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European Journal of Pharmacology 284 (1995) 43–50

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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 ε-opioid receptors

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Received 23 March 1995; revised 24 May 1995; accepted 9 June 1995

Abstract

Our laboratory has previously shown that intracerebroventricular (i.c.v.) administration of β-endorphin suppresses brain and liver ornithine decarboxylase activity (ODC; a growth regulatory enzyme) in preweanling rats. This investigation examined, in 6-day-old rats, the relative participation of brain μ-, δ- and ε-opioid receptors in β-endorphin's ODC effects, by comparing tissue ODC responses to β-endorphin given alone i.c.v. and in the presence of D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP; μ-opioid receptor antagonist), *N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI-174,864; δ-opioid receptor antagonist) or β-endorphin-(1–27) (ε-opioid receptor antagonist). Administration of 0.5 μg of β-endorphin alone significantly decreased brain and liver ODC activity 4 h after injection, and the effect was completely blocked by coinjection of CTOP (0.075 μg) but not by ICI-174,864 (0.75 or 3.75 μg) or β-endorphin-(1–27) (3.75 or 7.5 μg). The blockade of endogenous opioid:opioid receptor interactions by either CTOP (at doses > 0.075 μg) or ICI-174,864 alone was accompanied by increased levels of basal ODC activity. The results obtained demonstrate that i.c.v. β-endorphin downregulates ODC expression in central as well as in peripheral tissues by interacting with brain μ-opioid receptors, but not with δ- or ε-opioid receptors or μ/δ-opioid receptor complexes. Also, they indicate that endogenous opioid systems have a tonic inhibitory influence on ODC activity which is mediated, at least in part, by μ- and δ-opioid receptors.

Keywords: Opioid; Growth; Development; Brain; Liver; (Intracerebroventricular)

1. Introduction

Ornithine decarboxylase (ODC; E.C. 4.1.1.17) 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 cellular growth, multiplication and differentiation (Heby, 1981; Pegg and McCann, 1982; Slotkin and Bartolome, 1986). Due to its extremely short half-life (10–20 min), ODC activity is susceptible to rapid and profound change. Each tissue has a characteristic ODC ontogenetic pattern, and perturbations of these patterns are invariably associated with subsequent alterations in tissue maturation and/or function (Slotkin, 1979).

We have shown that intracerebral administration of β-endorphin produces a marked decrease in ODC activity throughout the body in preweanling, but not adult rats (Bartolome et al., 1986, 1991; Greer et al., 1991). These and other findings (Bartolome et al., 1991, 1994) support the hypothesis that endogenous central nervous system (CNS) β-endorphin plays an important role in controlling postnatal development, in part, by downregulating tissue ODC expression.

Although β-endorphin has been known for two decades, the opioid receptor type(s) through which this potent endogenous opioid peptide acts to elicit its physiological functions is still not well established. At least three distinct types of opioid receptors are present in the mammalian brain, i.e. μ-, δ- and κ-opioid receptors (Martin, 1984; Chang, 1984; Simon, 1987; Goldstein and Naidu, 1989). According to ligand-receptor binding studies in brain membrane preparations, β-endorphin has about equal affinity for both μ- and δ-opioid receptors, but negligible affinity for κ-

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Elsevier Science B.V.
SSDI 0014-2999(95)00358-4

opioid receptors (Akil et al., 1981; Paterson et al., 1983). The rat vas deferens contains a distinct type of opioid receptor designated ϵ-opioid receptor, which is highly selective for β-endorphin (Lemaire et al., 1978; Schulz et al., 1981; Garzón et al., 1985), and increasing evidence now indicates that ϵ-opioid receptors also are present in the brain of many species, including the rat (Johnson et al., 1982; Chang et al., 1984; Nock et al., 1993).

Evidence to suggest the involvement of opioid receptors in opioid's actions is nearly always drawn from experiments using naloxone or naltrexone. While most actions of β-endorphin are naloxone-and/or naltrexone-reversible (indicating the participation of μ- or δ-opioid receptors), other studies have reported biological effects of this peptide which are not blocked by these potent opioid antagonists (indicating that they are not mediated by μ- or δ-opioid receptors) (Haynes, 1985; Shahabi et al., 1990). Previously, we have found that naloxone completely blocks the inhibitory effect of β-endorphin on brain ODC activity when coinjected i.c.v. into rat pups, but does not prevent the peptide from inhibiting liver ODC activity (Bartolome et al., 1986). Identical results were obtained in rats pups given naloxone s.c. instead of i.c.v. (Bartolome et al., 1991). These observations imply that CNS β-endorphin's control of ODC activity may occur via interactions with centrally located μ- or δ-opioid receptors (naloxone-sensitive), with putative brain ϵ-opioid receptors (poorly blocked by naloxone), or perhaps through non-opioid-mediated mechanisms.

