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Central administration of morphine inhibits brain and liver ornithine decarboxylase activity in neonatal rats: Involvement of transcription- and non-transcription-dependent mechanisms

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Abstract

This study examined whether the developmental deficits usually observed in infants born to opiate addicted mothers could involve effects on ornithine decarboxylase, a growth-controlling enzyme. Intracerebroventricular (i.c.v.) injection of a single dose of morphine (2 μg) to 6-day-old rats markedly decreased basal brain and liver ornithine decarboxylase activity as well as the increases in hepatic ornithine decarboxylase activity produced by subcutaneously (s.c.) administered insulin, an important trophic hormone. Centrally applied morphine acts supraspinally to downregulate peripheral ornithine decarboxylase activity, since s.c. administration of the same dose as used i.c.v. decreased neither basal liver ornithine decarboxylase levels nor tissue responsiveness to insulin. This does not imply that the opiate is unable to affect ornithine decarboxylase when applied systemically. In fact, a robust inhibition of both basal and induced liver ornithine decarboxylase activity was obtained in rat pups given 20 μg of morphine s.c. This larger dose is able to trigger the hepatic ornithine decarboxylase effects presumably by stimulating opiate receptors located at central sites after crossing the blood–brain barrier and penetrating into the brain. Concomitant administration of naloxone plus morphine i.c.v. prevented morphine from downregulating ornithine decarboxylase activity, confirming the participation of supraspinal opioid receptors in morphine ornithine decarboxylase actions. Finally, as was the case for insulin induced stimulation of ornithine decarboxylase activity, i.c.v. injection of morphine markedly diminished insulin induced stimulation of hepatic ornithine decarboxylase mRNA accumulation. In turn, contrary to the inhibition of basal ornithine decarboxylase activity, morphine did not lower basal hepatic ornithine decarboxylase mRNA levels when given alone. Thus, CNS morphine can apparently suppress tissue ornithine decarboxylase expression through both transcriptional and posttranscriptional mechanisms. The evidence obtained suggest that postnatal exposure to opiate drugs might detrimentally affect development by altering normal tissue ornithine decarboxylase ontogeny. © 1997 Elsevier Science B.V.

Keywords: Opiate; β-Endorphin; Insulin; Growth; Development; Ornithine decarboxylase mRNA; Intracerebroventricular

1. Introduction

Opiates constitute a family of analgesic compounds, both natural or synthetic, that have morphine-like activity. It is well documented that neonates born to opiate-addicted mothers, as well as to rodents treated with opiates during gestation, have significantly lower body weight and length than control infants, and a number of subtle to severe behavioral and physiological disturbances that might persist through infancy and adulthood (Zelson et al., 1971; Naeye et al., 1973; Chasnoff, 1985; Hutchings, 1991; Wilson, 1992; Zagon and McLaughlin, 1992). The mechanisms by which opiates produce these developmental deficits are as yet poorly understood. It is also unclear whether opiates affect maturational processes through a single defined mechanism. It has been reported that morphine may exert some detrimental effects on the developing brain by depressing DNA synthesis and, therefore, cellular proliferation (Kornblum et al., 1987; Hauser, 1992).

Opiate drugs are thought to exert their effects by binding to receptors on cell membranes of neurons whose endogenous ligands are opioid peptides such as β-endorphin. We have shown that intracerebroventricular (i.c.v.), but not subcutaneous (s.c.), injection of a single dose of β-endorphin rapidly and markedly lowers basal levels of ornithine decarboxylase activity in brain, liver,

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heart, kidney and lung in rat pups (Bartolome et al., 1986: Greer et al., 1991). Numerous studies by different groups of investigators, including our own, have documented that measurements of ornithine decarboxylase activity constitute an early, sensitive and reliable index of tissue maturation (Heby, 1981; Pegg and McCann, 1982; Slotkin et al., 1982; Slotkin and Bartolome, 1983, 1986; Russell, 1980, 1985). ornithine decarboxylase catalyzes the rate-limiting step in the synthesis of the polyamines putrescine, spermidine and spermine (Pegg and Williams-Ashman, 1968), which are essential for normal cell growth, multiplication and differentiation (Heby, 1981; Pegg and McCann, 1982). Centrally applied, β-endorphin not only decreases basal ornithine decarboxylase activity but also suppresses insulin induced increases in liver ornithine decarboxylase activity (Bartolome et al., 1991). Of importance, the ornithine decarboxylase effects are age specific in that they are restricted to the first three weeks of age. These and other observations by our laboratory (Bartolome et al., 1987, 1995a,b) support the hypothesis that endogenous CNS β-endorphin, in addition to its well known involvement in analgesic, endocrine and behavioral events, functions physiologically as a negative trophic factor early during development via downregulation of tissue ornithine decarboxylase activity.

In this investigation we have assessed in 6-day-old rats the effects of i.c.v. injection of morphine on basal levels of ornithine decarboxylase activity in brain and liver, and on hormonally-induced increases in liver ornithine decarboxylase activity, to determine whether the adverse influences of perinatal opiate exposure on developmental processes could involve opiate actions on tissue ornithine decarboxylase expression. Morphine is a preferential μ-opioid receptor agonist, and we have recently shown that CNS β-endorphin downregulates ornithine decarboxylase activity throughout the body in neonatal rats by interacting specifically with brain μ-opioid receptors, but not with δ- or -opioid receptors (Bartolome et al., 1995a).

