al., 2001). This was probably because its spinal analgesic effects were partially attributed to mechanisms other than mu receptor activation, such as norepi-

* Corresponding author. Tel.: +81 43 226 2030; fax: +81 43 226 2034.
E-mail address: shimoyama@faculty.chiba-u.jp (M. Shimoyama).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.014

nephrine reuptake inhibition and dynorphin release in the spinal cord, which likely acted synergistically with mu receptor activation to produce analgesia (Shimoyama et al., 2001; Szeto et al., 2003). Because dDAL has a low propensity to produce respiratory depression after intrathecal administration, it may be a candidate for a safe spinal opioid in patients.

dDAL is highly polar because it carries a 3+ net charge at physiologic pH (Schiller et al., 2000), and was not expected to cross the blood–brain barrier. However, we found that after subcutaneous (s.c.) administration, dDAL produced potent and long-lasting antinociceptive effects, and the effects were largely attributed to its effects in the central nervous system (Zhao et al., 2002). Furthermore, recent studies have shown that dDAL is readily cell-permeable (Zhao et al., 2003a) and rapidly crosses the blood–brain barrier after intravenous administration (unpublished results). The present study was designed to compare the respiratory effects of dDAL and morphine after subcutaneous administration. We utilized flow-through whole body plethysmography in order to continuously record respiratory variables in awake, unrestrained mice. In addition to recording ventilation of mice breathing room air, we compared the effects of dDAL and morphine on respiratory responses to hypoxia and hypercapnia. Surprisingly, we found a significant difference between the ventilatory effects of dDAL and morphine. Although there was no difference between the effects of dDAL and morphine on ventilatory response in room air or in response to hypercapnia, only dDAL inhibited ventilatory response to hypoxia. Furthermore, we found that the effect of dDAL on hypoxic respiratory response was not antagonized by the opioid antagonist, naloxone.

2. Materials and methods

Experiments adhered to the European Community guidelines for the use of experimental animals and were approved by the Institutional Animal Use Committee of Chiba University Graduate School of Medicine.

2.1. Animals and drugs

Adult male C57B/6 mice (25–30 g) were obtained from Charles River Japan. dDAL was synthesized by methods described elsewhere (Schiller et al., 2000). Morphine hydrochloride and naloxone hydrochloride were obtained from Takeda Pharmaceuticals (Osaka, Japan) and Sigma (St. Louis, MO, USA), respectively. All drugs were dissolved in saline. Drugs or saline were delivered s.c. at a volume of 0.1 ml/10 g mouse weight.

2.2. Whole body plethysmography

For respiratory measurement, we used double-chamber, flow-through whole body plethysmography as previously described (Onodera et al., 1997; Nakamura et al., 2003). This allowed continuous flow of fresh gas into the system. Thus continuous measurement of respiratory variables was possible in unanesthesized unrestrained mice without unintended decrease in oxygen or accumulation of carbon dioxide inside the chamber. Experiments were conducted at room temperature of 24–25 °C. Chamber temperature and humidity and ambient pressure were recorded during the experiment. Rectal temperature of each animal was measured at the end of the experiment. Plethysmographic signals were recorded as changes in pressure difference between the barometric chamber and the reference chamber. The signals were amplified and fed into a computer, and the amplitude and frequency (respiratory frequency) of the signals were analyzed using the Chart software (AD Instruments, Colorado Springs, USA). Tidal volume was calculated according to the equation proposed by Epstein et al. (1980). Minute volume was defined as the product of respiratory frequency and tidal volume. Active movements of the mouse (e.g., exploring behaviors) inside the chamber resulted in artifacts in the plethysmographic signals (active periods); thus periods without movement artifacts (quiet periods) were analyzed (see below). We have shown that data obtained by this method were consistent with data obtained by direct plethysmography and pneumotachography (Onodera et al., 1997).

2.3. Study 1: time course of respiratory effects

Each mouse was allowed a 1-h acclimatization period inside the barometric chamber prior to the study. Air was used as the fresh gas that was delivered into the chamber. At the end of the acclimatization period, prior to administration of any drug, baseline plethysmographic recordings were made for 10 min. Following baseline recordings, the mouse was taken out of the chamber and dDAL at a dose of 0.48 or 1.6 μmol/kg (3 or 10 times ED_{50} value for analgesia, respectively), morphine at a dose of 17.0 or 56.6 μmol/kg (3 or 10 times ED_{50} for analgesia, respectively), or saline was administered s.c. The analgesic ED_{50} values for both drugs were obtained in a previous study in mice (Zhao et al., 2002). The mouse was returned to the chamber and continuous recordings were made for the next 12.5 h. Recordings were analyzed at each hour post-injection. A period between 5 min prior to the hour and 5 min after the hour (total of 10 min) was set as the analysis period of the hour. Since only quiet states could be analyzed (see above), three 20-s quiet periods immediately following active periods within the 10-min analysis period were analyzed. The average respiratory frequency, tidal volume, and minute volume of each 20-s quiet period were calculated, and the means of the three values obtained for each parameter were designated as the respiratory frequency, tidal volume, and minute volume values of the

hour. When more than three 20-s quiet periods existed within an analysis period, the three closest to the hour were analyzed. Baseline values were obtained by the analyses of the last three quiet periods during baseline recording. Post-injection values were expressed as percentage of baseline values.

