ELSEVIER

European Journal of Pharmacology 392 (2000) 157–161

ejp

www.elsevier.nl/locate/ejphar

Short communication

Analysis of sex and gonadectomy differences in β-endorphin antinociception elicited from the ventrolateral periaqueductal gray in rats

Eliza K. Krzanowska, Richard J. Bodnar *

Department of Psychology and Neuropsychology Doctoral Sub-Program, Queens College, CUNY, 65-30 Kissena Blvd., Flushing, NY 11367, USA

Received 15 December 1999; received in revised form 26 January 2000; accepted 1 February 2000

Abstract

Male rats exhibit significantly greater antinociception following central administration of morphine than female rats. The present study examined potential differences in β-endorphin (5.2–26 μg) antinociception elicited from the ventrolateral periaqueductal gray in adult sham-operated and gonadectomized male and female rats. Male rats displayed significantly greater peak (30 min) tail-flick latencies across the entire range of β-endorphin doses administered into the ventrolateral periaqueductal gray than female rats tested during the estrous phase of the estrous cycle. Adult gonadectomy failed to appreciably change the pattern of this effect in either males of females. Thus, antinociception elicited from the ventrolateral periaqueductal gray by β-endorphin, like morphine, is sensitive to sex differences.

Keywords: Pain; Antinociception; Sex difference; Gonadectomy, adult; β-Endorphin

1. Introduction

The opioid agonists, morphine and β-endorphin, employ different anatomical and neurochemical pathways in exerting their supraspinal antinociceptive effects. Intrathecal administration of naloxone blocked β-endorphin, but not morphine antinociception (Tseng and Fujimoto, 1985). Spinal [Met^5]enkephalin is released following ventricular and hypothalamic administration of β-endorphin, but not morphine (Tseng and Wang, 1992; Tseng et al., 1985). Both antibodies to met-enkephalin and spinal δ-opioid receptor antagonists block β-endorphin, but not morphine antinociception (Tseng and Suh, 1989; Suh and Tseng, 1990a). Ventricular morphine and β-endorphin fail to develop antinociceptive cross-tolerance (Suh and Tseng, 1990b), and are differentially altered by pentobarbital anesthesia (Tseng and Tang, 1992). Morphine and β-endorphin only display additive antinociceptive effects following ventricular and intrathecal administration (Roerig et al., 1988). These differences are observed particularly within the ventrolateral periaqueductal gray in which barbiturate anesthesia respectively reduces and enhances morphine and β-endorphin analgesia (Smith et al., 1992a). Further, whereas β-endorphin antinociception in the ventrolateral periaqueductal gray is dependent upon a spinal opioid component, morphine antinociception is dependent upon spinal adrenoceptors and 5-HT receptors (Suh et al., 1988, 1989; Tseng and Tang, 1990; Tseng and Collins, 1991; Monroe et al., 1997). Moreover, morphine, but not β-endorphin antinociception elicited from the ventrolateral periaqueductal gray is reduced by either *N*-methyl-D-aspartate receptor, muscarinic receptor or nicotinic receptor antagonists administered into the rostral ventromedial medulla (Spinella et al., 1996, 1997, 1999). Although naltrexone and the μ-opioid receptor antagonist-selective somatostatin analogue, Cys^2, Tyr^3, Orn^5, Pen^7 amide, each blocked morphine and β-endorphin antinociception elicited from the ventrolateral periaqueductal gray, the slopes of the dose-inhibition curves were not parallel, suggesting involvement of distinct receptor subpopulations (Smith et al., 1992b; Monroe et al., 1996).

Male rats and mice typically display significantly greater magnitudes of systemic morphine-induced antinociception

* Corresponding author. Tel.: +1-718-997-3543; fax: +1-718-997-3257.

E-mail address: richard_bodnar@qc.edu (R.J. Bodnar).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00110-2

than female rats and mice (Badillo-Martinez et al., 1984; Kavaliers and Innis, 1987; Baamonde et al., 1989; Candido et al., 1992; Islam et al., 1993; Mogil et al., 1996; Cicero et al., 1996, 1997; Kest et al., 1999). This sex difference in morphine antinociception appears to be centrally-mediated since it is also observed following microinjections into the lateral ventricles (Kepler et al., 1989), the rostral ventromedial medulla (Boyer et al., 1998), and the ventrolateral periaqueductal gray (Krzanowska and Bodnar, 1999). Given the previously cited differences between these two opioid receptor agonists in mediating antinociception elicited from the ventrolateral periaqueductal gray, the present study evaluated whether β-endorphin antinociception elicited from the ventrolateral periaqueductal gray displayed the same sex differences as morphine on the tail-flick test in male and female rats receiving either sham surgeries or adult gonadectomy.