2. Materials and methods

2.1. Animal treatments

Lactating Sprague–Dawley female rats with 3- or 4-day-old litters (10 pups per litter; Charles River Laboratories, Raleigh, NC, USA) were randomized on arrival, housed in breeding cages in a vivarium maintained at 22°C with a 12 h light–dark cycle, and were provided food (Purina Lab chow, Ralston-Purina, St. Louis, MO, USA) and water ad libitum. To prevent the induction of stress caused by exposure to a novel environment, all animals were moved from the vivarium to the experimental room the evening before experimentation. To lessen maternal caretaking differences, pups from all litters were randomized and redistributed to the nursing mothers on the day of experimentation. In addition, animals from different treatment groups were assigned to each dam. All animals were killed at about the same time of the day to minimize circadian influences on the variables under investigation. Morphine, naloxone and insulin (all dissolved in saline) and growth hormone (dissolved in sodium bicarbonate 0.03 M/saline) were administered s.c. (1 μl/g body weight) or i.c.v. (5 μl/rat) at the doses indicated in the results section. Intracerebroventricular injections were performed according to the technique of Haley and McCormick (1957).

2.2. Ornithine decarboxylase activity

Tissues were quickly dissected, weighed and homogenized (Polytron) in 19 vols. (w/v) of ice-cold 10 mM Tris–HCl (pH 7.2), and ornithine decarboxylase activity was determined in the 26 000 $\times$ g at 20 min supernatant as previously reported (Slotkin and Bartolome, 1983). Essentially, the incubation medium contained 0.9 ml of supernatant and a final concentration of 1.8 mM dithiothreitol, 50 μM pyridoxal-5′-phosphate and 4.8 μM L-[1-^{14}C]ornithine in a total volume of 1 ml. Vials were capped with serum stoppers (into which plastic center wells containing paper filter wicks were suspended), and incubated 30 min at 37°C. The reaction was stopped with 0.5 ml of 10% trichloroacetic acid, and the $^{14}CO_2$ evolved was trapped with 0.2 ml of hyamine hydroxide (injected into the paper wicks) during a second 30-min incubation. Center wells were removed, placed in scintillation fluid and counted for radioactivity. Decarboxylation not attributable to ornithine decarboxylase was determined by running a parallel incubation in the presence of 5 mM α-difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase activity (Metcalf et al., 1978). Ornithine decarboxylase activity is the difference in radioactivity between samples incubated in the presence and absence of α-difluoromethylornithine. Ornithine decarboxylase activity is expressed as nmol $^{14}CO_2$ evolved/g of tissue weight per h.

2.3. Poly(A)$^+$ RNA isolation and Northern blot analysis

Total liver mRNA was extracted with acid guanidinium thiocyanate–phenol-chloroform according to Chomczynski and Sacchi (1987), and enriched for poly(A)$^+$RNA by Oligotex-dT chromatography following manufacturer's instructions (Qiagen, Santa Clarita, CA, USA). Polyadenylated RNA samples (5.0–7.5 μg/well) were denaturated with 6% formaldehyde and 50% formamide, and fractionated electrophoretically on 1.0% agarose-2.2 M formaldehyde gels. The gels were stained with ethidium bromide and photographed to assess the integrity of the RNA samples and to ensure that equal amounts of RNA had been applied to each lane of the gel. Separated RNAs were then transferred onto nitrocellulose membranes (Schleicher

and Schuell, Keene, NH, USA) by capillary blotting, and then linked to the membrane by baking it at 80°C for 1 h. Membranes were incubated overnight at 42°C in a prehybridization solution containing 6 × SSPE, 5 × Denhardt's solution, 50% formamide, 0.1% SDS, 1 mM EDTA and 100 μg/ml of denaturated salmon sperm DNA. To each ml of prehybridization solution were added 1×10^6 cpm of heat-denaturated ^{32}P-labelled ornithine decarboxylase probe, and hybridization was carried out overnight at 42°C. Membranes were washed two times (15 min each) in 2 × SSC, 0.1% SDS at room temperature, once in 0.1 × SSC, 0.1% SDS at 42°C for 30 min, and once with the same solution at 65°C for 5–10 min. Membranes were then exposed to Kodak X-OMAT AR film at −70°C for 2–5 days using an intensifying screen. Exposure time was such that the images produced by the blots were within the linear response range of the film. Intensity of the hybridization signals was determined by laser densitometric scanning of autoradiograms using a Hewlett Packard ScanJet3c Scanner equipped with a ScanJet3c/T transparency adapter (Palo Alto, CA, USA) and Sigma Scan/Image software version 1.2 (Jandel, San Rafael, CA, USA). The ornithine decarboxylase probe consisted of a 2.2-kb *Eco*RI fragment of mouse ornithine decarboxylase (Wen et al., 1989), kindly provided by Dr. P. Blackshear (Duke University, Durham, NC, USA). The probe was radiolabeled with 5′-[α-^{32}P]dCTP to a specific activity of 1 to 2×10^6 cpm/μg, using a Primed-It II Random Primer DNA Labeling kit, and a NucTrap push column (Stratagene, La Jolla, CA, USA) to separate non-incorporated deoxyribonucleoside triphosphates from the radiolabeled ornithine decarboxylase probe.

2.4. Drugs and reagents

L-[1-^{14}C]Ornithine monohydrochloride (57.2 mCi/mmol) and 5′-[α-^{32}P]dCTP (3000 Ci/mmol) were obtained from Dupont NEN (Boston, MA, USA), morphine sulfate from the National Institute of Drug Abuse (Research Triangle Park, NC, USA), naloxone from Sigma (St. Louis, MO, USA), insulin (Iletin) from Eli Lilly (Indianapolis, IN, USA), and growth hormone from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (Baltimore, MD, USA). All other reagents were analytical grade.

2.5. Statistical analysis

Statistical comparisons utilized one- or two-way analysis of variance (ANOVA; data log-transformed whenever variance was heterogeneous), followed by Student–Newman–Keuls test for pairwise multiple comparison where appropriate. Significance was accepted at the level of $P < 0.05$.

3. Results

Intracerebroventricular (i.c.v.) administration of morphine to 6-day-old rats produced a dose-dependent decrease in brain ($F_{4,122} = 60.2$; $P < 0.001$) and liver ($F_{4,128} = 21.8$; $P < 0.001$) ornithine decarboxylase activity (Fig. 1). The effect could be seen at i.c.v. doses of morphine as low as 0.5 μg. Enzyme levels in animals given the higher dose were reduced to approximately 38% and 16% of control values in the brain and liver, respectively.