2.4. Study 2: effect on body temperature

Since measurement of rectal temperature of a mouse during the above experiment would excite the mouse, resulting in changes in ventilation and continuous active movement, we only measured the temperature of the mouse at the end of the experiment and used the data in the calculation of tidal volume. However, at this time point, the effect of the administered drugs had already disappeared. Systemic administration of opioids may cause decrease in body temperature, which will result in calculated tidal volumes of smaller values than the actual tidal volumes. Thus, to examine the possibility of change in body temperature and to determine the extent of its effect, in a separate study, we measured the changes in body temperature after the administration of dDAL and morphine. Rectal temperature was measured prior to and after the administration of dDAL or morphine (10 times ED_{50}) at the time of peak effect of each drug (2 h and 1 h post-injection, respectively) determined from the above study. In the saline group, post-administration temperature was measured at both time points. The experiment was conducted at room temperature of 24–25 °C.

2.5. Study 3: effects on respiratory responses to hypercapnia and hypoxia

Effects of dDAL and morphine at three times ED_{50} on respiratory responses to hypercapnic (5% CO_2) and hypoxic (15% O_2) challenges were examined. Following acclimatization and baseline recording, dDAL, morphine, or saline was administered s.c. and recording was restarted. The start of a testing period was set at 2 h after drug administration for dDAL and 1 h for morphine. These time points were determined from the peak effects of the drugs obtained in study 1. The first 10-min period of the testing period was designated as the pre-hypercapnic challenge period. At the end of this period, the fresh gas flow was switched from air to a gas mixture of 5% CO_2/21% O_2 in N_2 base. Five minutes were allowed for the gas in the chamber to be completely displaced. The next 5 min was designated as the hypercapnic challenge period. Following the hypercapnic challenge, fresh gas flow was switched back to air and 20 min was allowed to pass. The latter 10 min of this period was designated as the pre-hypoxic challenge period. Next, the fresh gas flow was switched to a gas mixture of 15% O_2 and 85% N_2. Following a 5-min period for displacement, a hypoxic challenge period was recorded for the next 5 min. Last three quiet periods during the pre-challenge periods were analyzed in the same manner as in the study above. Since the animals were not actively moving during challenge periods, the first 20 s of the first, second, and third 1-min periods of each challenge period were analyzed. The mean of the three minute volume values of the pre-challenge period and that of the challenge period were calculated.

2.6. Study 4: effects of naloxone

Effects of dDAL and morphine at 10 times ED_{50} on respiratory response to hypoxic challenge (10% O_2) in the presence of naloxone were examined. Mice were assigned to one of four groups. Following acclimatization and baseline recordings, mice of one group were given dDAL, those of another group were given morphine, and those of the remaining two groups were given saline. Each mouse was returned to the barometric chamber and recording was restarted. Mice that had been given morphine received an injection of naloxone at 10 mg/kg, s.c. at 1 h after the first injection (MOR–NAL group). Mice that had received dDAL and those in one of the groups that had received saline were given naloxone at 10 mg/kg, s.c. (dDAL–NAL group and SAL–NAL group, respectively) at 2 h after the first injection. Mice of the remaining group that had received saline were given saline again at 2 h after the first injection (SAL–SAL group). A 10-min period prior to the second injection was designated as the post-first drug analysis period. Again, each mouse was returned to the barometric chamber and recording was restarted. Twenty minutes after the second injection, a hypoxic challenge was initiated by switching the fresh gas flow from air to a gas mixture of 10% O_2 and 90% N_2. A 10-min period prior to the challenge was designated as the post-second drug (pre-challenge) analysis period. Five minutes were allowed for the gas in the chamber to be completely displaced. The next 5 min were designated as the hypoxic challenge period. The timeline of the experiment was determined by a preliminary study that showed that the depressant effects of the same doses of morphine and dDAL on minute volume were completely reversed by 10 mg/kg naloxone during a period of 10–30 min post-injection. Three 20-s quiet periods during baseline recording, the post-first drug, and post-second drug (pre-challenge) periods, and the hypoxic challenge period were analyzed as above. The mean of the three minute volume values for each period was calculated.

