Brian K. Tsang MD,*
Zhi He MD,*
Wichai Wongchanapai MD,†
Ing K. Ho PhD,†
John H. Eichhorn MD*

Visceral analgesic tolerance to intrathecal butorphanol in rats

Purpose: Recent experimental data suggest that intrathecal (it) κ -opioid agonists produce profound visceral analgesia. This study investigated the development of visceral analgesic tolerance to it butorphanol, a potent κ -agonist that has fewer side effects than commonly used it opioids. Understanding of this tolerance could make it butorphanol more effective in treating chronic visceral pain.

Methods: This was a randomized, controlled animal study involving 80 Sprague-Dawley rats. Rats implanted with lumbar it catheters were infused either with it saline or butorphanol ($52 \text{ nmol}\cdot\text{hr}^{-1}$) for 96 hr. Six hours afterwards, each rat was challenged once with one of the differing it butorphanol doses to construct dose-response curves. Visceral analgesia was evaluated by the abdominal writhing responses to the acetic acid injected intraperitoneally. The time of the first writhes and the total number of writhes were recorded.

Results: For both the saline- and butorphanol-infused groups, a higher challenge dose of it butorphanol produced longer time for the first writhes to occur ($P < 0.01$, one-way ANOVA), and fewer writhes occurring within 30 min ($P < 0.01$, one-way ANOVA). However, the dose response curves of the butorphanol-infused groups were shifted rightward ($P < 0.001$, partial F test).

Conclusion: The challenge doses of it butorphanol produced dose-dependent visceral analgesia in both the saline- and butorphanol-infused groups, confirming its efficacy. The butorphanol-infused groups showed dose-response shifts, demonstrating the development of tolerance to this visceral analgesia.

Objectif : Des données expérimentales récentes laissent croire que des agonistes opioïdes intrathécaux (it) produisent une analgésie viscérale profonde. L'étude actuelle examine le développement de la tolérance analgésique viscérale au butorphanol it, un agoniste puissant qui présente moins d'effets secondaires que les opioïdes it utilisés habituellement. La connaissance de cette tolérance pourrait améliorer l'efficacité du butorphanol it pour le traitement de la douleur viscérale chronique.

Méthode : Il s'agit d'une étude animale, randomisée et contrôlée portant sur 80 rats Sprague-Dawley à qui on a implanté des cathéters lombaires permettant une perfusion de solution salée ou de butorphanol it ($52 \text{ nmol}\cdot\text{hr}^{-1}$) pendant 96 h. Six heures après cette perfusion, chaque rat a été soumis une fois à l'une des différentes doses de butorphanol it afin de produire des courbes doses-réponses. L'analgésie viscérale a été évaluée par les réactions de contraction abdominale à l'injection intrapéritonéale d'acide acétique. Le moment de la première contraction et le nombre total de contractions ont été enregistrés.

Résultat : Dans les deux groupes, ayant reçu une perfusion de solution salée ou de butorphanol, une plus forte dose provocatrice de butorphanol it a entraîné une première contraction plus tardive ($P < 0.01$, analyse de variance simple) et moins de contractions se produisant pendant les 30 premières min ($P < 0.01$, analyse de variance simple). Cependant, les courbes doses-réponses des groupes ayant reçu une perfusion de butorphanol étaient déplacées vers la droite ($P < 0.001$, test F partiel).

Conclusion : Les doses provocatrices de butorphanol it ont produit une analgésie viscérale proportionnelle à la dose administrée et ce, pour tous les groupes, qu'ils aient reçu une perfusion de solution salée ou de butorphanol, confirmant ainsi l'efficacité de ce dernier. Dans les groupes qui ont reçu une perfusion de butorphanol, on a observé des déplacements des courbes doses-réponses, ce qui indique le développement d'une tolérance à cette analgésie viscérale.

From the Departments of Anesthesiology* and Pharmacology & Toxicology,† University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA.

Address correspondence to: Brian K. Tsang MD, Department of Anesthesiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA. Phone: 601-984-5950; Fax: 601-984-5959; E-mail: bktsang@anesthesia.umsmed.edu

Supported in part by the University of Mississippi Medical Center Biomedical Research Grant 59910 (BKT), and by Preferred Medical Products and Bristol-Myers Squibb

Accepted for publication July 8, 1998.