To establish whether centrally-administered morphine suppresses ornithine decarboxylase activity in central and peripheral tissues by interacting with opioid receptors in the CNS, similar studies were undertaken in animals treated with naloxone, an opioid antagonist. As shown in Fig. 2, i.c.v. administration of 10 μg of naloxone together with 2 μg of morphine completely prevented morphine from decreasing both brain ($F_{1,38} = 6.75$; $P < 0.02$) and liver ($F_{1,68} = 13.12$; $P < 0.001$) ornithine decarboxylase basal levels. Naloxone given alone did not significantly alter ornithine decarboxylase activity in either tissue.

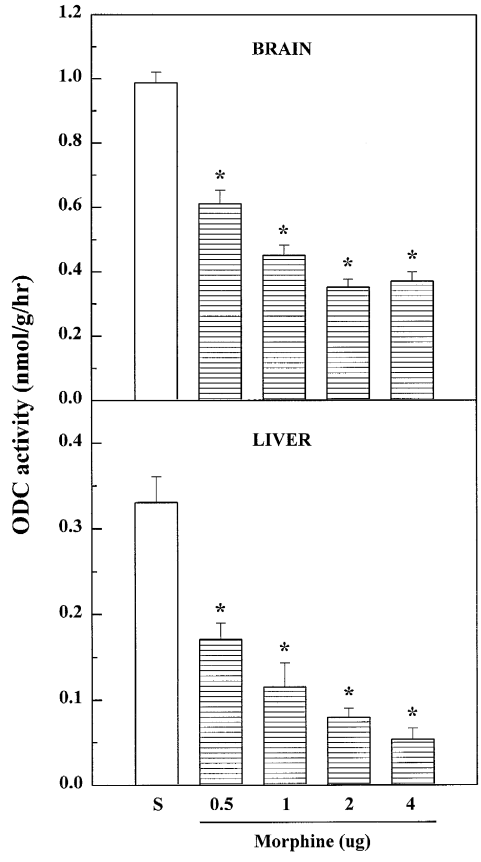

Fig. 1. Dose–response effect of central administration of morphine on basal brain and liver ornithine decarboxylase levels. Six-day-old rats were injected i.c.v. with morphine at the doses indicated above or with saline (S), and tissue ornithine decarboxylase activity was assayed 4 h later. Data represent the means ± S.E.M. of 14–44 individual determinations per group, per tissue. * $P < 0.05$ vs. their respective S groups (Student–Newman–Keuls following one-way ANOVA).

To test whether i.c.v. morphine is able to suppress tissue ornithine decarboxylase responsiveness to trophic stimuli, separate studies were performed in animals administered insulin (an important throphic hormone particularly during early development). As previously reported (Bartolome et al., 1991), s.c. injection of 20 IU of insulin/kg body weight produced a robust increase in liver ornithine decarboxylase activity 4 h after injection ($F_{1,121} = 178.09$; $P < 0.01$) (Fig. 3). More importantly, i.c.v. morphine (2 μg) given 15 min prior to insulin administration markedly inhibited insulin's ability to increase liver ornithine decarboxylase activity ($F_{1,121} = 50.11$; $P < 0.01$) (Fig. 3). Similarly to the inhibitory effect of morphine on basal tissue ornithine decarboxylase levels, i.c.v. injection of naloxone together with morphine effectively prevented morphine from suppressing liver ornithine decarboxylase responsiveness to insulin ($F_{1,121} = 26.22$; $P < 0.11$) (Fig. 3).

To determine whether the downregulation of liver ornithine decarboxylase activity by CNS morphine may involve opioid actions on ornithine decarboxylase gene transcription, we assessed the effects of morphine on liver ornithine decarboxylase mRNA expression. Two distinct species of ornithine decarboxylase mRNA, a 2.2-kb band and 2.6-kb band, were noted (Fig. 4, top panel). In agreement with the literature the shorter form was more abundance than the longer (Berger et al., 1984; Pohjanpelto et al., 1985). Densitometric analysis was performed to quantitate the autoradiographic data of the shorter form. Consistent with our previous work (Bartolome et al., 1995b), s.c. administration of 20 IU of insulin/kg body weight markedly increased liver ornithine decarboxylase mRNA accumulation (about 3-fold) (Fig. 4). More importantly, as was the case for ornithine decarboxylase activity, pretreatment with morphine i.c.v. completely prevented insulin from stimulating liver ornithine decarboxylase mRNA accumulation (Fig. 4). On the other hand, in contrast to ornithine decarboxylase activity, morphine given alone did not significantly alter liver ornithine decarboxylase mRNA basal levels (Fig. 4).

To establish whether centrally administered morphine inhibits liver ornithine decarboxylase activity by activating mechanisms within the brain, separate studies were performed in rat pups given morphine peripherally. In contrast to the central route of administration, s.c. injection of 2 μg of morphine decreased neither basal liver ornithine decarboxylase levels nor tissue ornithine decarboxylase responsiveness to insulin (Fig. 5). However, significant decreases in both basal and induced ornithine decarboxylase values were obtained in animals given 20 μg of morphine (Fig. 5).