2.7. Statistical analysis

Data were analyzed using one-way or two-way analysis of variance (ANOVA) for repeated measures and post-hoc analysis by Student–Newman Keuls test, unless stated otherwise. A P value less than 0.05 was considered significant.

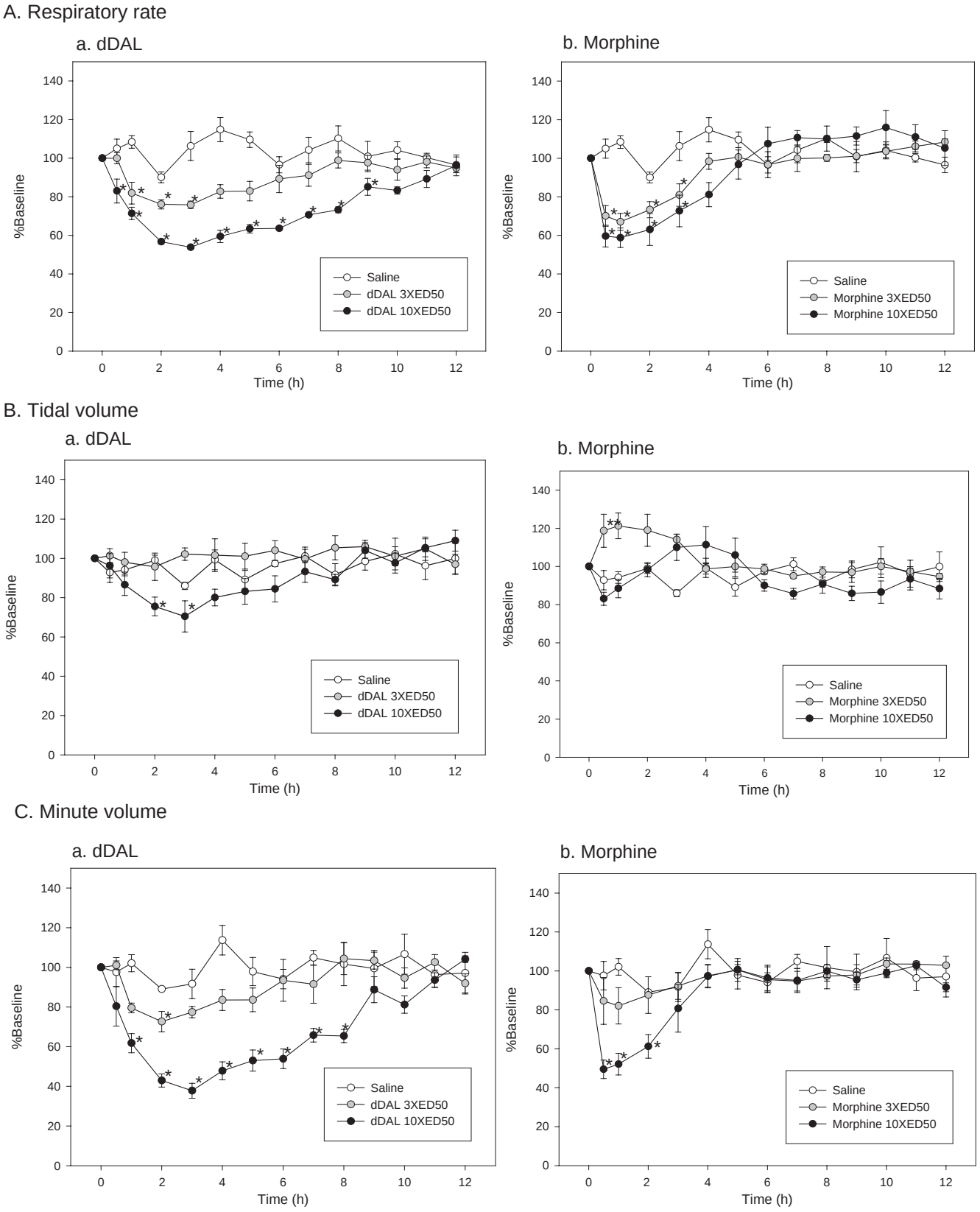

Fig. 1. Time courses of the effects of dDAL and morphine at equipotent analgesic doses (3 or 10 times ED_{50}) or saline on respiratory frequency, tidal volume, and minute volume in mice breathing room air. All values are expressed as percentage of baseline values and are shown as mean±S.E. *Significantly different from saline control.