INTRATHECAL (*it*) opioids produce a dose-dependent and naloxone-reversible analgesia through the actions on opioid receptors in the dorsal horn of the spinal cord.^{1,2} Recently, *it* opioids delivered via implanted microinfusion pumps have gained popularity in the treatment of patients with chronic nonmalignant pain.^{2,3} While clinicians mostly use μ -opioid agonists for *it* infusion, animal data have shown *it* selective κ -opioid agonists produce profound visceral analgesia.^{1,4,5} While these selective agonists are not clinically available, butorphanol, a mixed agonist/antagonist opioid with particularly high κ -receptor affinity, has been used both spinally and epidurally to treat pain patients.⁶⁻⁹ In contrast to μ - and δ -opioid receptors, activities at the κ -opioid receptors are not associated with severe respiratory depression.^{10,11} Mixed agonist-antagonist analgesics, including butorphanol, offer the potential benefit of greater receptor site selectivity, while diminishing the incidence of adverse sequelae such as respiratory depression.^{11,12} Furthermore, spinal butorphanol has also been reported to cause neither pruritus nor urinary retention.¹³

Thus, *it* butorphanol may have an important role in the future treatment of patients with chronic visceral pain. As with systemic administration, prolonged *it* administration of opioids has been associated with the development of analgesic tolerance.¹⁴ For chronic pain patients, the problem of analgesic tolerance can be formidable and troubling, as they may require *it* opioids for many decades. In this study, we have established a model for studying the development of visceral analgesic tolerance to *it* butorphanol. Further investigation using this model could lead to better understanding of the tolerance development which, in turn, could lead to more effective use of *it* butorphanol in treating chronic visceral pain.

Methods

Animals and Materials

Adult male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN, USA), weighing 250-300 g at the time of surgery, were housed in group cages with water and food pellets freely available, and maintained on a 12-hr light/12-hr dark cycle in the animal room. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center. A total of 80 animals was used. Polyurethane microspinal catheters (28 gauge outer diameter) and butorphanol were gifts from Preferred Medical Products, a division of Ballard Medical Products

(Lewiston, NY, USA) and from the Bristol-Myers Squibb Corporation (Princeton, NJ, USA), respectively. All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA) unless otherwise specified.

Intrathecal Catheter Implantation

The surgical procedure for implanting the microspinal catheter has been previously described.^{15,16} Briefly, while spontaneously breathing halothane 1-3% in oxygen 100% via a face mask, the rat was mounted in a conventional stereotaxic instrument. With the overlying skin shaved and sterilized, the posterior superficial neck muscles were separated to give a clear visual field of the atlanto-occipital membrane. The catheter was inserted through a small incision at the centre of the membrane and gently advanced caudally 8.5 cm down to the lumbar subarachnoid space. The muscles and the skin wound were closed.

The external end of the *it* catheter was connected to a 4-cm long Tygon tubing (0.38 mm inner diameter, Cole-Palmer, Chicago, IL, USA), which was plugged by a stainless steel wire. The external tubing was shielded by a plastic protector sutured at the top of the head to prevent damage by the animal. After surgery, the rats were given 30,000 u procaine penicillin G *sc* to prevent infection, then housed individually to minimize the risk of dislocation of the implanted catheter by other animals.

Osmotic Minipump Implantation

The rats recovered for one week before an osmotic minipump (Alzet 2001, Alza Corp. Palo Alto, CA, USA) was attached.¹⁷ Under halothane anaesthesia, the minipump was implanted *sc* between the scapulae and connected to the *it* catheter by inserting the tip of the tubular flow-moderator of the minipump into the distal end of the Tygon tubing (whose proximal end was in turn connected to the indwelling *it* catheter). Solutions added to the minipumps before the implantation were passed through 0.2 μ m millipore sterilizing filters. To minimize the dead space in the indwelling catheter, 7 μ l test solution was used to flush and fill the catheter prior to connection to the minipump.

Intrathecal Drug Administration

Butorphanol was dissolved in sterile physiological saline. Animal groups were randomly assigned to receive a continuous *it* infusion for 96 hr via the osmotic minipump at a rate of 1 μ l·hr⁻¹ butorphanol (52 nmol· μ l⁻¹) to induce tolerance, or to receive *it* infusion of saline as controls. Six hours after the *it* infusions were terminated, the saline-infused rats were

assigned to four different groups to receive *it* challenge doses of butorphanol (1.6, 6.5, 26 or 65 nmol·10 μ l⁻¹·rat⁻¹). The butorphanol-infused rats were assigned to another four different groups to receive *it* challenge doses of butorphanol 130, 260, 350 or 520 nmol·10 μ l⁻¹·rat⁻¹. The challenge dosage ranges were selected (based on previous pilot studies) to give adequate analgesic responses for the control and butorphanol-tolerant animals. There were 8-11 animals in each group, and each animal received only one *it* challenge dose to eliminate possible interaction between different doses of drugs.