2. Methods

2.1. Subjects, surgeries and histological procedures

Male and female albino Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, 70 days of age) were housed individually in the Queens College Vivarium, and were maintained on a 12 h light/12 h dark cycle with Purina rat chow and water available ad libitum. Sham-operated and gonadectomy groups, matched for preoperative body weights for each sex, were anesthetized with a combination of chlorpromazine (3 mg/kg, i.p.) and ketamine (100 mg/kg, i.m.). Castrations were performed by removing the testes and testicular fat following a 2-cm midscrotal incision, while ovariectomies were performed by removing the ovaries and ovarian fat through a dorsal approach (e.g. Kepler et al., 1989). In sham surgery, the organs were exposed, but not removed. Two weeks following gonadal surgeries, all rats were reanesthetized, and a stainless steel guide cannula (26-gauge, Plastics One, Roanoke, VA) was stereotaxically (Kopf Instruments, Tujunda, CA) implanted into the ventrolateral periaqueductal gray: incisor bar (−5 mm), 0.3–0.6 mm anterior to the lambda suture, 1.5–2.0 mm lateral to and angled 12° toward the sagittal suture, and 6.5–7.0 mm from the top of the skull. The cannula was secured to anchor screws with dental acrylic. One month after gonadal surgeries and just prior to behavioral testing, gonadectomy-induced body weight changes were assessed. Body weights of castrated male rats were significantly less than sham-operated controls, while body weights of ovariectomized female rats were significantly more than sham-operated controls. After testing, all animals were deeply anesthetized, and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. Secondary sexual organs of male (seminal vesicles) and female (uterus) rats, were dissected, dried and weighed. The brains were removed, blocked, and cut coronally in 40 μm sections through the ventrolateral periaqueductal gray. The tissue, stained with cresyl violet, was examined by an observer unfamiliar with the behavioral data. Only animals with confirmed cannula placements were included in the data analysis.

2.2. Drugs

All intracranial microinjections (0.5 μl) were infused using a Hamilton microsyringe through polyethylene tubing and a stainless steel internal cannula (33-gauge, Plastics One) with all testing taking place at 2–8 h into the light cycle. β-endorphin (Peninsula Laboratories, Belmont, CA) was dissolved in 0.9% normal saline. Each drug treatment was separated by at least 1 week to minimize any potential tolerance effects, and rats received a maximum of four microinjection conditions including control treatment.

2.3. Nociceptive test

A radiant heat source (IITC, Woodland Hills, CA) was mounted 8 cm above and 3–9 cm proximal to the tip of the rat's tail; removal of the tail activated the photocell and determined the latency (0.01 s accuracy). The thermal intensity of the radiant heat source was set to produce baseline tail-flick latencies between 2 and 3.5 s. To avoid tissue damage, a trial was automatically terminated if a response did not occur within 12 s. Each session consisted of three latency determinations at different points on the tail at 10-s intertrial intervals. Baseline latencies were determined for at least 4 days to ensure stability of responding, with all animals displaying consistent latencies in baseline and vehicle testing.

2.4. Procedures

Sham-operated ($n = 8$) and castrated ($n = 7$) male, and sham-operated ($n = 8$) and ovariectomized ($n = 8$) female rats, matched for baseline latencies, received β-endorphin at doses of 0, 5.2, 6.5, 13 and 26 μg in the ventrolateral periaqueductal gray in counterbalanced order. This dose range was chosen because it was equimolar to the dose range used for morphine previously (Krzanowska and Bodnar, 1999). Tail-flick latencies were determined 30, 60, 90 and 120 min after each microinjection which samples the time course of peak agonist action of β-endorphin in the ventrolateral periaqueductal gray (Smith et al., 1992a,b; Monroe et al., 1996, 1997; Spinella et al., 1999). Although the phase of the estrous cycle failed to previously alter antinociception elicited by ventricular morphine (Kepler et al., 1989), the present study minimized potential estrous effects by only testing sham-operated females during the estrous phase of their cycle. On the test day, vaginal smears were taken 0–1 h into the light cycle; experimental tests occurred 1–7 h later. This procedure does not alter

baseline nociceptive measures after this interval (see: Krzanowska and Bodnar, 1999).