Finally, to find out whether centrally-administered mor-

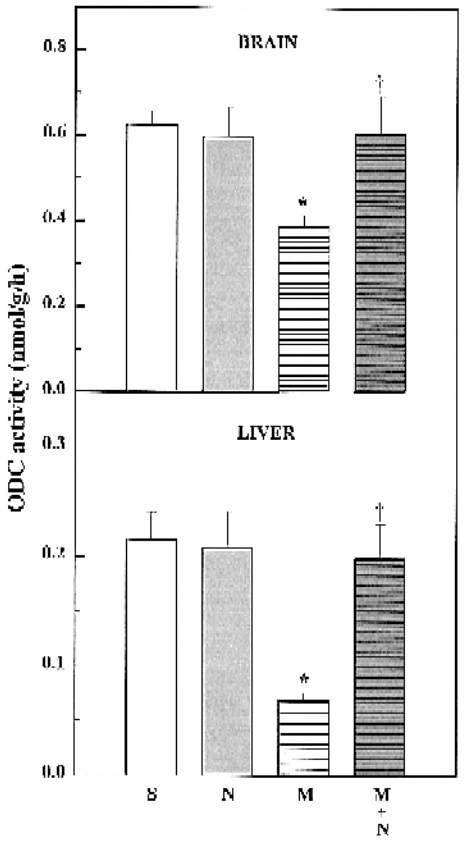

Fig. 2. Effects of naloxone (N) on morphine-induced decreases in basal brain and liver ornithine decarboxylase levels. Six-day-old rats were injected i.c.v. with either saline (S), 10 μg N, 2 μg morphine (M) or M + N, and tissue ornithine decarboxylase activity was assayed 4 h later. Data represent the means ± S.E.M. of 6 to 16 individual determinations per group (brain), and 10–25 individual determinations per group (liver). * $P < 0.05$ vs. respective S groups, and † $P < 0.05$ vs. respective M groups (Student–Newman–Keuls following two-way ANOVA).

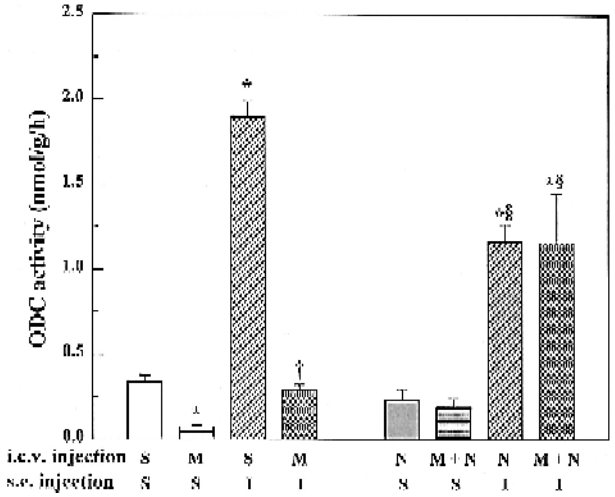

Fig. 3. Effects of morphine (M) and naloxone (N) on insulin (I)-induced increases in liver ornithine decarboxylase activity. Six-day-old rats were injected i.c.v. with either 2 μg of M, 10 μg of N, M plus N or saline (S) 15 min before receiving 20 IU of I/kg body wt. or S s.c., and ornithine decarboxylase activity was determined 4 h after the second injection. Data represent the means ± S.E.M. of 9–23 determinations per group. * $P < 0.05$ vs. S–S group, † $P < 0.05$ vs. S–I group, and § $P < 0.05$ vs. N–S group (Student–Newman–Keuls following two-way ANOVA).

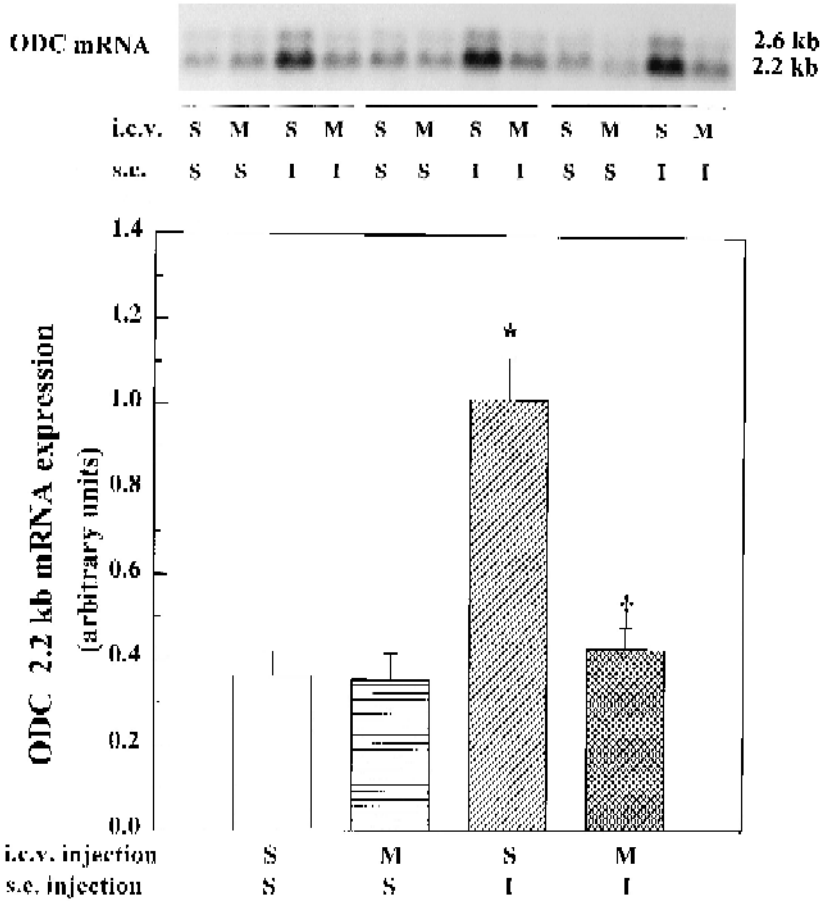

Fig. 4. (Top) Effects of central administration of morphine (M) on basal ornithine decarboxylase mRNA levels, and on insulin (I)-induced increases in ornithine decarboxylase mRNA levels in the liver of 6-day-old rats. Animals ($n = 3$ per group) were injected i.c.v. with 2 μg M or with saline (S) 15 min before receiving 20 IU I/kg body wt. or S s.c., and liver ornithine decarboxylase mRNA levels were measured 4 h after the second injection. Each blot is the result obtained from one individual animal. (Bottom) Densitometric analysis of autoradiogram shown in the top panel. Data represent the means ± S.E.M. * $P < 0.05$ vs. S–S group, and † $P < 0.05$ vs. S–I group (Student–Newman–Keuls following two-way ANOVA).

phine is able to suppress liver ornithine decarboxylase responsiveness to trophic hormones other than insulin, additional studies were undertaken in rat pups treated with growth hormone. As shown in Fig. 6, s.c. administration of 5 mg of growth hormone/kg body weight profoundly increased liver ornithine decarboxylase activity in 6-day-old rats, yet it failed to do so in pups pretreated i.c.v. with morphine.