The current studies were undertaken to identify, in 6-day-old rats, the precise opioid receptor type(s) through which CNS β-endorphin acts to regulate ODC expression in central and peripheral tissues. More specifically, by using selective opioid receptor antagonists this investigation assessed the relative contribution of brain μ-, δ- and ϵ-opioid receptors in the decreases in brain and liver ODC activity produced by intracerebroventricular (i.c.v.) administration of β-endorphin. The liver was selected as a peripheral tissue, because hepatic ODC is highly sensitive to changes in CNS β-endorphin function (Bartolome et al., 1986).

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 shipped by climate-controlled truck, housed in breeding cages in a vivarium maintained at 22°C with a 12-h light-dark cycle, and were allowed free access to food (Purina Lab Chow, Ralston-Purina, St. Louis, MO, USA) and water. 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 minimize maternal caretaking differences, pups from all litters were randomized and redistributed to the nursing mothers at this time. In addition, on the experimental day, pups from different treatment groups were assigned to each dam. All animals were killed at about the same time of the day to eliminate circadian influences on the variables under investigation. At 6 days of age, rat pups were injected i.c.v. with 0.5 μg of β-endorphin (dissolved in 10 μl of saline) alone, together with selected opioid receptor antagonists or with saline, and animals were killed by decapitation 4 h later. Intracerebroventricular injections were performed according to the technique of Haley and McCormick (1957). Experiments were replicated at least once to verify reproducibility.

2.2. Ornithine decarboxylase activity

The brains and livers were quickly dissected, weighed and homogenized (Polytron) in 19 vols. (w/v) of ice-cold 10 mM Tris-HCl (pH 7.2), and ODC activity was determined in the 26000 $\times g$ at 20 min supernatant as previously reported (Bartolome et al., 1991). Essentially, the incubation medium contained 0.9 ml of supernatant and final concentrations 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 ODC was determined by running a parallel incubation in the presence of 5 mM α-difluoromethylornithine, a specific, irreversible inhibitor of ODC activity (Metcalf et al., 1978). ODC activity is expressed as nmol of $^{14}CO_2$ evolved/g of tissue weight/h.

2.3. Drugs and reagents

L-[1-^{14}C]Ornithine monohydrochloride (57.2 mCi/mmol) was obtained from DuPont NEN (Boston, MA, USA), β-endorphin (β-lipotropin-(61–91) human), β-endorphin-(1–27) human, [*N*-MePhe^3,D-Pro^4]morphiceptin (PL017) and D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP) from Peninsula Laboratories (Belmont, CA, USA), *N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI-174,864) from Cambridge Research Biochemicals (Wilmington, DE, USA), naloxone

hydrochloride from Sigma Chemical Corp. (St. Louis, MO, USA), and hyamine hydroxide (scintillation grade) from Research Products International (Mount Prospect, IL, USA). All reagents were analytical grade.

2.4. 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 or Dunnett's tests for multiple comparisons, where appropriate. Significance was accepted at the level of $P < 0.05$. Probability at the level of $P < 0.055$ was also noted.

3. Results

To establish whether CNS β-endorphin-induced suppression of tissue ODC activity is mediated by μ-opioid receptors, 6-day-old rat pups were injected