Visceral Analgesic Test

Acetic acid abdominal writhing tests were carried out to determine the suppression of the visceral pain induced by acetic acid, a chemical irritant to the viscera (visceral peritoneum).^{18,19} This test is specific for visceral pain because the parietal peritoneum (innervated by the spinal somatic nerves) lacks chemo receptors to respond to the diluted acetic acid. Rats were given 10 ml·kg⁻¹ acetic acid 0.6%, intraperitoneally, 15 min after the challenge dose of *it* butorphanol. An abdominal writhe was defined as a characteristic stretching of the hind limbs, arching of the back, rolling on one side, or contraction of the abdominal muscles. The time of the first writhe displayed and the total number of writhes occurring in 30 min were recorded. For the purpose of calculation, rats that did not show any writhes within 30 min were given the time of 30 min as the time of the first writhe. The rats were sacrificed at the end of the test period.

Statistical Analysis

The dose-response regression curves were constructed and analyzed for parallelism by computer-assisted pharmacologic calculations.²⁰ Data consisting of the time of the first writhe and the total number of writhes were analyzed by the one-way analysis of variance (ANOVA). The partial F test was used to compare the dose-response curves between the saline- and butorphanol-infused groups. Statistical significance was set at the level of $P < 0.05$.

Results

In Figures 1 and 2, each data point represents the mean $\pm$ SEM (standard error of means) of 8-11 animals. For both the saline- and butorphanol-infused groups, a higher challenge dose of *it* butorphanol produced longer time for the first writhe to occur ($P < 0.01$, one-way ANOVA), and fewer writhes occurring within 30 min ($P < 0.01$, one-way ANOVA).

There was a positive correlation of the time of the

FIGURE 1 The visceral analgesic responses, measured by the time of the first writhe, of the saline-infused and butorphanol-infused animals upon differing doses of intrathecal butorphanol challenges. Each data point represented the mean $\pm$ SEM (standard error of means) of 8-11 animals. Linear regression was constructed to yield the correlation coefficient, r .

FIGURE 2 The visceral analgesic responses, measured by the number of the writhes within 30 min, of the saline-infused and butorphanol-infused animals upon differing doses of intrathecal butorphanol challenges. Each data point represented the mean $\pm$ SEM (standard error of means) of 8-11 animals. Linear regression was constructed to yield the correlation coefficient, r .

first writhe with the log doses of the *it* butorphanol challenges for both the saline-infused groups ($r = 0.905$) and the butorphanol-infused groups ($r = 0.947$) (Figure 1). There was no difference (parallelism test) in the slopes of the dose-response curves between the saline-infused and the butorphanol-infused groups. However, the dose-response curve of the butorphanol-infused groups was shifted rightward (i.e., occurring at much higher dose range) than that

of the saline-infused groups ($P < 0.001$, partial F test).

There were linear but negative correlations of the total number of writhes with the log doses of the *it* butorphanol challenges for both the saline-infused groups ($r = -0.998$) (Figure 2) and the butorphanol-infused groups ($r = -0.961$). The slope of the butorphanol-infused dose-response curve was not different from that of the saline-infused group (parallelism test), but the curve was shifted rightward ($P < 0.001$, partial F test).

Discussion

The data from the saline-infused animals, the measures of both the time of the first writhes and the total number of writhes established that *it* administration of butorphanol (with high κ -agonist activity) produced a dose-dependent visceral analgesia against the chemical irritation induced by the injected acetic acid. The relatively large volume of dilute acetic acid (0.6%) produced diffuse low level stimulation to both visceral and parietal peritoneum, provoking mostly visceral pain, but minimal somatic pain. The activation of somatic pain fibres in the parietal peritoneum generally requires a more focal, intense stimulation.²¹ Our result agrees with the suggestions of others that experimental κ -receptor agonists have high efficacy for visceral analgesia.^{4,22,23} The use of *it* opioid infusion continues to evolve in the treatment of patients with nonmalignant pain syndromes, including chronic visceral pain syndromes such as chronic pancreatitis pain or pelvic pain of visceral origin. Perhaps, *it* butorphanol, given its visceral analgesic efficacy, could become valuable in the treatment of patients with chronic visceral pain.