4. Discussion

Our identification in the developing rat pup of key growth-regulatory processes that are suppressed by central (but not peripheral) administration of β-endorphin prompted us to explore the possibility that similar mechanisms might be operating to produce the developmental deficits commonly observed in infants born to mothers addicted to opiates. Specifically, we injected morphine i.c.v. to 6-day-old rats and measured brain and liver ornithine decarboxylase activity 4 h after injection. We found that i.c.v. doses of morphine as low as 0.5 μg/rat significantly decreased both brain and liver ornithine decarboxylase activity. As noted earlier, ornithine decarboxylase plays a fundamental role in the regulation of normal development. Numerous studies have shown that each tissue has a characteristic ornithine decarboxylase ontogenetic pattern, and perturbation of these patterns in response to environmental manipulations are invariably associated with subsequent alterations in tissue maturation and/or function (Slotkin, 1979; Russell, 1980; Slotkin et al., 1982; Slotkin and Bartolome, 1986). Accordingly, the evidence obtained suggest that the detrimental effects of opiate drugs on developing organisms result, at least in part, from actions on tissue ornithine decarboxylase expression.

In addition to its involvement in biochemical processes governing basal cellular metabolic activity, ornithine decarboxylase is also considered to function as an intermediate in cellular effects of agents known to stimulate growth (Slotkin and Bartolome, 1983). Thus, both basal ornithine decarboxylase activity and ornithine decarboxylase's ability to respond to trophic stimuli are important elements for

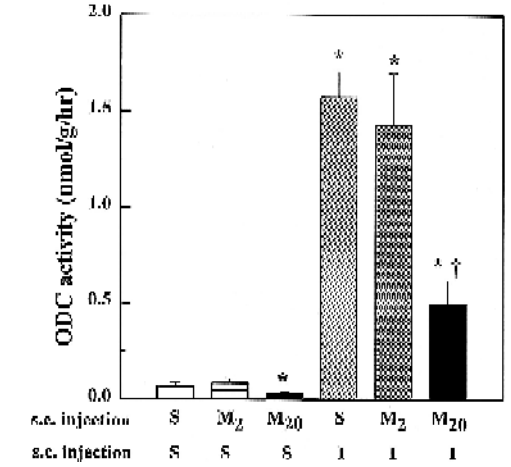

Fig. 5. Effects of peripheral administration of morphine (M) on basal ornithine decarboxylase activity and on insulin (I)-induced increases in ornithine decarboxylase activity in the liver of 6-day-old rats. Animals were injected s.c. with 2 or 20 μg M or saline (S) 15 min before receiving 20 IU I/kg body wt. or S s.c., and liver ornithine decarboxylase activity was assayed 4 h after the second injection. Data represents the means ± S.E.M. of 4–6 individual determinations per group. * $P < 0.05$ vs. S–S group, and † $P < 0.05$ vs. S–I group (Student–Newman–Keuls following two-way ANOVA).

the maintenance of normal development. This concept does not imply that all trophic factors act by a common signal transduction pathway, but only indicates that ornithine decarboxylase is part of a cascade of post receptor events that ultimately affects cell growth and division. It is therefore possible that the low ornithine decarboxylase levels found in tissues of rat pups treated with morphine involve actions on ornithine decarboxylase reactivity to endogenous trophic signals. We tested this possibility by measuring liver ornithine decarboxylase responses to s.c. administration of insulin. Insulin is a potent hepatotrophic hormone (Butcher et al., 1978), and evidence suggest that this occurs in part through its ability to stimulate ornithine decarboxylase activity (Piik et al., 1978). We found that liver ornithine decarboxylase responsiveness to insulin was markedly decreased in animals pretreated i.c.v. with morphine, as compared to control values. Morphine also abolished growth hormone induced stimulation of liver ornithine decarboxylase activity, indicating that this is a generalized opiate effect.

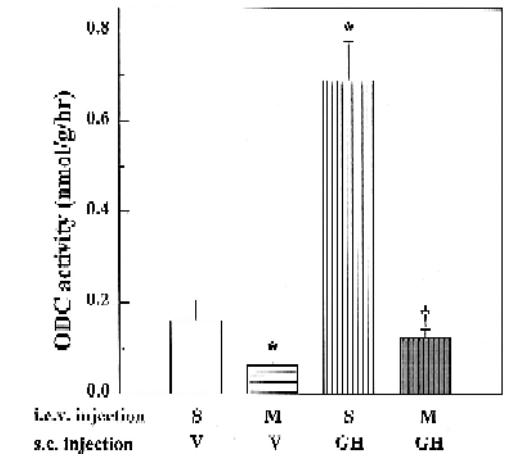

Fig. 6. Effects of morphine (M) on growth hormone (GH) induced increases in liver ornithine decarboxylase activity. Six-day-old rats were injected i.c.v. with 2 μg M or saline (S) 15 min before receiving 5 mg GH/kg body wt. or vehicle (V; $NaHCO_3$ 0.03 M) s.c., and ornithine decarboxylase activity was determined 4 h after the second injection. Data represent the means ± S.E.M. of 7 or 8 determinations per group. * $P < 0.05$ vs. S–V group, and † $P < 0.05$ vs. S–GH group (Student–Newman–Keuls following two-way ANOVA).