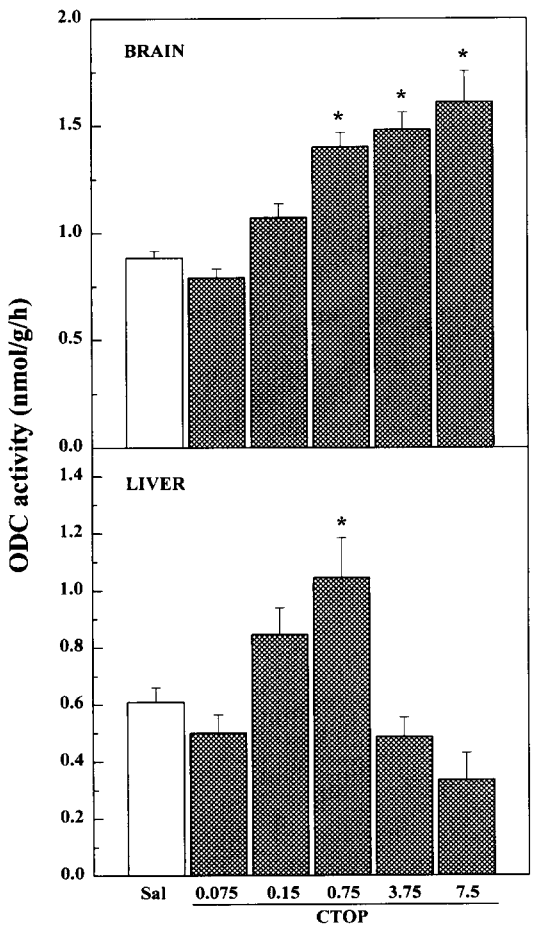

Fig. 1. Effect of different doses (μg, i.c.v.) of CTOP on basal tissue ODC levels. Data represent the means ± S.E.M. of 9–50 individual determinations per group, per tissue. * $P < 0.05$ vs. their respective saline (Sal) groups (Dunnett's).

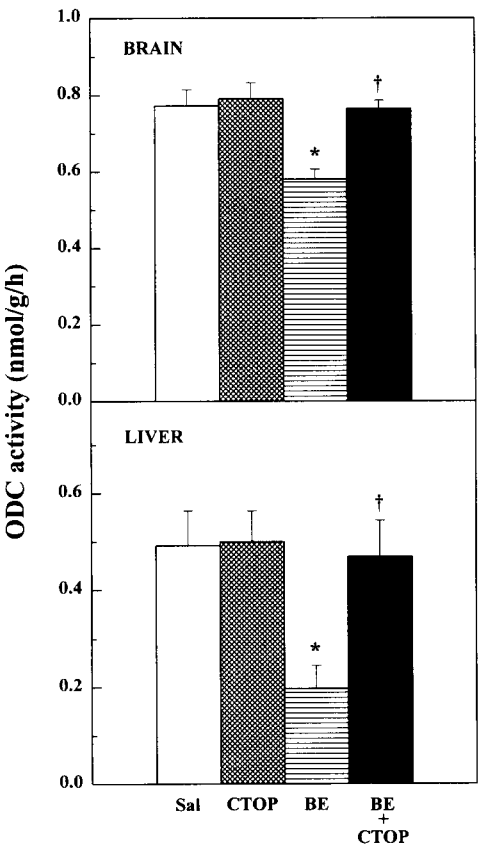

Fig. 2. Effect of CTOP (0.075 μg, i.c.v.) on β-endorphin-(BE; 0.5 μg, i.c.v.) induced decrease of tissue ODC activity. Data represent the means ± S.E.M. of 9–12 individual determinations per group, per tissue. * $P < 0.05$ vs. their respective saline (Sal) groups, and † $P < 0.05$ vs. their respective BE groups (Student-Newman-Keuls).

i.c.v. with β-endorphin, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2 (CTOP), a potent and highly selective μ-opioid receptor antagonist (Gulya et al., 1986; Hawkins et al., 1989), or the combination of the two, and brain and liver ODC activity were measured 4 h after injection. It has been reported that 1.0 μg of CTOP given i.c.v. blocks β-endorphin-induced increase of dopamine release in the nucleus acumbens (Spanagel et al., 1990) and reinforcing (Bals-Kubik et al., 1990) and antitransit effects of the peptide (Shook et al., 1988). We administered 0.075 μg of CTOP instead, because in preliminary studies (Fig. 1) we found that higher doses increased basal tissue ODC levels by themselves, which could confound interpretation of the results obtained. As shown in Fig. 2, i.c.v. administration of 0.5 μg of β-endorphin significantly decreased brain and liver ODC activity. More importantly, coinjection of 0.075 μg of CTOP (a dose which did not affect basal ODC activity when given alone) with β-endorphin effectively prevented β-endorphin from inhibiting both brain ($F(1,40) = 5.25$; $P < 0.05$) and liver ($F(1,43) = 4.82$; $P < 0.05$) ODC activity.