3. Results

3.1. Time course of respiratory effects of dDAL and morphine

Respiratory frequency after dDAL administration was significantly and dose-dependently decreased compared to saline administration (Fig. 1A-a). The effect peaked at 2–3 h after administration and lasted 9 h with the higher dose. Tidal volume was significantly decreased only after the higher dose of dDAL (Fig. 1B-a). Minute volume was dose-dependently decreased by dDAL (Fig. 1C-a). Morphine also dose-dependently decreased respiratory frequency (Fig. 1A-b), with the effect peaking at 1 h after administration and lasting 3 h. On the other hand, morphine at the lower dose did not decrease tidal volume, but rather, tidal volume was increased during the first 2 h when the decrease of respiratory frequency was the most prominent (Fig. 1B-b). At the higher dose, no significant change in tidal volume was observed. Minute volume was significantly decreased by morphine at the higher dose but not by the lower dose (Fig. 1C-b). The minute volume values at peak effect after the administration of three times ED_{50} of dDAL (2 h post-injection) and morphine (1 h post-injection) were not significantly different from each other. Similarly, minute volume values at peak effect after the administration of 10 times ED_{50} of dDAL (3 h post-injection) and morphine (0.5 h post-injection) were not significantly different from each other. These comparisons were made by Student's *t* tests.

3.2. Effects of dDAL and morphine on body temperature

No significant decrease in rectal temperature resulted from the administration of dDAL and morphine at doses of 10 times ED_{50} (Table 1).

3.3. Effects of dDAL and morphine on hypercapnic and hypoxic respiratory responses

Minute volume increased in response to a 5% CO_2 challenge in mice given saline or three times the ED_{50} doses of dDAL or morphine. The minute volume values during hypercapnic challenges were not different among the three groups (Fig. 2A). In response to 15% O_2, minute volume increased in mice given saline or morphine (three times ED_{50}). In contrast, mice given dDAL (three times ED_{50}) showed no increase in minute volume in response to 15% O_2 (Fig. 2B). The minute volume of the dDAL group during hypoxic challenge was significantly smaller than those of the saline and morphine groups.

Table 1
Effect of dDAL and morphine on rectal temperature

	Baseline (°C)	1 h (°C)	2 h (°C)
Saline (*n*=6)	37.0±0.23	36.6±0.16	36.7±0.13
dDAL (*n*=6)	37.3±0.19	–	37.1±0.23
Morphine (*n*=6)	36.9±0.34	36.7±0.43	–

Data are shown as mean±S.E. No significant difference was present between the saline group and the dDAL group at baseline and 2 h post-injection, and between the saline group and the morphine group at baseline and 1 h post-injection.

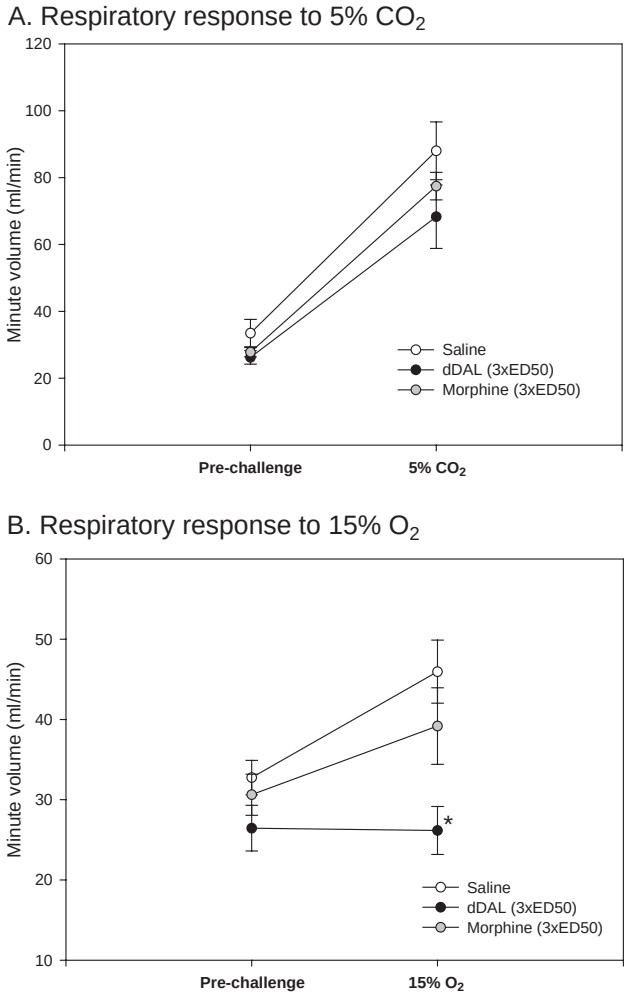

Fig. 2. Respiratory response of mice to 5% CO_2 challenge (A) and 15% O_2 challenge (B) after the administration of dDAL or morphine at three times ED_{50} for analgesia, or saline. Minute volumes are shown as mean+S.E. The number of animals in each group is six. *Significantly different from saline control and morphine groups.