The results obtained demonstrate that centrally administered morphine can suppress ornithine decarboxylase activity in developing rat tissues by inhibiting cell metabolic activity (as indicated by the subnormal basal ornithine decarboxylase levels), and/or by reducing tissue ornithine decarboxylase sensitivity to 'classical' trophic factors (as indicated by the decreased liver ornithine decarboxylase sensitivity to insulin and growth hormone). Morphine's ability to inhibit liver ornithine decarboxylase responses to both insulin and growth hormone suggests further that the opiate prevents ornithine decarboxylase stimulation by acting at a signal transduction step beyond the plasma membrane hormone receptors. Also, decreased tissue sensitivity to peptide hormones resulting from loss of receptors has characteristics that are inconsistent with the rapid onset of the effect seen in this study (Kahn et al., 1973; Sherman et al., 1977). The changes in ornithine decarboxylase could be blocked by the concomitant i.c.v. injection of morphine plus naloxone (and opioid antagonist), indicating that CNS morphine influences ornithine decarboxylase expression in central as well as in peripheral tissues by interacting with brain opioid receptors.

Ornithine decarboxylase activity can be modulated at multiple levels, including ornithine decarboxylase mRNA transcription and/or stability, translation of ornithine decarboxylase mRNA, degradation of the ornithine decarboxylase protein enzyme, and enzyme inhibitors/activators (Kahana and Nathans, 1985; Hölttä and Pohjanpelto, 1986; Katz and Kahana, 1987). Whereas more than one mode of control of ornithine decarboxylase activity may act within a tissue in response to a particular stimuli, in other tissues a single regulatory mechanism may be involved (Slotkin and Bartolome, 1983). Using Northern blotting analysis, we measured the amount of liver ornithine decarboxylase mRNA to determine whether the suppression of basal and/or induced ornithine decarboxylase activity by morphine could be explained by actions of the opiate at the level of ornithine decarboxylase gene transcription.

As was the case for insulin induced stimulation of ornithine decarboxylase activity, i.c.v. administration of morphine markedly inhibited insulin induced stimulation of hepatic ornithine decarboxylase mRNA accumulation. The magnitude of the effect closely resembles that of the opiate on ornithine decarboxylase activity. Given the strong correlation between ornithine decarboxylase mRNA levels

and ornithine decarboxylase activity, and having previously shown that insulin heightens hepatic ornithine decarboxylase activity by increasing the number of ornithine decarboxylase mRNA transcripts (Bartolome et al., 1995b), it appears quite likely that morphine prevents insulin from stimulating hepatic ornithine decarboxylase activity by interfering with insulin's ability to activate ornithine decarboxylase gene transcription. In turn, contrary to the inhibition of basal ornithine decarboxylase activity, morphine failed to decrease basal liver ornithine decarboxylase mRNA levels when given alone, suggesting the involvement of translational or posttranslational processes in opiate induced suppression of steady-state (unstimulated) ornithine decarboxylase activity. These observations lend further support to the postulated existence of at least two distinctly different mechanisms through which stimulation of brain μ-opioid receptors can downregulate peripheral ornithine decarboxylase in neonatal rats (Bartolome et al., 1995b). While the inhibition of stimulated hepatic ornithine decarboxylase activity by morphine apparently reflects a decrease in ornithine decarboxylase gene transcription, the inhibition of basal ornithine decarboxylase activity seems to involve posttranscriptional mechanisms.

In developing rats, the blood–brain barrier (BBB) does not fully mature until the end of the third postnatal week (Johanson, 1980). Therefore, it is conceivable that the deficits in ornithine decarboxylase activity seen in the liver of rat pups given morphine centrally could result from a direct action of morphine on target organs after leakage from its intracerebral site of injection into systemic blood. However, this possibility seems unlikely since, in contrast to the central route of administration, s.c. injection of the same dose of morphine as used i.c.v. (2 μg) was unable to decrease basal liver ornithine decarboxylase levels or to reverse insulin's evoked increases of ornithine decarboxylase activity. Thus, the effects of i.c.v. morphine on peripheral ornithine decarboxylase appear to be triggered by signals that originate within the brain. Similarly, we have previously shown that central, but not peripheral, administration of β-endorphin downregulates ornithine decarboxylase activity in peripheral tissues of rat pups (Bartolome et al., 1986, 1995b).

The finding that morphine acts supraspinally to downregulate ornithine decarboxylase activity does not imply that the opiate is unable to affect ornithine decarboxylase when applied systemically. In contrast to β-endorphin, passage of morphine through the BBB is well recognized (Oldendorf et al., 1972) and, as indicated above, neonatal rats exhibit anatomically immature BBB. In fact, a robust inhibition of both basal and induced liver ornithine decarboxylase activity was obtained in animals administered 20 μg of morphine s.c., a dose one order of magnitude higher than the dose given i.c.v. This larger s.c. dose of morphine triggers the peripheral ornithine decarboxylase effects presumably by stimulating μ-opioid receptors located at central sites after crossing the BBB and penetrating into the brain. Incidentally, this dose of morphine (equivalent to 20 μmol/kg body weight) is considered analgesic for rats, because the LD_{50} of morphine in rats is about 500 μmol/kg (Christensen et al., 1976).

Remarkably, the above described actions of morphine on tissue ornithine decarboxylase activity and ornithine decarboxylase mRNA levels exactly mirror those we have obtained in rat pups of the same age centrally administered β-endorphin (Bartolome et al., 1995b). Taken together, the evidence obtained strongly suggests that morphine and morphine-like drugs exert their detrimental effects in developing humans and experimental animals, at least in part, by exacerbating the effects of endogenous opioid peptides on ornithine decarboxylase. As previously discussed, during development CNS β-endorphin (or a β-endorphin-like peptide) normally seem to regulate growth by tonically inhibiting tissue ornithine decarboxylase expression (Bartolome et al., 1995a). Interestingly, in contrast to the inhibitory effect of exogenous administration of opiates on brain DNA synthesis, endogenous opioids do not appear to tonically depress DNA synthesis (Kornblum et al., 1987).