To test whether substances known to activate μ-opioid receptors other than β-endorphin also are able to lower tissue ODC activity, we injected i.c.v. [*N*-MePhe^3,D-Pro^4]morphiceptin (PL017), a selective μ-opioid receptor agonist (Chang et al., 1983), to a separate group of rat pups, and measured brain and liver ODC activity. Similarly to β-endorphin, administration of a relatively small dose of PL017 (0.05 μg) significantly decreased ODC activity and, as was the case for β-endorphin, coinjection of CTOP (0.075 μg) with PL017 completely blocked the effect in the brain ($F(1,43) = 3.9$; $P < 0.055$) as well as in the liver ($F(1,43) = 5.06$; $P < 0.05$) (Fig. 3).

There is some evidence indicating that β-endorphin may produce certain opioid responses by interacting with a μ/δ-opioid receptor complex in the CNS (Schoffelmeer et al., 1988; Bals-Kubik et al., 1990). Thus, to find out whether δ-opioid receptors may also be involved in β-endorphin's ODC actions we assessed the effect of *N,N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI-174,864), a highly selective δ-opioid receptor antagonist (Cotton et al., 1984; Cowan et al., 1985) on

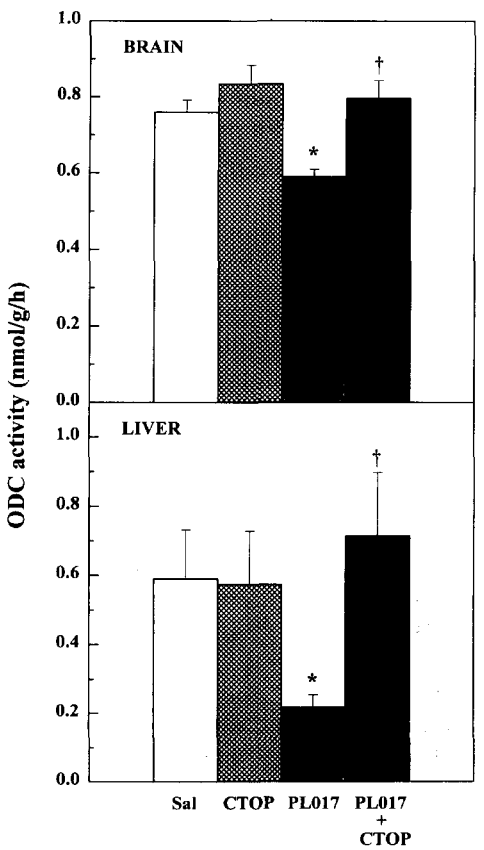

Fig. 3. Effect of PL017 (0.05 μg) administered alone i.c.v. or together with CTOP (0.075 μg) on tissue ODC activity. Data represent the means ± S.E.M. of 11 or 12 individual determinations per group, per tissue. * $P < 0.05$ vs. their respective saline (Sal) groups, and † $P < 0.05$ vs. their respective PL017 groups (Student-Newman-Keuls).

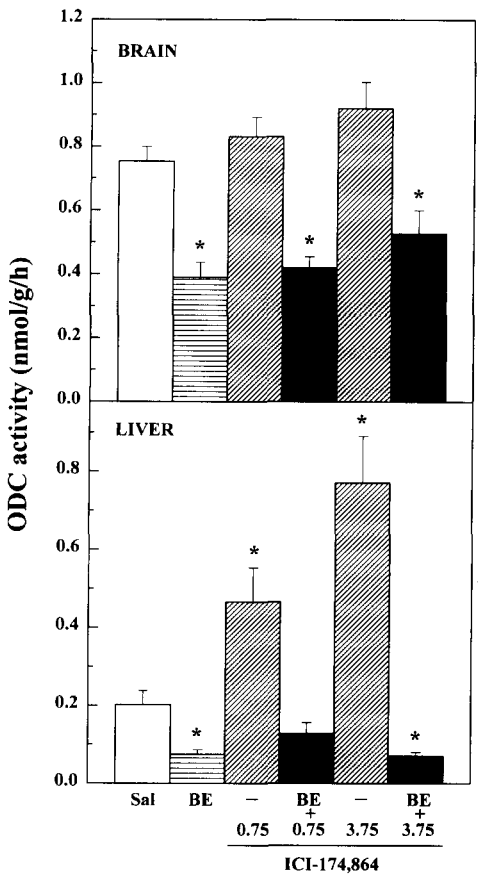

Fig. 4. Effect of ICI-174,864 (0.75 or 3.75 μg, i.c.v.) on β-endorphin-(BE; 0.5 μg, i.c.v.) induced decrease of tissue ODC activity. Data represent the means ± S.E.M. of 13–17 individual determinations per group, per tissue. * $P < 0.05$ vs. their respective saline (Sal) groups (Student-Newman-Keuls).