The osmotic minipump used in this study delivers infusion at a rate of merely $1 \mu\text{L}\cdot\text{hr}^{-1}$. Yaksh and Rudy have demonstrated that, after an hour, even a relatively large *it* bolus ($5 \mu\text{L}$) of dye,¹⁴ C-urea or³ H-naloxone does not diffuse more than 2 cm away from the tip of the *it* catheter.¹⁵ Therefore, we believe that *it* butorphanol delivered via the minipump stimulated only the spinal, but not the supraspinal, opioid receptors. There is an abundance of various opioid receptors found in the region of the substantia gelatinosa of the spinal cord dorsal horn, where most afferent pain fibres terminate.¹ With the high κ - and moderate δ -agonist activities of butorphanol,²⁴ it is likely that spinal and opioid receptors are mainly responsible in mediating the visceral analgesia demonstrated in this study. Further studies are warranted to confirm this hypothesis.

When the dose-response curves (for either the time of the first writhes or the total number of writhes) of the butorphanol-infused groups were compared with

those of the saline-infused groups, the rightward shift was obvious. This indicated that the development of tolerance to visceral analgesia. Being a mixed opioid agonist-antagonist, butorphanol was believed previously to have a lower potential for tolerance and dependence development than morphine.¹¹ However, recent data have shown that analgesic tolerance developed with prolonged intracerebroventricular administration of butorphanol, involving both κ - and δ -opioid receptors in the brain.²⁵⁻²⁷ Thus, it is reasonable to suggest that the visceral analgesic tolerance to *it* butorphanol demonstrated in this study involved κ - and δ -opioid receptors in the spinal cord. However, more studies are indicated.

The development of visceral analgesic tolerance to continuous *it* butorphanol infusion may pose a troublesome problem for chronic pain patients who could benefit from such therapy. A recent retrospective study found a slow but steady dose escalation in noncancer patients receiving *it* opioids, with an average increase of daily dose of 6-fold in two years.³ Since most patients with nonmalignant sources of pain generally have a stable, nonprogressive course of disease, the development of analgesic tolerance probably accounts for most, if not all, of the necessary dose escalation. Therefore, analgesic tolerance is a serious problem for these patients as they are likely to require *it* opioids for a prolonged time.

Rawal *et al.* have demonstrated that, if a bolus dose of butorphanol was injected *it* in sheep, there were severe behavioural side effects (loud vocalization and limb paralysis) and spinal cord histological damages.²⁸ In contrast, the rats in the current study showed no behavioural side effects. Rawal's results could be a species-specific effect, but the butorphanol doses used in their study (0.075 and $0.375 \text{ mg}\cdot\text{kg}^{-1}$) were much higher than the doses used in this study ($\mu\text{g}\cdot\text{kg}^{-1}$). Even a high dose of *it* sufentanil produced behaviour and histological side effects in Rawal's study.²⁸ Thus, the apparent toxicity of *it* butorphanol demonstrated by Rawal in sheep could be dose-related. Several clinical studies using epidural butorphanol and one using *it* butorphanol did not report behavioural side effects such as increased pain or limb paralysis.^{6,7,10} While further toxicity studies using clinically relevant doses and carried out in different species would be beneficial, current clinical experiences fail to demonstrate any overt neurotoxicity from epidural or *it* butorphanol.

The development of tolerance to visceral analgesia induced by *it* butorphanol is shown in this study. Further studies are warranted to delineate how opioid receptors and secondary messenger systems are involved in the development of the tolerance to *it*

butorphanol. With better understanding of the mechanisms of the tolerance development, it is hoped that, in the future, novel pharmacological interventions might be rationally developed to reduce or eliminate this undesirable analgesic tolerance. Then, *it* butorphanol could become maximally effective and most valuable in the treatment of patients with chronic visceral pain.