2.5. Statistics

Three-way analyses of variance were performed on latency scores with sex and gonadal status as a between-groups factor, and drug doses and test times as within-groups factors. To assess sex and gonadectomy differences at peak antinociceptive test times (30 min) across β-endorphin doses, antinociceptive difference scores were obtained by subtracting baseline latencies from the latencies determined following each dose of β-endorphin. Tukey planned comparisons ($P < 0.05$) were used to discern significant sex and gonadectomy effects.

3. Results

3.1. Histological verification

Weights of the seminal vesicles were reduced by 80% in castrated relative to sham-operated males, while uterine weights were reduced by 67% in ovariectomized relative to sham-operated females. Histological placements were localized within the ventrolateral periaqueductal gray in all four groups, and cannula placements were found as far rostral as the level of the III cranial nerve, and as far caudal as the dorsal raphe nucleus (Fig. 1). Patterns of placements were highly similar in all sex and gonadectomy groups.

3.2. β-Endorphin antinociception

Significant differences were observed among sex and gonadectomy groups ($F(3,33) = 3.41$, $P < 0.029$), among β-endorphin doses ($F(4,44) = 98.91$, $P < 0.0001$), across the 2 h time course ($F(3,33) = 92.69$, $P < 0.0001$), and for all interactions between and among these variables. Baseline tail-flick latencies failed to differ among the four groups. β-endorphin in the ventrolateral periaqueductal gray significantly increased latencies in sham male rats following the 5.2 (30 min), 6.5 (30 min), 13 (30–120 min) and 26 (30–120 min) μg doses, in castrated male rats following the 6.5 (30–60 min), 13 (30–90 min) and 26 (30–120 min) μg doses, in sham female rats following the 13 (30–90 min) and 26 (30–120 min) μg doses, and in ovariectomized female rats following the 13 (30–90 min) and 26 (30–120 min) μg doses. Peak effects in all groups occurred 30 min after β-endorphin administration, and specific sex and gonadectomy differences were assessed at this time point. Significant differences in antinociceptive difference scores were observed between male and female rats ($F(1,7) = 40.32$, $P < 0.0004$) and across β-endorphin doses ($F(3,21) = 11.69$, $P < 0.0001$), but not between sham-operated and gonadectomized animals ($F(1,7) =$ 0.02, n.s.) or for any of the interaction terms. As indicated in Fig. 2, male rats displayed significantly greater magni-