β-endorphin-induced inhibition of ODC activity. As depicted in Fig. 4, i.c.v. administration of β-endorphin together with 0.75 μg of ICI-174,864, a dose that has been shown to block δ-opioid receptor-mediated effects of β-endorphin (Tseng et al., 1985; Gulya et al., 1986), or even a dose 5 times higher did not prevent β-endorphin from suppressing ODC activity. On the other hand, similar to CTOP, when either dose of ICI-174,864 was given alone a marked increase in basal ODC levels was obtained in the liver (Fig. 4). This ICI-174,864-induced increase of hepatic ODC levels was effectively inhibited by β-endorphin.

Finally, to account for the possibility that centrally located ϵ-opioid receptors may contribute to β-endorphin-induced inhibition of tissue ODC expression, we administered the peptide i.c.v. together with 3.75 or 7.5 μg of β-endorphin-(1–27), a putative ϵ-opioid receptor antagonist (Hammonds et al., 1984) and measured ODC activity. These doses of β-endorphin-(1–27) have been reported to block opioid responses in rodents evoked by i.c.v. doses of β-endorphin equivalent

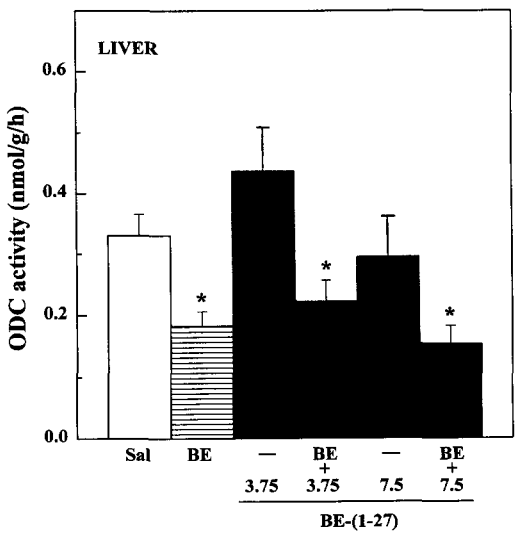

Fig. 5. Effect of β-endorphin-(1–27) (BE-(1–27); 3.75 or 7.5 μg, i.c.v.) on β-endorphin-(BE; 0.5 μg, i.c.v.) induced decrease of liver ODC activity. Data represent the means ± S.E.M. of 11–28 individual determinations per group. * $P < 0.05$ vs. saline (Sal) group (Student-Newman-Keuls).

to that used in the current studies (Suh et al., 1988). The liver was used as a target tissue for these additional experiments because hepatic ODC is far more sensitive to i.c.v. β-endorphin, compared to the brain. As shown in Fig. 5, coinjection of β-endorphin-(1–27) with β-endorphin did not affect β-endorphin's ability to inhibit liver ODC activity. In contrast to CTOP and ICI-174,864, administration of β-endorphin-(1–27) alone had no overall effect on basal liver ODC levels.

4. Discussion

In this investigation, we have utilized selective opioid receptor antagonists to identify, in the brain, the specific opioid receptor type(s) through which i.c.v. administered β-endorphin acts to decrease brain and liver ODC activity. It was found that the highly selective μ-opioid receptor antagonist CTOP (Gulya et al., 1986; Hawkins et al., 1989) completely prevented β-endorphin from inhibiting brain and liver ODC activity when coinjected i.c.v. into 6-day-old rat pups. This finding strongly suggest that β-endorphin's ODC effects are mediated by μ-opioid receptors. Also, they are in line with reports showing CTOP to effectively antagonize other effects of supraspinally administered β-endorphin, including analgesia (Smith et al., 1992), inhibition of gastrointestinal transit (Shook et al., 1988) and electrically evoked release of [^{3}H]NE from neocortical slices (Schoffelmeer et al., 1991).