3.4. Non-opioid effects of dDAL on hypoxic respiratory response

In room air, minute volume values of mice given dDAL or morphine at 10 times ED_{50} were significantly decreased compared to those given saline but were not different from each other (Table 2). After the injection of naloxone, minute volumes of both groups (dDAL–NAL and MOR–NAL groups) were not different from saline control (SAL–SAL group). This indicated that the respiratory depressant effects of dDAL and morphine under room air condition were completely reversed by naloxone. Naloxone by itself (SAL–NAL group) did not affect minute volume in room air or the

Table 2
Minute volume of mice given saline, dDAL, morphine, and/or naloxone in room air or 10% oxygen

First drug–second drug	Baseline (ml/min)	Post-first drug (ml/min)	Post-second drug (ml/min)	10% O_2 (ml/min)
SAL–SAL group (n=6)	34.9±1.4	34.1±1.2	35.4±1.7	65.7±5.7
SAL–NAL group (n=6)	33.2±2.1	32.1±1.5	32.4±1.6	63.1±4.8
dDAL–NAL group (n=7)	32.6±1.5	13.6±1.4[a]	34.6±1.8	48.7±2.5[b]
MOR–NAL group (n=6)	34.4±1.8	15.8±0.7[a]	34.7±1.3	64.4±3.8

SAL=saline; dDAL=[Dmt[1]]DALDA; MOR=morphine; NAL=naloxone. [Dmt[1]]DALDA and morphine were given at equipotent doses of 10 times analgesic ED_{50}. Minute volumes are shown as mean±S.E.
[a] Significantly different from SAL–SAL and SAL–NAL groups.
[b] Significantly different from SAL–SAL, SAL–NAL, and MOR–NAL groups.

hypoxic response. The hypoxic response in the MOR–NAL group was not different from control groups (SAL–SAL and SAL–NAL groups). In contrast, mice in the dDAL–NAL group had a significantly lower response to hypoxia as compared to the control groups.

4. Discussion

In the present study, we examined the respiratory effects of equipotent analgesic doses (3 times ED_{50} and 10 times ED_{50}) of dDAL and morphine after subcutaneous administration. We demonstrated that dDAL dose-dependently decreased respiratory frequency and, at the higher dose, also decreased the tidal volume of unanesthesized, unrestrained mice. The result was a decrease of approximately 60% in minute volume at the higher dose. Decrease in body temperature was unlikely a factor since rectal temperature did not change at peak effect of the drug (Table 1). The duration of the respiratory depression was 8–9 h and reflected the prolonged action of dDAL after s.c. administration as previously reported (Zhao et al., 2002). Morphine at equipotent doses similarly decreased respiratory frequency in a dose-dependent manner. However, in contrast to the action of dDAL on tidal volume, we observed an increase in tidal volume at the lower dose of morphine. This increase in tidal volume was likely a compensatory effect to preserve minute ventilation and/or prevent hypoxemia, and was lost at the higher dose of morphine. Minute volume at peak effect of either dose of morphine was not different from the equipotent dose of dDAL, although the onset and duration of effect were much shorter with morphine than with dDAL. We have shown that the elimination half-life of dDAL is four times longer compared to morphine (Szeto et al., 2001).

We previously reported that dDAL administered intrathecally showed a lower propensity to produce late respiratory depression compared to morphine in rats (Shimoyama et al., 2001). This was likely due to the very low dose of dDAL required to produce equipotent analgesia. In fact, the potency of spinal dDAL was 3000 times that of spinal morphine (Shimoyama et al., 2001), while the affinity to the mu receptor was only 34.6 times that of morphine (Zhao et al., 2003b). The extraordinary high potency of dDAL was likely a result of other actions of dDAL such as norepinephrine reuptake inhibition and the release of dynorphin that enhanced the mu analgesic effect in the spinal cord (Shimoyama et al., 2001; Szeto et al., 2003). Thus the proportion of direct mu opioid effect contributing to the analgesic effect was markedly smaller for dDAL than morphine, and thus spinal dDAL produced less opioid respiratory depression than morphine at equipotent doses. In the present study, we found that systemic administration of dDAL produced a similar decrease in minute volume as morphine at equipotent doses in mice breathing room air. This respiratory depression by dDAL observed in room air was due to opioid activity, since it was completely reversed by naloxone (Table 2). These findings imply that the analgesic effect of systemic dDAL is largely attributed to actions via opioid receptors and that enhancement by non-opioid systems that was suggested to occur in spinal dDAL analgesia seems to play a negligible role.