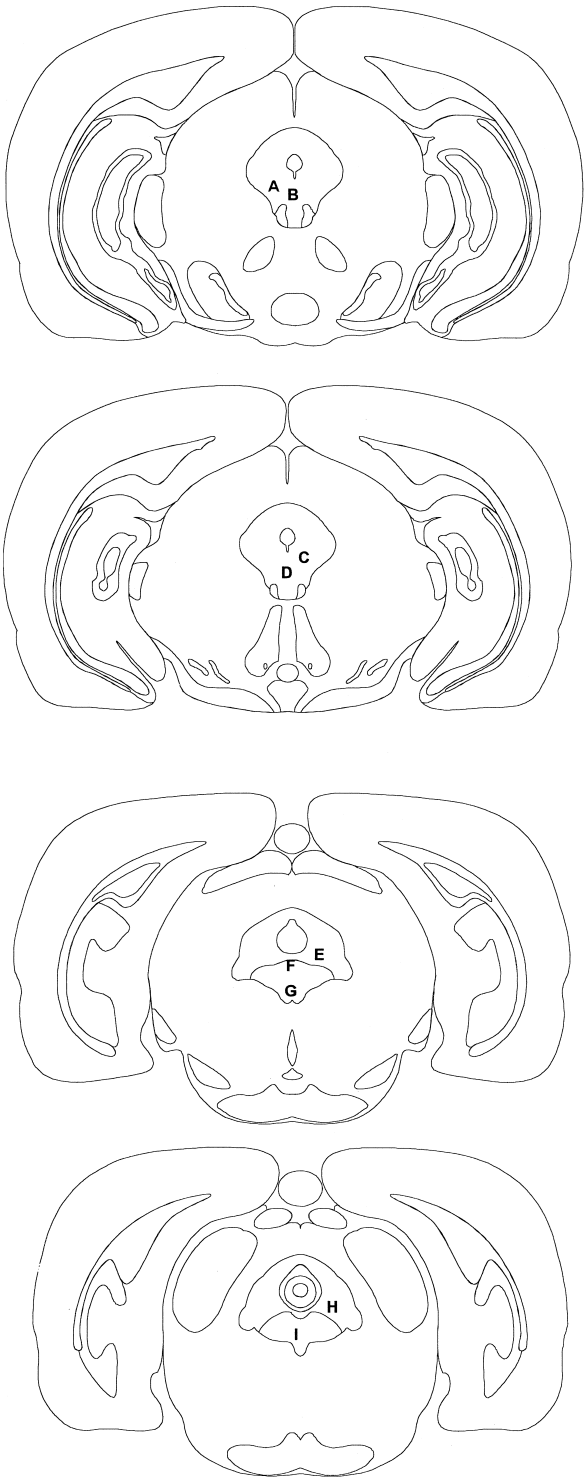

Fig. 1. Histological verification of ventrolateral periaqueductal gray cannula placements. There was considerable overlap among sham males (SM), castrated males (CM), sham females (SF) and ovariectomized females (OF): (A) CM (1), SF (1), OF (1); (B) SM (1), CM (1); (C) SM (1), CM (1), SF (2), OF (1); (D) SM (3), CM (2), SF (1); (E) OF (2); (F) CM (1), SF (1), OF (2); (G) SM (1), CM (1); (H) SM (2), CM (1), SF (1), OF (2); (I) SM (1), CM (1), SF (2), OF (2).

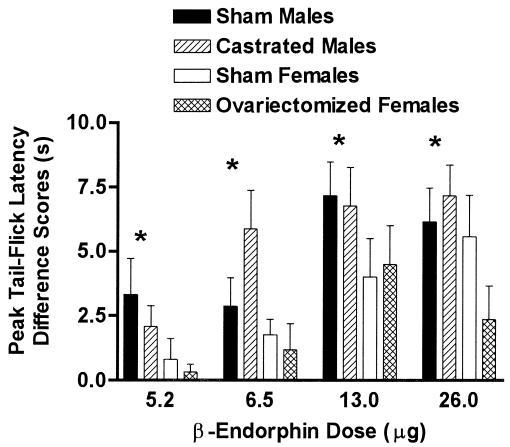

Fig. 2. Alterations (mean ± S.E.M.) in the magnitude of peak (30 min) β-endorphin-induced antinociception on the tail-flick test following microinjections into the ventrolateral periaqueductal gray of sham-operated male rats, castrated male rats, sham-operated female rats tested during the estrous phase of the estrous cycle and ovariectomized female rats. Peak antinociceptive difference scores for each β-endorphin dose in each of the four groups were ascertained by subtracting each agonist score from its corresponding baseline score. It should be noted that baseline tail-flick latencies did not differ among groups. The asterisks denote a significant difference between sham-operated and castrated male rats relative to sham-operated and ovariectomized female rats at each of the β-endorphin doses. Sham-operated male rats and castrated male rats failed to differ from each other in β-endorphin-induced antinociception, and sham-operated female rats and ovariectomized female rats failed to differ from each other in β-endorphin-induced antinociception.

tudes of peak antinociception than female rats following the 5.2 ($F(1,7) = 8.03$, $P < 0.025$), 6.5 ($F = 10.43$, $P < 0.015$), 13 ($F = 7.22$, $P < 0.031$) and 26 ($F = 7.74$, $P < 0.027$) μg doses of β-endorphin administered into the ventrolateral periaqueductal gray.

