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Effects of Chronic Morphine Treatment on β-Endorphin-Related Peptides in the Caudal Medulla and Spinal Cord

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Abstract: The effects of chronic morphine treatment on β-endorphin (βE)-immunoreactive (βE-ir) peptide levels were determined in the rat caudal medulla and different areas of the spinal cord. Seven days of morphine pelleting had no effect on total βE-ir peptides in the caudal medulla. In contrast, it significantly increased βE-ir peptide concentrations in the cervical and thoracic regions of the spinal cord compared with placebo-pelleted controls, whereas in the lumbosacral region this trend did not reach statistical significance. Injections of the opiate receptor antagonist naloxone 1 h before the rats were killed had no effect on the morphine-induced increases in the cord. Chromatographic analyses revealed that enzymatic processing of βE-related peptides in the spinal cord seemed unaffected by the morphine and/or naloxone treatments. In light of previous data showing that morphine down-regulates βE biosynthesis in the hypothalamus, the present results suggest that the regulation of βE-ir peptides in the spinal cord is distinct from that found in other CNS areas. These data provide support for previous results suggesting that βE-expressing neurons may be intrinsic to the spinal cord. **Key Words:** Opiate—Endogenous opioid—Proopiomelanocortin—Morphine—Nucleus tractus solitarius. **Bronstein D. M. et al.** Effects of chronic morphine treatment on β-endorphin-related peptides in the caudal medulla and spinal cord. *J. Neurochem.* **60,** 2304–2307 (1993).

The endogenous opioid peptide β-endorphin (βE) is found in numerous CNS regions and has been implicated in a wide variety of physiological and behavioral functions (Akil et al., 1984). βE is synthesized as part of the larger precursor protein, proopiomelanocortin (POMC). In addition to the opioid-active 31-amino-acid peptide, βE_{1-31}, POMC produces several other βE-related peptides, including β-lipotropin, βE_{1-27}, and βE_{1-26}, and their acetylated derivatives, *N*-Ac-βE_{1-31}, *N*-Ac-βE_{1-27}, and *N*-Ac-βE_{1-26}.

Different CNS regions contain unique mixes of βE-immunoreactive (βE-ir) peptides. For example, whereas βE_{1-31} accounts for 50–75% of the total βE-ir activity in most rostral brain structures, it represents only 25% of the total βE-ir activity in the nucleus tractus solitarius (NTS) (Dores et al., 1986) and as little as 10% in the sacral portions of the spinal cord (Gianoulakis and Angelogianni, 1989). The regional differences in POMC peptide products are due, in part, to their originating from separate POMC cell groups. βE-ir activity found in forebrain and midbrain areas is derived almost exclusively from perikarya in the arcuate nucleus of the hypothalamus (Watson et al., 1977; Bloom et al., 1978; Krieger et al., 1980). In more caudal areas of the CNS, βE-ir activity originates primarily from a small number of cells scattered around the NTS (Schwartzberg and Nakane, 1983; Khachaturian et al., 1985; Palkovits and Eskay, 1987; Palkovits et al., 1987).

βE-ir activity has been detected in nerve fibers in the spinal cord (Khachaturian et al., 1985; Tsou et al., 1986), but it has generally been assumed that these fibers derived from supraspinal sources as POMC-immunoreactive cell bodies have never been observed in the cord itself. Recently, however, several lines of evidence raise the possibility that βE-ir peptides might be expressed in cells intrinsic to the spinal cord: (a) The pattern of POMC processing in the spinal cord, i.e., higher-molecular-weight species make up a relatively large percentage of the total βE-ir activity, is distinct from that found in the arcuate or NTS POMC systems. (b) Our laboratory demonstrated that βE-ir activity persisted in lower spinal cord levels even after complete transections at higher spinal levels (Gutstein et al., 1992). (c) POMC mRNA has recently been detected in the spinal cord (Plantinga et al., 1992). If, in fact, spinal cord POMC cells exist, it is reasonable to believe that they might be regulated differ-