It is noteworthy that, while the dose of CTOP (0.075 μg) used in the above experiments did not significantly alter basal tissue ODC levels when given alone, administration of higher doses (0.15–7.5 μg) produced a marked increase in brain ODC activity. One likely explanation for this effect can be that CTOP prevents endogenous β-endorphin and possibly other naturally occurring opioid peptides from interacting with μ-opioid receptors, and thereby from decreasing tissue ODC activity. Thus, CTOP-evoked increases in brain ODC levels could actually reflect an inhibition of endogenous opioid function. This is particularly important as, contrary to the general thought that opioid systems (i.e. opioid peptides and opioid receptors) are normally quiescent under basal conditions, it suggests that they exert a tonic inhibitory influence on ODC and that it occurs, at least in part, via μ-opioid receptors. A more complex dose-response relationship seems to exists regarding CTOP : μ-opioid receptor interactions and liver ODC activity. Whereas intermediate doses of CTOP (0.15 and 0.75 μg) produced an increase in hepatic ODC levels, higher doses (3.75 and 7.5 μg) produced a mild, though consistent, fall in enzyme levels. The bases for these disparate liver ODC responses to CTOP remain obscure. One possibility is that, at high doses, CTOP might act as partial μ-opioid receptor agonist. The finding that only liver ODC levels showed a decrease could reflect a higher sensitivity of μ-opioid receptors controlling peripheral ODC to μ-opioid receptor agonists vs. those controlling central ODC. The fact that the inhibitory effect of β-endorphin on liver ODC activity was relatively larger compared to the brain is consistent with this idea. Alternatively, μ-opioid receptors influencing brain ODC may be physically located in brain regions more distant from the site (i.c.v.) of CTOP and β-endorphin injection than those influencing liver ODC, and therefore, more difficult to reach by the drug.

If brain μ-opioid receptors do indeed play a prominent role in mediating the tonic inhibition of ODC activity throughout the body by endogenous opioids, one might expect that central administration of substances with known selectivity towards this opioid receptor type, other than β-endorphin, also should be able to lower tissue ODC activity. We tested this hypothesis by injecting (i.c.v.) PL017, a highly preferential μ-opioid receptor agonist (Chang et al., 1983), to a separate group of rat pups. As predicted, PL017 significantly decreased brain and liver ODC activity. Furthermore, as was the case with β-endorphin, coinjection of CTOP with PL017 abolished the ODC effect of PL017 in both tissues. The comparable antagonism of PL017 and β-endorphin by the μ-opioid receptor antagonist CTOP confirms that the two are acting at the same site, and shows that β-endorphin stimulates μ-opioid receptors to inhibit ODC activity.

Emerging evidence indicates that μ- and δ-opioid receptors do not always exist as separate entities

throughout the CNS, but they may be physically associated and functionally coupled to form a μ/δ-opioid receptor complex in discrete brain regions (Smith et al., 1983; Schoffelmeer et al., 1988; Bals-Kubik et al., 1990). This macromolecular structure is visualized as consisting of two sites, one binding μ-opioid receptor ligands and the other binding δ-opioid receptor ligands. More importantly, it has been proposed that β-endorphin may exert some effects by activating both μ- and δ-sites simultaneously, and that they can be inhibited if either site is blocked. Consistent with this concept, the blockade of either μ- or δ-opioid receptors has been recently shown to be sufficient to fully abolish the stimulation of dopamine release in the nucleus accumbens (Spanagel et al., 1990) and the reinforcing effects (Bals-Kubik et al., 1990) induced by β-endorphin in rats. In our studies, the possibility therefore exists that i.c.v. β-endorphin decreases tissue ODC activity via functionally linked μ- and δ-opioid receptors. If so, ICI-174,864, a highly selective δ-opioid receptor blocker (Cotton et al., 1984; Cowan et al., 1985), should be as effective as CTOP in antagonizing β-endorphin's ODC effects. However, unlike CTOP, ICI-174,864 failed to reverse β-endorphin-induced suppression of ODC activity at doses which have been shown to block δ-opioid receptor-mediated responses of the peptide (Shook et al., 1988; Spanagel et al., 1990; Bals-Kubik et al., 1990). Therefore, it seems clear that β-endorphin acts upon one distinct i.e. μ- but not δ-opioid receptor site to inhibit ODC activity. It should be pointed out, however, that the inability of ICI-174,864 to affect tissue ODC responses to β-endorphin does not necessarily exclude δ-opioid receptors from mediating ODC effects of other endogenous opioids. In fact, in these experiments, ICI-174,864 profoundly elevated hepatic ODC basal levels when given alone. This observation indicates that δ-opioid receptors may also be involved in the tonic regulation of hepatic ODC activity, since ICI-174,864 by itself enhanced basal ODC levels (presumably by blocking endogenous opioids: δ-opioid receptors interactions). Thus, during normal development, ODC activity may be controlled, at least in part, by multiple CNS endogenous opioid systems acting through distinct opioid receptor types.