4. Discussion

Marked sex differences have been observed in antinociception induced by morphine and μ-opioid receptor agonists following microinjection into either the lateral ventricles, rostral ventromedial medulla or the ventrolateral periaqueductal gray (Kepler et al., 1989, 1991; Boyer et al., 1998; Krzanowska and Bodnar, 1999). The present study demonstrated that β-endorphin antinociception elicited from the ventrolateral periaqueductal gray was sensitive to sex differences on the tail-flick test with male rats displaying significantly greater peak antinociceptive effects than female rats over the entire dose range. Whereas male rats displayed latencies close to cut-off values following β-endorphin doses as low as 13 μg in the ventrolateral periaqueductal gray, female rats displayed only moderate degrees of antinociception across the β-endorphin dose range. Adult gonadectomy, which alters the activational effects of gonadal hormones, failed to significantly alter the magnitude in male or female rats. This relative lack of gonadectomy effects differs from morphine antinociception elicited from the ventrolateral periaqueductal gray in which ovariectomized females displayed significantly greater levels of morphine antinociception relative to sham-operated females tested during the estrous phase (Krzanowska and Bodnar, 1999). Since the present β-endorphin doses were equimolar to those employed for morphine antinociception previously (Krzanowska and Bodnar, 1999), these data strongly suggest similar patterns of sex differences for these two opioid agonists on the tail-flick test. These effects are of interest since the tail-flick test is the most commonly-used nociceptive measure to distinguish the different neurochemical substrates of β-endorphin and morphine antinociception (e.g. Suh et al., 1989; Tseng and Tang, 1990; Smith et al., 1992a,b; Monroe et al., 1996, 1997; Spinella et al., 1996, 1997, 1999).

Acknowledgements

This research was supported in part by PSC/CUNY Grants 69256 and 61291 to RJB.

References

Baamonde, A.I., Hidalgo, A., Andres-Trelles, F., 1989. Sex-related differences in the effects of morphine and stress on visceral pain. Neuropharmacology 28, 967–970.

Badillo-Martinez, D., Kirchgessner, A.L., Butler, P.D., Bodnar, R.J., 1984. Monosodium glutamate and morphine analgesia: test-specific effects. Neuropharmacology 23, 1141–1149.

Boyer, J.S., Morgan, M.M., Craft, R.M., 1998. Microinjection of morphine into the rostralventromedial medulla produces greater antinociception in male compared to female rats. Brain Res. 796, 315–318.

Candido, J., Lufty, K., Billings, B., Sierra, V., Duttaroy, A., Inturrisi, C.E., 1992. Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation. Pharmacol. Biochem. Behav. 42, 685–692.

Cicero, T.J., Nock, B., Meyer, E.R., 1996. Gender-related differences in the antinociceptive properties of morphine. J. Pharmacol. Exp. Ther. 279, 767–773.

Cicero, T.J., Nock, B., Meyer, E.R., 1997. Sex-related differences in morphine's antinociceptive activity: relationship to serum and brain morphine concentrations. J. Pharmacol. Exp. Ther. 282, 939–944.

Islam, A.K., Cooper, M.L., Bodnar, R.J., 1993. Interactions among aging, gender and gonadectomy effects upon morphine antinociception in rats. Physiol. Behav. 54, 45–53.

Kavaliers, M., Innis, D.G.L., 1987. Sex and day/night differences in opiate-induced responses of insular wild deer mice, *Peromyscus maniculatis triangularis*. Pharmacol. Biochem. Behav. 27, 477–482.

Kepler, K.L., Kest, B., Kiefel, J.M., Cooper, M.L., Bodnar, R.J., 1989. Roles of gender, gonadectomy and estrous phase in the analgesic effects of intracrebroventricular morphine in rats. Pharmacol. Biochem. Behav. 34, 119–127.

Kepler, K.L., Standifer, K.M., Paul, D., Pasternak, G.W., Kest, B., Bodnar, R.J., 1991. Differential gender effects upon central opioid analgesia. Pain 45, 87–95.

Kest, B., Wilson, S.G., Mogil, J.S., 1999. Sex differences in supraspinal morphine analgesia are dependent on genotype. J. Pharmacol. Exp. Ther. 289, 1370–1375.

Krzanowska, E., Bodnar, R.J., 1999. Morphine antinociception elicited from the ventrolateral periaqueductal gray is sensitive to sex and gonadectomy differences in rats. Brain Res. 821, 224–230.

Mogil, J.S., Kest, B., Sadowski, B., Belknap, J.K., 1996. Differential genetic mediation of sensitivity to morphine in genetic models of opiate antinociception: influence of nociceptive assay. J. Pharmacol. Exp. Ther. 276, 532–544.

Monroe, P.J., Hawranko, A.A., Smith, D.L., Smith, D.J., 1996. Biochemical and pharmacological characterization of multiple beta-endorphinergic antinociceptive systems in the rat periaqueductal gray. J. Pharmacol. Exp. Ther. 276, 65–93.

Monroe, P.J., Smith, D.L., Smith, D.J., 1997. Beta-endorphin dose-dependently activates multiple spinopetal antinociceptive pathways following its microinjection into the rat periaqueductal gray: comparison with those activated by morphine. Analgesia 3, 21–26.