On the other hand, results of the hypercapnic and hypoxic challenge tests revealed a specific difference between dDAL and morphine. After the administration of dDAL or morphine at the dose of three times ED_{50}, the minute volume values prior to hypercapnic or hypoxic challenge were not significantly different from each other or from that of saline. Minute volume increased in response to 5% CO_2 in all three groups and was not different among the groups. In contrast, in response to hypoxia of 15% O_2, minute volume was significantly lower in mice given dDAL than those given morphine, which was not different from saline control. These data indicated that dDAL specifically suppressed the response to hypoxia at this lower dose. Effects of the higher doses (10 times ED_{50}) of the opioids were not examined because, at these doses, there was a marked decrease in minute volume compared to saline, which would likely have resulted in hypercapnia and hypoxemia prior to the challenge tests confounding the results.

Such specificity made us suspect that the decrease in hypoxic response we observed after dDAL administration was not due to depressant opioid actions of dDAL on the respiratory center in the brain, since the respiratory center was responsive to CO_2 challenge under the influence of dDAL at the lower dose. To test whether the effect of dDAL on hypoxic response was due to a non-opioid action, we examined the effect of the opioids on hypoxic response in the presence of naloxone. A higher dose (10 times ED_{50}) of

dDAL or morphine and a higher level of hypoxia than in study 2 were used to make the possible effect clearer. After the administration of dDAL or morphine at the dose of 10 times ED_{50}, the minute volume values prior to the administration of naloxone were not significantly different from each other but were significantly lower than those after saline. The administration of naloxone increased the minute volume values of mice given dDAL (dDAL+naloxone) or morphine (morphine+naloxone) back to baseline level so that they were not different from saline control. However, the respiratory response to 10% O_2 assessed by increase in minute volume in mice given dDAL+naloxone was significantly diminished, while the response in mice given morphine+naloxone was not different from control. These results indicate that the depressant effect of dDAL on hypoxic response was due to a non-opioid action that was not present in morphine.

A likely site of this non-opioid action of dDAL would be the carotid body, which responds to hypoxia by stimulating ventilation. The mechanism by which dDAL may inhibit carotid body response to hypoxia is not known. We have shown that dDAL inhibits norepinephrine uptake (Shimoyama et al., 2001). Dopamine and norepinephrine are released from the carotid body glomus cells in response to hypoxia (Gonzalez et al., 1994; Fidone et al., 1997; Eyzaguirre and Abudara, 1999), but their definite roles in the sensory discharges of the carotid sinus nerve remain unclear. Catecholamines also act at receptors on glomus cells and inhibit chemical secretion (Benot and Lopez-Barneo, 1990; Almaraz et al., 1997). Thus one possible mechanism by which dDAL may produce suppressive effects on the carotid body is through its inhibitory effects on norepinephrine reuptake. Although there are multiple theories of how the carotid body senses hypoxia, the mitochondria in glomus cells may play a central role in oxygen sensing. Hypoxia, which affects oxidative phosphorylation, causes mitochondrial depolarization (Duchen and Biscoe, 1992a,b; Roy et al., 2002). By inhibiting the activity of cytochrome oxidase (cyt aa3), hypoxia also leads to increased mitochondrial generation of reactive oxygen species (ROS) (Semenza, 1999). These conditions favor the opening of the permeability transition pore in mitochondria, with release of mitochondrial Ca^{2+} (Hunter et al., 1976). This release of intracellular Ca^{2+} stores can lead to influx of Ca^{2+}, and the rapid rise in cytoplasmic Ca^{2+} concentration will result in neurosecretion and sensory excitation. We recently reported that dDAL targets mitochondria, scavenges ROS, and is very effective in preventing permeability transition (Zhao et al., 2004). The suppressive action of dDAL on mitochondrial permeability transition, if exerted on carotid body cells, may result in the attenuation of the carotid body response to hypoxia. Such effects of dDAL on the mitochondria are also likely to be protective against hypoxic damage to cells. dDAL has been shown to have significant cardioprotective actions against reperfusion injury (Wu et al., 2002).

In summary, systemic administration of dDAL resulted in opioid receptor-mediated dose-dependent depression of ventilation. Although the effect was long-lasting compared to morphine, this reflected the duration of action of the drug, since the analgesic effect of dDAL is four times longer compared to morphine. Similar precautions regarding respiratory depression should therefore be taken with the systemic use of dDAL as with any other opioid analgesic. In addition, dDAL attenuated the ventilatory response to hypoxia through a non-opioid action. The inhibition of the peripheral chemoreflex does not appear to have a significant impact on the respiratory effect of dDAL under normal conditions since the reduced minute volume observed in animals breathing room air was completely reversed by naloxone. Nonetheless, it does have significance in hypoxic conditions and may be of concern in such clinical situations. The relationship between dDAL's protective effects on the mitochondria and the suppressive effect on hypoxic respiratory response is very much unclear, and further studies are needed to determine the overall effects of dDAL during hypoxia. On the other hand, reduced sensitivity of the peripheral chemoreflex loop may have a stabilizing influence on ventilation, and this may have clinical significance in the treatment of sleep apneas, particularly in those cases that are associated with an increased ventilatory sensitivity to oxygen (Khoo et al., 1991; Longobardo et al., 1982). The decrease in hypoxic sensitivity by acetazolamide, mediated by an inhibitory effect on the carotid bodies, has been associated with beneficial effects in some patients with sleep-disordered breathing (Teppema and Dahan, 2004; Younes et al., 2001). Thus dDAL and preferably dDAL analogs that lack opioid activity may have beneficial effects in patients with sleep-disordered breathing.