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Abbreviations used: CSC, cervical spinal cord; βE, β-endorphin; βE-ir, β-endorphin-immunoreactive; MN, morphine/naloxone; MS, morphine/saline; NTS, nucleus tractus solitarius; PN, placebo/naloxone; POMC, proopiomelanocortin; PS, placebo/saline; RIA, radioimmunoassay; SSC, lumbosacral spinal cord; TSC, thoracic spinal cord.

ently from the arcuate and/or NTS POMC systems. This was recently found to be the case, as βE biosynthesis appeared to be up-regulated by chronic naltrexone treatment in arcuate POMC neurons, whereas βE-ir peptide levels in the NTS and spinal cord remained unchanged (Bronstein et al., 1993).

In light of previous reports that chronic morphine down-regulates POMC peptide biosynthesis in the rostral arcuate system (Mocchetti et al., 1989; Bronstein et al., 1990), the present study was undertaken to assess the effects of chronic morphine administration on βE-ir peptides in the caudal medulla and spinal cord.

MATERIALS AND METHODS

Male Sprague–Dawley rats were implanted with morphine (75 mg per pellet) or placebo pellets over a 7-day period (one pellet on the first day and three more on the fourth day) and then received an intraperitoneal injection of saline or naloxone (1 mg/kg) 1 h before they were killed (Bronstein et al., 1990). The four treatment groups were designated as placebo/saline (PS), morphine/saline (MS), placebo/naloxone (PN), and morphine/naloxone (MN). The caudal medulla, including the NTS (hereafter designated as NTS), and different spinal cord regions [cervical, thoracic, and lumbosacral (CSC, TSC, and SSC, respectively)] were collected on ice and frozen at −80°C until assayed for βE-ir activity. Mean ± SE tissue weights (mg) for the NTS, CSC, TSC, and SSC were 80 ± 2, 147 ± 6, 193 ± 9, and 142 ± 7, respectively. Peptides were extracted and quantified by radioimmunoassay (RIA) as described previously (Akil et al., 1979). Because the antibody used recognized any peptide containing the midportion sequence of βE_{1-31} (amino acids 17–27), RIAs of crude brain or spinal cord extracts provided a measure of total βE-ir activity. Concentrations of different molecular-weight βE-ir peptides in the NTS, CSC, and TSC were determined by chromatographic separation on Sephadex G-50 columns followed by RIA of the collected fractions (Bronstein et al., 1990). Results were analyzed by two-way ANOVA, with pelleting (placebo or morphine) and injection (saline or naloxone) as the two independent variables.

RESULTS

The data presented here were collected from two independent experiments that gave qualitatively identical effects. Seven days of morphine pelleting had no effect on total βE-ir peptide concentrations in the NTS (Fig. 1). In contrast, it significantly increased βE-ir peptide concentrations in the CSC and TSC ($F = 4.19$, $p < 0.05$; and $F = 5.06$, $p < 0.05$, respectively). In both regions, total βE-ir peptide concentrations were roughly 50% higher in morphine- than in placebo-treated animals. Morphine tended to elevate βE-ir activity in the SSC as well, but this increase (~30%) did not reach statistical significance ($F = 1.59$, $p = 0.21$). Injections of naloxone to placebo-treated animals did not alter total βE-ir peptide levels in any region, nor did it affect the morphine-induced increases in βE-ir activity in the spinal cord.