Roerig, S.C., Fujimoto, J.M., Tseng, L.F., 1988. Comparisons of descending pain inhibitory pathways activated by beta-endorphin and morphine as characterized by supraspinal and spinal antinociceptive interactions in mice. J. Pharmacol. Exp. Ther. 247, 1107–1113.

Smith, D.J., Robertson, B., Monroe, P.J., 1992a. Antinociception from the administration of beta-endorphin into the periaqueductal gray is enhanced while that of morphine is inhibited by barbiturate anesthesia. Neurosci. Lett. 146, 143–146.

Smith, D.J., Robertson, B., Monroe, P.J., Leedham, J.A., Cabral, J.D.Y., 1992b. Opioid receptors mediating antinociception from beta-endorphin and morphine in the periaqueductal gray. Neuropharmacology 31, 1137–1150.

Spinella, M., Cooper, M.L., Bodnar, R.J., 1996. Excitatory amino acid antagonists in the rostral ventromedial medulla inhibit mesencephalic morphine analgesia in rats. Pain 64, 545–552.

Spinella, M., Schaefer, L.A., Bodnar, R.J., 1997. Ventral medullary mediation of mesencephalic morphine analgesia by muscarinic and nicotinic cholinergic receptor antagonists in rats. Analgesia 3, 119–130.

Spinella, M., Znamensky, V., Moroz, M., Ragnauth, A., Bodnar, R.J., 1999. Actions of NMDA and cholinergic receptor antagonists in the rostral ventromedial medulla upon beta-endorphin analgesia elicited from the ventrolateral periaqueductal gray. Brain Res. 829, 151–159.

Suh, H.H., Fujimoto, J.M., Tseng, L.F., 1989. Differential mechanisms mediating beta-endorphin-and morphine-induced analgesia in mice. Eur. J. Pharmacol. 168, 61–70.

Suh, H.H., Tseng, L.F., 1990a. Delta but not mu-opioid receptors in the spinal cord are involved in antinociception induced by beta-endorphin given intracerebroventricularly in mice. J. Pharmacol. Exp. Ther. 253, 981–986.

Suh, H.H., Tseng, L.F., 1990b. Lack of antinociceptive cross-tolerance between intracerebroventricularly administered beta-endorphin and morphine or DPDPE in mice. Life Sci. 46, 759–765.

Suh, H.H., Tseng, L.F., Li, C.H., 1988. Beta-endorphin (1–27) antagonizes beta-endorphin-, but not morphine-, DPDPE-and U50488H-induced analgesia in mice. Neuropharmacology 27, 957–963.

Tseng, L.F., Collins, K.A., 1991. Different mechanisms mediating tail-flick inhibition induced by beta-endorphin, DAMGO and morphine from ROb and GiA in anesthetized rats. J. Pharmacol. Exp. Ther. 257, 530–538.

Tseng, L.F., Fujimoto, J.M., 1985. Differential actions of intrathecal naloxone on blocking the tail-flick inhibition induced by intraventricular beta-endorphin and morphine in rats. J. Pharmacol. Exp. Ther. 232, 74–79.

Tseng, L.F., Higgins, M.J., Hong, J.S., Hudson, P.M., Fujimoto, J.M., 1985. Release of immunoreactive met-enkephalin from the spinal cord by intraventricular beta-endorphin but not morphine in anesthetized rats. Brain Res. 343, 60–69.

Tseng, L.F., Suh, H.H., 1989. Intrathecal [Met5]-enkephalin antibody blocks analgesia induced by intracerebroventricular beta-endorphin but not morphine in mice. Eur. J. Pharmacol. 173, 171–176.

Tseng, L.F., Tang, R., 1990. Different mechanisms mediate beta-endorphin-and morphine-induced inhibition of the tail-flick response in rats. J. Pharmacol. Exp. Ther. 252, 546–551.

Tseng, L.F., Tang, R.R., 1992. Pentobarbital attenuates antinociception induced by i.c.v. morphine but not beta-endorphin in the mouse. Eur. J. Pharmacol. 214, 175–180.

Tseng, L.F., Wang, Q., 1992. Forebrain sites differentially sensitive to beta-endorphin and morphine for analgesia and release of met-enkephalin in the pentobarbital-anesthetized rat. J. Pharmacol. Exp. Ther. 261, 1028–1036.