Acknowledgments

This study was supported in part by a Grant-in-Aid for Scientific Research (15591617) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan (M.S.); a Grant-in-Aid for Cancer Research (Study Group on Palliative Care and Psycho-Oncology in Cancer Treatment) from the Ministry of Health and Welfare, Japan (N.S.); and a Multi-Center Program Project Grant (DA08924) from the National Institute on Drug Abuse, NIH, USA (H.H.S. and P.W.S.).

References

Almaraz, L., Perez-Garcia, M.T., Gomez-Nino, A., Gonzalez, C., 1997. Mechanisms of α_2-adrenoceptor-mediated inhibition in rabbit carotid body. Am. J. Physiol. 272, C628–C637 (Cell Physiol. 41).

Bailey, P.L., Rhondeau, S., Schafer, P.G., Lu, J.K., Timmins, B.S., Foster, W., Pace, N.L., Stanley, T.H., 1993. Dose-response pharmacology of intrathecal morphine in human volunteers. Anesthesiology 79, 49–59.

Benot, A.R., Lopez-Barneo, J., 1990. Feedback inhibition by Ca^{2+} currents by dopamine in glomus cells of the carotid body. Eur. J. Neurosci. 2, 802–812.

Duchen, M.R., Biscoe, T.J., 1992a. Mitochondrial function in type I cells isolated from rabbit arterial chemoreceptors. J. Physiol. 450, 13–31.

Duchen, M.R., Biscoe, T.J., 1992b. Relative mitochondrial membrane potential and $[Ca^{2+}]$I in type I cells isolated from the rabbit carotid body. J. Physiol. 450, 33–61.

Epstein, R.A., Epstein, M.A., Haddad, G.G., Mellins, R.B., 1980. Practical implementation of the barometric method for measurement of tidal volume. J. Appl. Physiol. 49, 1107–1115.

Etches, R.C., Sandler, A.N., Daley, M.D., 1989. Respiratory depression and spinal opioids. Can. J. Anaesth. 36, 165–185.

Eyzaguirre, C., Abudara, V., 1999. Carotid body glomus cells: chemical secretion and transmission (modulation?) across cell–nerve ending junctions. Respir. Physiol. 115, 135–149.

Fidone, S.J., Gonzalez, C., Almaraz, L., Dinger, L., 1997. Cellular mechanisms of peripheral chemoreceptor function. In: Crystal, R.J., West, J.B., Barnes, P.J., Weibel, E.R. (Eds.), The Lung. Lippincott-Raven, Philadelphia, PA, pp. 1725–1746.

Gonzalez, C., Almaraz, L., Obeso, A., Rigual, R., 1994. Carotid body chemoreceptors: from natural stimuli to sensory discharges. Physiol. Rev. 74, 829–898.

Gray, P.A., Rekling, J.C., Bocchiaro, C.M., Feldman, J.L., 1999. Modulation of respiratory frequency by peptidergic input to rhythmogenic neurons in the preBotzinger complex. Science 286, 1566–1568.

Hunter, D.R., Haworth, R.A., Southard, J.H., 1976. Relationship between configuration, function, and permeability in calcium-treated mitochondria. J. Biol. Chem. 251, 5069–5077.

Khoo, M.C.K., Gottschalk, A., Pack, A.I., 1991. Sleep-induced periodic breathing and apnea: a theoretical study. J. Appl. Physiol. 70, 2014–2024.

Kozaki, Y., Tadaki, E., Kumazawa, T., 2000. Morphine inhibits resting respiration, but it attenuates reflexive respiratory suppression in anesthetized cat through kappa-receptor. Jpn. J. Physiol. 50, 615–624.

Longobardo, G.S., Gothe, B., Goldman, M.D., Cherniack, N.S., 1982. Sleep apnea considered as a control system instability. Respir. Physiol. 50, 311–333.