Different-sized βE-related peptides that contributed to the total βE-ir activity found in crude extracts were separated by gel filtration chromatography and then quantified

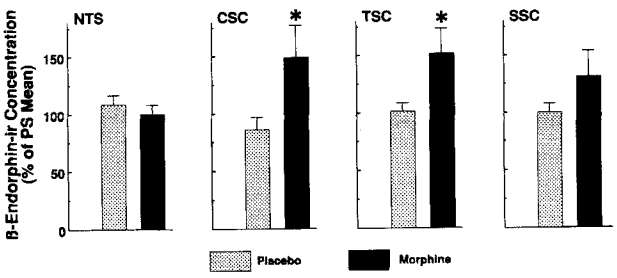

FIG. 1. Effect of chronic morphine treatment on βE-ir peptide concentrations in the caudal medulla (NTS) and spinal cord (CSC, TSC, and SSC). Rats were implanted subcutaneously with morphine (75 mg) or placebo pellets over a 7-day period. One hour before the animals were killed, they received injections of naloxone (1 mg/kg) or saline vehicle. Caudal medulla and spinal cord regions were assayed for total βE-ir peptide by RIA. Data were collected from two independent experiments and expressed as percentages of the PS mean value from each experiment. Absolute concentrations of βE-ir peptide in the NTS, CSC, TSC, and SSC for PS-treated animals were 7.0 ± 0.9, 6.9 ± 2.1, 2.1 ± 0.3, and 0.66 ± 0.11 fmol/mg of tissue, respectively. As naloxone treatment had no effect in any region, the vehicle and naloxone groups have been pooled, i.e., PS + PN = placebo and MS + MN = morphine, to illustrate more clearly morphine versus placebo differences. Data are mean ± SEM (bars) values of 16–27 animals.

by RIA. Three major peaks of βE-ir activity were detected. The molecular masses of these peaks corresponded to β-lipotropin (11 kDa), βE_{1-31} (3.5 kDa), and $\beta E_{1-27}/\beta E_{1-26}$ (3.0 kDa). In agreement with previous data (Dores et al., 1986; Gianoulakis and Angelogianni, 1989; Gutstein et al., 1992), the relative heights of the three peaks varied in the regions analyzed (Fig. 2). There was progressively less processing of POMC precursor to βE-related peptides as one descended from the NTS to the TSC, i.e., more caudal spinal cord regions contained higher proportions of larger-molecular-weight βE-ir species compared with the NTS or CSC. This pattern of POMC processing is in contrast to that seen in the hypothalamus, where virtually all POMC is processed to βE_{1-31}- and $\beta E_{1-27}/\beta E_{1-26}$-size material. Chronic morphine treatment, with or without naloxone, had no appreciable effect on the chromatographic profiles in any of the regions examined.

DISCUSSION

The results of this study demonstrate that chronic morphine treatment increases βE-ir peptide concentrations in the spinal cord but not the caudal medulla. To our knowledge, this is the first demonstration that βE-ir peptides in the spinal cord can be pharmacologically regulated. These results may prove significant in two respects. First, the finding that 7 days of morphine pelleting increases βE-ir activity in the spinal cord but not in the NTS or in other more rostral brain regions (Bronstein et al., 1990; Berglund et al., 1989; Mocchetti et al., 1989) indicates that spinal βE-ir activity may be uniquely regulated by opiates. This would support previous data (Gutstein et al., 1992; Platinga et al., 1992) suggesting the existence of POMC cells intrinsic to the spinal cord. Second, the present data demonstrate that βE-ir peptide levels in the spinal cord are influenced by opiate