As noted earlier, morphine produces its pharmacologic effects by preferentially stimulating μ-opioid receptors, and our previous work clearly demonstrated that CNS β-endorphin downregulates ornithine decarboxylase activity in central as well as in peripheral tissues by acting upon brain μ-opioid receptors, but not with δ- or -opioid receptors (Bartolome et al., 1995a). There is evidence to suggest brain μ-opioid receptor function in infant rats is refractory to developing tolerance to chronic exposure to opiates relative to adult animals (Windh et al., 1995). Tolerance is a state of diminished responsiveness to repeated drug administration. This implies that the developing organism could experience a more intense opiate action than an adult exposed to a similar dose. Consequently lack of tolerance of cellular ornithine decarboxylase response system(s) would make neonates particularly vulnerable to the detrimental developmental consequences of opiates. In addition, morphine is eliminated primarily by liver metabolism and has a much longer elimination half-life in the human neonate than in the older child or adult. This is caused by a low hepatic clearance value, which increases with age (Olkkola et al., 1988).

Finally, it is noteworthy that, in humans, newborn babies of non-opiate-addicted mothers have plasma levels of β-endorphin about 4 times higher than their mothers, but they decrease to adult values by postnatal day 5 (Csontos et al., 1979; Moss et al., 1982). In contrast, plasma levels of β-endorphin in newborn infants of opiate-addicted mothers increase dramatically on postnatal day 2 (up to 1,000-fold the adult values) and remain supranormal at 40 days of age, even in those infants showing good clinical control of withdrawal syndrome (Panerai et al., 1983). Elevated concentrations of β-endorphin have also been found in the hypothalamus and

pituitary gland of foetuses of opiate-addicted mothers (Gianoulakis, 1986) and in foetuses and newborns of morphine-treated rats (Bianchi et al., 1988). It is therefore quite possible that the developmental deficits associated with maternal opiate use or abuse may be the result of continuous overstimulation of opioid receptors caused by the repeated administration of opiate drugs, as well as by an increase in endogenous opioid activity.

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References

Bartolome, J.V., Bartolome, M.B., Daltner, L.A., Evans, C.J., Barchas, J.D., Kuhn, C.M., Schanberg, S.M., 1986. Effects of β-endorphin on ornithine decarboxylase in tissues of developing rats: A potential role for this endogenous neuropeptide in the modulation of tissue growth. Life Sci. 38, 2355.

Bartolome, J.V., Bartolome, M.B., Harris, E.B., Schanberg, S.M., 1987. Nα-acetyl-β-endorphin stimulates ornithine decarboxylase activity in preweanling rat pups: Opioid and non-opioid-mediated mechanisms. J. Pharmacol. Exp. Ther. 240, 895.

Bartolome, J.V., Bartolome, M.B., Lorber, B.A., Schanberg, S.M., 1991. CNS β-endorphin regulation of insulin-induced ornithine decarboxylase expression in liver of neonatal rats. Mol. Cell. Neurosci. 2, 1.

Bartolome, J.V., Chang, K.-J., Bartolome, M.B., 1995a. The inhibition of ornithine decarboxylase activity in developing rat tissues by central nervous system β-endorphin is mediated by μ-opioid receptors, but not by δ- or -opioid receptors. Eur. J. Pharmacol. 284, 43.

Bartolome, J.V., Wang, S., Bartolome, M.B., 1995b. Transcription-dependent and -independent regulation of hepatic ornithine decarboxylase activity by CNS β-endorphin in rat pups. Mol. Brain Res. 33, 149.

Berger, F.G., Szymanski, P., Read, E., Watson, G., 1984. Androgen-regulated ornithine decarboxylase mRNAs of mouse kidney. J. Biol. Chem. 259, 7941.

Bianchi, M., Marini, A., Sacerdote, P., Cocco, E., Brini, A., Panerai, A.E., 1988. Effect of chronic morphine on plasma and brain β-endorphin and methionine enkephalin in pregnant rats and in their fetuses or newborn. Neuroendocrinology 47, 89.

Butcher, M.L.R., Patel, U., Cohen, S., 1978. Hormonal factors concerned with liver regeneration. In: Porter, R., Whelan, J. (Eds.), Hepatotrophic Factors. CIBA Foundation Symposium 55. Elsevier, New York, p. 95.

Chasnoff, I.J., 1985. Effects of maternal narcotic vs. nonnarcotic addiction on neonatal neurobehavior and infant development. In: T.M. Pinker (Ed.), Consequences of Maternal Drug Abuse. National Institute on Drug Abuse, Washington, DC, p. 84.

Chomczynski, P., Sacchi, N., 1987. Single-step method for RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156.

Christensen, H.E., Fairchild, E.J., Caroll, B.S., Lewis, R.J., 1976. Registry of Toxic Effects of Chemical Substances. U.S. Department of Health Education and Welfare, Baltimore, MD, p. 729.

Csontos, K., Rust, M., Höllt, V., Mahr, W., Kromer, W., Teschemacher, H.J., 1979. Elevated plasma β-endorphin levels in pregnant women and their neonates. Life Sci. 25, 835.

Gianoulakis, G., 1986. Effect of maternally administered opiates on the development of the beta-endorphin system in the offspring. NIDA Res. Monogr. 75, 595.

Greer, N.L., Schanberg, S.M., Bartolome, J.V., 1991. Effect of central administration of β-endorphin on lung ornithine decarboxylase activity in developing rats. Pediatr. Res. 29, 182.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12, 12.

Hauser, K.F., 1992. Morphine regulates DNA synthesis in rat cerebellar neuroblasts in vitro. Dev. Brain Res. 70, 291.