McNicol, E., Horowicz-Mehler, N., Fisk, R.A., Bennett, K., Gialeli-Goudas, M., Chew, P.W., Lau, J., Carr, D., 2003. American Pain Society. Management of opioid side effects in cancer-related and chronic noncancer pain: a systematic review. J. Pain 4, 231–256.

Morin-Surun, M.P., Boudinot, E., Gacel, G., Champagnat, J., Roques, B.P., Denavit-Saubie, M., 1984. Different effects of mu and delta opiate agonists on respiration. Eur. J. Pharmacol. 98, 235–240.

Nakamura, A., Fukuda, Y., Kuwaki, T., 2003. Sleep apnea and effect of chemostimulation on breathing instability in mice. J. Appl. Physiol. 94, 525–532.

O'Mahony, S., Coyle, N., Payne, R., 2001. Current management of opioid-related side effects. Oncology (Huntingt.) 15, 61–73.

Onodera, M., Kuwaki, T., Kumada, M., Masuda, Y., 1997. Determination of ventilatory volume in mice by whole body plethysmography. Jpn. J. Physiol. 47, 317–326.

Roy, A., Li, J., Al-Mehdi, A.-B., Mokashi, A., Lahiri, S., 2002. Effect of acute hypoxia on glomus cell E_m and ψ_m as measured by fluorescence imaging. J. Appl. Physiol. 93, 1987–1998.

Santiago, T.V., Edelman, N.H., 1985. Opioids and breathing. J. Appl. Physiol. 59, 1675–1685.

Schiller, P.W., Nguyen, T.M., Berezowska, I., Depuis, S., Weltrowska, G., Chung, N.N., Lemieux, C., 2000. Synthesis and in vitro opioid activity profiles of DALDA analogues. Eur. J. Med. Chem. 35, 895–901.

Semenza, G.L., 1999. Perspectives on oxygen sensing. Cell 98, 281–284.

Shimoyama, M., Shimoyama, N., Zhao, G.-M., Schiller, P.W., Szeto, H.H., 2001. Antinociceptive and respiratory effects of intrathecal H-Tyr-D-Arg-Phe-Lys-NH_2 (DALDA) and [Dmt^1]DALDA. J. Pharmacol. Exp. Ther. 297, 364–371.

Stott, D.G., Pleuvry, B.J., 1991. Relationship between analgesia and respiratory depression for mu opioid receptor agonists in mice. Br. J. Anaesth. 67, 603–607.

Szeto, H.H., Lovelace, J.L., Fridland, G., Soong, Y., Fasolo, J., Wu, D., Desiderio, D.M., Schiller, P.W., 2001. In vivo pharmacokinetics of selective mu-opioid peptide agonists. J. Pharmacol. Exp. Ther. 298, 57–61.

Szeto, H.H., Soong, Y., Wu, D., Qian, X., Zhao, G.-M., 2003. Endogenous opioid peptides contribute to antinociceptive potency of intrathecal [Dmt^1]DALDA. J. Pharmacol. Exp. Ther. 305, 696–702.

Teppema, L.J., Dahan, A., 2004. Low-dose acetazolamide reduces the hypoxic ventilatory response in the anesthetized cat. Respir. Physiol. Neurobiol. 140, 43–51.

Wu, D., Soong, Y., Zhao, G.-M., Szeto, H.H., 2002. A highly potent peptide analgesic that protects against ischemia–reperfusion-induced myocardial stunning. Am. J. Physiol., Heart Circ. Physiol. 283, H783–H791.

Younes, M., Ostrowski, M., Thompson, W., Leslie, C., Shewchuk, W., 2001. Chemical control stability in patients with obstructive sleep apnea. Am. J. Respir. Crit. Care Med. 163, 1181–1190.

Zhao, G.M., Wu, D., Soong, Y., Shimoyama, M., Berezowska, I., Schiller, P.W., Szeto, H.H., 2002. Profound spinal tolerance after repeated exposure to a highly selective μ-opioid peptide agonist: role of δ-opioid receptors. J. Pharmacol. Exp. Ther. 302, 188–196.

Zhao, K., Luo, G., Zhao, G.M., Schiller, P.W., Szeto, H.H., 2003a. Transcellular transport of a highly polar 3+ net charge opioid tetrapeptide. J. Pharmacol. Exp. Ther. 304, 425–432.

Zhao, G.M., Qian, X., Schiller, P.W., Szeto, H.H., 2003b. Comparison of [Dmt^1]DALDA and DAMGO in binding and G protein activation at μ, δ, and κ opioid receptors. J. Pharmacol. Exp. Ther. 307, 947–954.

Zhao, K., Zhao, G.M., Wu, D., Soong, Y., Birk, A.V., Schiller, P.W., Szeto, H.H., 2004. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. J. Biol. Chem. 279, 34682–34690.