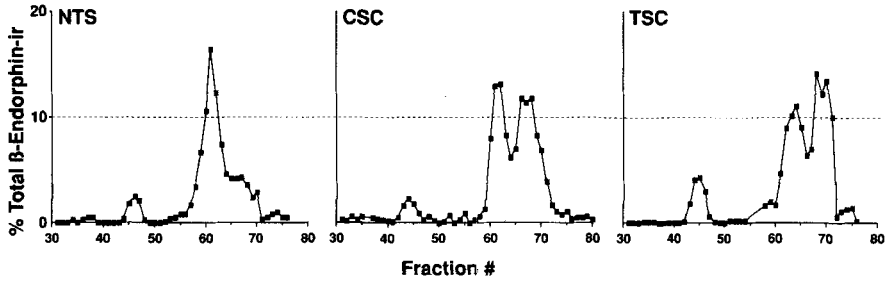

FIG. 2. Representative chromatographic profiles of βE-ir peptide in the NTS and spinal cord of control (PS) animals. Animals were treated and tissue was collected as described in Fig. 1. Crude extracts from two to four animals were pooled and chromatographed on a Sephadex G-50 column developed with 1% formic acid containing 0.1% bovine serum albumin. Collected fractions were assayed for βE-ir activity. Data are expressed as percentages of the total βE-ir activity detected (total βE-ir content was determined by summating βE-ir peptide levels from each of the individual fractions).

receptor activation. This is consistent with a possible role for βE-ir peptides in various opioid-mediated functions in the spinal cord, e.g., analgesia. Previously, chronic naltrexone treatment was shown not to alter βE-ir peptide concentrations in the spinal cord (Bronstein et al., 1993), suggesting that spinal cord βE-ir activity was not under tonic inhibitory control by endogenous opioids. Thus, if βE-ir activity is to have a physiological role in opiate-mediated processes in the spinal cord, it may first require the activation of endogenous opioid-containing neurons.

It is presently not known whether the effects of morphine on spinal cord βE-ir peptides were direct, i.e., on putative POMC cells in the spinal cord, or indirect, i.e., mediated by supraspinal or intraspinal neurons. It is also not clear whether the increase in total βE-ir activity represents an induction of βE biosynthesis subsequent to increased neuronal activation or an inhibition of release and accumulation of peptide subsequent to decreased neural activity. In other POMC cells, such as those in the pituitary gland or arcuate nucleus, additional parameters of POMC biosynthesis, e.g., POMC mRNA levels, rates of POMC translation or processing, and βE release, provide information that helps distinguish whether biosynthesis has been stimulated or repressed. In the case of the spinal cord, however, these measures are currently technically infeasible or extremely difficult owing to the fact that expression levels are low (see Platinga et al., 1992). We were able to analyze different molecular-weight βE-ir forms and found that morphine, alone or in combination with naloxone, did not appear to alter the relative amounts of different βE-ir peptides in the NTS or spinal cord. This differs from the situation in the hypothalamus where 3 days of morphine treatment increased total βE-ir activity primarily because of a selective increase in $\beta E_{1-27}/\beta E_{1-26}$ concentrations (Bronstein et al., 1990) and where an 8-day treatment with naltrexone decreased total βE-ir activity in large part because of a decrease in $\beta E_{1-27}/\beta E_{1-26}$ levels (Bronstein et al., 1993).

The present results, together with those from previous studies (Mocchetti et al., 1989; Bronstein et al., 1990, 1993), suggest that hypothalamic, NTS, and spinal cord βE species are differentially regulated by chronic opiate agonist or antagonist treatment. The rostral POMC system, with somata localized in and around the arcuate nucleus, appears to be down-regulated by opiate agonists and up-regulated by opiate antagonists, suggesting a tonic inhibitory control of these cells by one or more of the endogenous opioid peptides. POMC neurons in the NTS appear unaffected by either opiate agonist or antagonist treatment. Finally, spinal cord βE-ir activity is elevated by chronic morphine treatment but unaffected by chronic naltrexone treatment. The differential regulation of βE synthesis by opiate receptor ligands provides support for the hypothesis that there may be an intrinsic POMC system in the spinal cord in addition to those originating in the arcuate nucleus and NTS. This could have important implications for a possible role of βE-ir peptides in antinociceptive and/or autonomic functions at the spinal level.

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REFERENCES

Akil H., Watson S. J., Barchas J. D., and Li C. H. (1979) Beta-endorphin immunoreactivity in rat and human blood: radioimmunoassay, comparative levels, and physiological alterations. *Life Sci.* **24,** 1659–1666.