References

- 1 *Pasternak GW*. Pharmacological mechanisms of opioid analgesics. *Clin Neuropharmacol* 1993; 16: 1–18.
- 2 *Coombs DW, Saunders RL, Gaylor MS, et al*. Relief of continuous chronic pain by intraspinal narcotics infusion via an implanted reservoir. *JAMA* 1983; 250: 2336–9.
- 3 *Paice JA, Penn RD, Shott S*. Intraspinal morphine for chronic pain: a retrospective, multicenter study. *J Pain Symptom Manage* 1996; 11: 71–80.
- 4 *Schmauss C, Yaksh TL*. *In vivo* studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of μ , δ and κ receptors with visceral chemical and cutaneous thermal stimuli in the rat. *J Pharmacol Exp Ther* 1984; 228: 1–12.
- 5 *Millan MJ*. Kappa-opioid receptors and analgesia. *Trends Pharmacol Sci* 1990; 11: 70–6.
- 6 *Tsai SK, Lee TY, Mok MS, Lippman M*. Subarachnoid administration of butorphanol for the relief of postoperative pain. *Anesth Sinica* 1985; 23: 121–3.
- 7 *Abboud TK*. Epidural and intrathecal administration of opioids in obstetrics. *Acute Care* 1988; 12(Suppl 1): 17–21.
- 8 *Camann WR, Loferski BL, Fanciullo GJ, Stone ML, Datta S*. Does epidural administration of butorphanol offer any clinical advantage over the intravenous route? A double-blind, placebo-controlled trial. *Anesthesiology* 1992; 76: 216–20.
- 9 *Gould DB*. Epidural butorphanol better fentanyl, morphine options (Letter). *Ann Thorac Surg* 1995; 59: 1039–40.
- 10 *Abboud TK, Moore M, Zhu J, et al*. Epidural butorphanol or morphine for the relief of post-cesarean section pain: ventilatory responses to carbon dioxide. *Anesth Analg* 1987; 66: 887–93.
- 11 *Sosnowski M, Yaksh TL*. Spinal administration of receptor-selective drugs as analgesics: new horizons. *J Pain Symptom Manage* 1990; 5: 204–13.
- 12 *Jacobs AM, Youngblood F*. Opioid receptor affinity for agonist-antagonist analgesics. *J Am Podiatr Med Assoc* 1992; 82: 520–4.
- 13 *Palacios QT, Jones MM, Hawkins JL, et al*. Post-Caesarean section analgesia: a comparison of epidural butorphanol and morphine. *Can J Anaesth* 1991; 38: 24–30.
- 14 *Yaksh TL*. The spinal pharmacology of acutely and chronically administered opioids. *J Pain Symptom Manage* 1992; 7: 356–61.
- 15 *Yaksh TL, Rudy TA*. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 17: 1031–6.
- 16 *Tsang BK, He Z, Ma T, Ho IK, Eichhorn JH*. Decreased paralysis and better motor coordination with microspinal versus PE10 intrathecal catheters in pain study rats. *Anesth Analg* 1997; 84: 591–4.
- 17 *Loomis CW, Milne B, Cervenka FW*. Determination of cross tolerance in rat spinal cord using intrathecal infusion via sequential mini-osmotic pumps. *Pharmacol Biochem Behav* 1987; 26: 131–9.
- 18 *Hayashi G, Takemori AE*. The type of analgesic-receptor interaction involved in certain analgesic assays. *Eur J Pharmacol* 1971; 16: 63–6.
- 19 *Porreca F, Mosberg HI, Omnaas JR, Burks TF, Cowan A*. Supraspinal and spinal potency of selective opioid agonists in the mouse writhing test. *J Pharmacol Exp Ther* 1987; 240: 890–4.
- 20 *Tallaride RJ, Murray RB*. Parallel lines 1: test for parallelism. In: Tallaride RJ, Murray RB. *Manual of Pharmacologic Calculations*, 2nd ed. New York: Springer-Verlag New York Inc, 1987: 19.
- 21 *Guyton AC*. Somatic sensations: II. pain, headache, and thermal sensations. In: Guyton AC. *Textbook of Medical Physiology*, 8th ed. Philadelphia: W.B. Saunders Company, 1991: 526.
- 22 *Tung AS, Yaksh TL*. *In vivo* evidence for multiple opiate receptors mediating analgesia in the rat spinal cord. *Brain Res* 1982; 247: 75–83.
- 23 *Pircio AW, Gylis JA, Cavanagh RL, Buyniski JP, Bierwagen ME*. The pharmacology of butorphanol, a 3,14-dihydroxymorphinan narcotic antagonist analgesic. *Arch Int Pharmacodyn* 1976; 220: 231–57.
- 24 *Horan PJ, Ho IK*. Comparative pharmacological and biochemical studies between butorphanol and morphine. *Pharmacol Biochem Behav* 1989; 34: 847–54.
- 25 *Peachey JE*. Clinical observations of agonist-antagonist analgesic dependence. *Drug Alcohol Depend* 1987; 20: 347–65.
- 26 *Feng YZ, Narita M, Tseng YT, Hoskins B, Ho IK*. Crosstolerance between butorphanol and morphine in rats. *Pharmacol Biochem Behav* 1994; 49: 657–61.
- 27 *Feng YZ, Tseng YT, Jaw SP, Hoskins B, Ho IK*. Tolerance development to butorphanol: comparison with morphine. *Pharmacol Biochem Behav* 1994; 49: 649–55.
- 28 *Rawal N, Nuutinen L, Raj PP, et al*. Behavioral and histopathologic effects following intrathecal administration of butorphanol, sufentanil, and nalbuphine in sheep. *Anesthesiology* 1991; 75: 1025–34.