Heby, O., 1981. Role of polyamines in the control of cell proliferation and differentiation. Differentiation 19, 1.

Hölttä, E., Pohjanpelto, P., 1986. Control of ornithine decarboxylase in Chinese hamster ovary cells by polyamines. J. Biol. Chem. 261, 9502.

Hutchings, D.E., 1991. Opiates during pregnancy: Neurobehavioral effects in the offspring. Adv. Biosci. 80, 285.

Johanson, C.E., 1980. Permeability and vascularity of the developing brain: Cerebellum vs cerebral cortex. Brain Res. 190, 3.

Kahana, C., Nathans, D., 1985. Translational regulation of mammalian ornithine decarboxylase by polyamines. J. Biol. Chem. 260, 15390.

Kahn, C.R., Neville, D.M. Jr, Roth, J., 1973. Insulin-receptor interaction in the obesehyperglycemic mouse. J. Biol. Chem. 248, 244.

Katz, A., Kahana, C., 1987. Transcriptional activation of mammalian ornithine decarboxylase during stimulated growth. Mol. Cell. Biol. 7, 2641.

Kornblum, H.I., Loughlin, S.E., Leslie, F.M., 1987. Effects of morphine on DNA synthesis in neonatal rat brain. Dev. Brain Res. 31, 45.

Metcalf, B.W., Bey, P., Danzin, C., Jung, M.J., Casara, P., Vevert, J.P., 1978. Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and products analogues. J. Am. Chem. Soc. 100, 2551.

Moss, I.R., Conner, H., William, B.A., Yee, F.H., Iorio, P., Scarpelli, E.M., 1982. Human β-endorphin-like immunoreactivity in the perinatal/neonatal period. J. Pediatr. 101, 443.

Naeye, R.L., Blanc, W., Leblanc, W., Khatamee, M.A., 1973. Fetal complications of maternal heroin addiction: Abnormal growth, infections, and episodes of stress. J. Pediatr. 83, 1055.

Oldendorf, W.H., Hyman, S., Braun, L., Oldendorf, S.Z., 1972. Blood–brain barrier. Penetration of morphine, codeine, heroin and methadone after carotid injection. Science 176, 984.

Olkkola, K.T., Maunuksela, E.-L., Korpela, R., Rosenberg, P.H., 1988. Kinetics and dynamics of postoperative intravenous morphine in children. Clin. Pharmacol. Ther. 44, 128.

Panerai, A.E., Martini, A., DiGiulio, A.M., Fraioli, F., Vegni, C., Pardi, G., Marini, A., Mantegazza, P., 1983. Plasma β-endorphin, β-lipotropin, and met-enkephalin concentrations during pregnancy in normal and drug-addicted women and their newborn. J. Clin. Endocrinol. Metab. 57, 537.

Pegg, A.E., McCann, P.P., 1982. Polyamine metabolism and function. Am. J. Physiol. 243, C212.

Pegg, A.E., Williams-Ashman, H.G., 1968. Biosynthesis of putrescine in the prostate gland of the rat. Biochem. J. 100, 533.

Piik, K., Poso, H., Janne, J., 1978. Reversible inhibition of rat lvier regeneration by 1,3-diamino-2-propanol, an inhibitor of ornithine decarboxylase. FEBS Lett. 89, 307.

Pohjanpelto, P., Höltta, E., Jänne, O.A., Knuutila, S., Alitalo, K., 1985. Amplification of ornithine decarboxylase gene in response to polyamine deprivation in Chinese hamster ovary cells. J. Biol. Chem. 260, 8532.

Russell, D.H., 1980. Ornithine decarboxylase as a biological and pharmacological tool. Pharmacology 20, 117.

Russell, D.H., 1985. Ornithine decarboxylase: A key regulatory enzyme in normal and neoplastic growth. Drug Metab. Rev. 16, 1.

Sherman, B.M., Stagner, J.I., Zamudio, R., 1977. Regulation of lactogenic hormone binding in rat liver by steroid hormones. Endocrinology 100, 101.

Slotkin, T.A., 1979. Ornithine decarboxylase as a tool in developmental neurobiology. Life Sci. 24, 1623.

Slotkin, T.A., Bartolome, J., 1983. Ornithine decarboxylase: Marker of neuroendocrine and neurotransmitter actions. Methods Enzymol. 103, 59.

Slotkin, T.A., Bartolome, J., 1986. Role of ornithine decarboxylase and the polyamines in nervous system development: A review. Brain Res. Bull. 17, 307.

Slotkin, T.A., Seidler, F.J., Trepanier, P.A., Whitmore, W.L., Lerea, L., Barnes, G.A., Weigel, S.J., Bartolome, J., 1982. Ornithine decarboxylase and polyamines in tissues of the neonatal rat: Effects of α-difluoromethylornithine, a specific, irreversible inhibitor of ornithine decarboxylase. J. Pharmacol. Exp. Ther. 222, 741.

Wen, L., Huang, J.-K., Blackshear, P.J., 1989. Rat ornithine decarboxylase gene. J. Biol. Chem. 264, 9016.

Wilson, G.S., 1992. Heroin use during pregnancy: Clinical studies of long-term effects. In: Sonderegger, T.B. (Ed.), Perinatal Substance Abuse. The Johns Hopkins University Press, Baltimore, MD, p. 224.

Windh, R.T., Little, P.J., Kuhn, C.M., 1995. The ontogeny of μ opiate tolerance and dependence in the rat: Antinociceptive and biochemical studies. J. Pharmacol. Exp. Ther. 273, 1361.

Zagon, I.S., McLaughlin, P.J., 1992. The perinatal opioid syndrome: Laboratory findings and clinical implications. In: Sonderegger, T.B. (Ed.), Perinatal Substance Abuse. The Johns Hopkins University Press, Baltimore, MD, p. 207.

Zelson, C., Rubio, E., Wasserman, E., 1971. Neonatal narcotic addiction: 10 year observation. Pediatrics 48, 178.