Akil H., Watson S. J., Young E., Lewis M. E., Khachaturian H., and Walker J. M. (1984) Endogenous opioids: biology and function. *Annu. Rev. Neurosci.* **7,** 223–255.

Berglund L. A., Millington W. R., and Simpkins J. W. (1989) Gonadal steroid and chronic morphine treatment do not change the posttranslational processing of β-endorphin in the rat brain. *Life Sci.* **44,** 591–601.

Bloom F., Battenberg E., Rossier J., Ling N., and Guillemin R. (1978) Neurons containing beta-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. *Proc. Natl. Acad. Sci. USA* **75,** 1591–1595.

Bronstein D. M., Przewlocki R., and Akil H. (1990) Effects of morphine treatment on pro-opiomelanocortin systems in rat brain. *Brain Res.* **519,** 102–111.

Bronstein D. M., Day N. C., Gutstein H. B., Trujillo K. A., and Akil H. (1993) Pre- and posttranslational regulation of β-endorphin biosynthesis in the CNS: effects of chronic naltrexone treatment. *J. Neurochem.* **60,** 40–49.

Dores R. M., Jain M., and Akil H. (1986) Characterization of the forms of β-endorphin and α-MSH in the caudal medulla of the rat and guinea pig. *Brain Res.* **377,** 251–260.

Gianoulakis C. and Angelogianni P. (1989) Characterization of β-endorphin peptides in the spinal cord of the rat. *Peptides* **10,** 1049–1054.

Gutstein H. B., Bronstein D. M., and Akil H. (1992) β-Endorphin processing and cellular origins in rat spinal cord. *Pain* **51,** 241–247.

Khachaturian H., Lewis M. E., Tsou K., and Watson S. J. (1985) β-Endorphin, α-MSH, ACTH, and related peptides, in *Handbook of Chemical Neuroanatomy, Vol. 4: GABA and Neuropeptides in the CNS, Part I* (Björklund A. and Hökfelt T., eds), pp. 216–272. Elsevier, Amsterdam.

Krieger D. T., Liotta A. S., Brownstein M. J., and Zimmerman E. A. (1980) ACTH, β-lipotropin, and related peptides in brain, pituitary, and blood. *Recent Prog. Horm. Res.* **36,** 277–344.

Mocchetti I., Ritter A., and Costa E. (1989) Down-regulation of pro-opiomelanocortin synthesis and beta-endorphin utilization in hypothalamus of morphine-tolerant rats. *J. Mol. Neurosci.* **1,** 33–38.

Palkovits M. and Eskay R. L. (1987) Distribution and possible origin of β-endorphin and ACTH in discrete brainstem nuclei of rats. *Neuropeptides* **9,** 123–137.

Palkovits M., Mezey E., and Eskay R. L. (1987) Pro-opiomelanocortin-derived peptides (ACTH/β-endorphin/α-MSH) in brainstem baroreceptor areas of the rat. *Brain Res.* **436,** 323–328.

Plantinga L. C., Verhaagen J., Edwards P. M., Schrama L. H., Burbach J. P. H., and Gispen W. H. (1992) Expression of the pro-opiomelanocortin gene in dorsal root ganglia, spinal cord and sciatic nerve after sciatic nerve crush in the rat. *Mol. Brain Res.* **16,** 135–142.

Schwartzberg D. G. and Nakane P. K. (1983) ACTH-related peptide containing neurons within the medulla oblongata of the rat. *Brain Res.* **276,** 351–356.

Tsou K., Khachaturian H., Akil H., and Watson S. J. (1986) Immunocytochemical localization of pro-opiomelanocortin-derived peptides in the adult rat spinal cord. *Brain Res.* **378,** 28–35.

Watson S. J., Barchas J. D., and Li C. H. (1977) Beta-lipotropin: localization in cells and axons in rat brain by immunocytochemistry. *Proc. Natl. Acad. Sci. USA* **74,** 5155–5158.