If endogenous opioids do interact with μ- and δ-opioid receptors to tonically inhibit tissue ODC expression (a key growth-promoting enzyme) in infant rats, one can anticipate that interruption of opioid: opioid receptor interactions during the first postnatal weeks should alter development. In fact, it has been shown that daily administration of naltrexone (which shows high affinity for both μ- and δ-opioid receptors) from birth, in a dose regimen that produces a continuous blockade of opioid receptors, dramatically increases body and brain size in young rats, increases the number of brain cells, and accelerates the appearance of physical characteristics and spontaneous motor/sensorimotor behaviour (Hauser et al., 1987).

In the periphery, β-endorphin has been proposed to interact with a β-endorphin-selective receptor type commonly referred to as the ϵ-opioid receptor (Schulz et al., 1981; Garzón et al., 1985; Illes et al., 1987). While there is now evidence indicating that ϵ-opioid receptors also are present in the brain of many species, including the rat (Johnson et al., 1982; Chang et al., 1984; Nock et al., 1993), their role in CNS β-endorphin function remains controversial. For example, by selectively stimulating centrally located ϵ-opioid receptors supraspinally administered β-endorphin has been hypothesized to produce antinociceptive effects in mice, which can be blocked by coinjection of β-endorphin-(1–27) (a putative ϵ-opioid receptor antagonist), but not by naloxone or CTOP (Suh et al., 1988; Suh and Tseng, 1990). However, Smith et al. (1992) provided evidence indicating that the receptor mediating the antinociceptive response to β-endorphin injected into the periaqueductal gray region of awake rats is pharmacologically distinct from the ϵ-opioid receptor. Furthermore, Kamei et al. (1993) reported that the antitussive effect of i.c.v. β-endorphin was not blocked by β-endorphin-(1–27). In our studies we have found that, in contrast to CTOP, β-endorphin-(1–27) affected neither liver ODC responses to β-endorphin when given together nor basal ODC levels when given alone. It therefore seems clear that centrally located ϵ-opioid receptors contribute neither to the ODC effects of i.c.v. administered β-endorphin nor to the tonic control of ODC activity by endogenous opioid in rat pups.

In an earlier study (Bartolome et al., 1986), we reported that naloxone (a preferential μ-opioid receptor antagonist) prevented β-endorphin from inhibiting ODC activity in the brain but not in the liver when coinjected i.c.v. into rats of the same age used in the present experiments. From those findings we concluded that brain ODC responses to β-endorphin are mediated by 'classical' opioid receptors, while hepatic ODC responses to the peptide occur through central mechanisms independent of its opioid character. However, the current more extensive studies with more potent and selective opioid receptor antagonists do not support this conjecture. We now know that β-endorphin acts upon the same opioid receptor type, i.e. μ-opioid receptors, to influence both brain and liver ODC and, therefore, one would expect both tissue ODC responses to i.c.v. β-endorphin to be naloxone-reversible. At present we have no explanation for this discrepancy. The failure of naloxone to block the liver ODC effect cannot be attributed to an insufficient dose since, in that study, we found that higher doses actually decreased basal liver ODC levels when given alone (which would have invalidated the results obtained). It needs to be mentioned that naloxone not

always has been shown to effectively block μ-opioid receptor-mediated effects of opioids. For instance, morphine (a typical μ-opioid receptor agonist) inhibits forskolin-stimulated adenylate cyclase activity in the embryonic chick brain, but this effect is not reversed by naloxone (Sakellaridis and Vernadakis, 1986).

In summary, the results obtained indicate that i.c.v. β-endorphin suppresses ODC expression in both central and peripheral tissues of infant rats by specifically acting upon brain μ-opioid receptors. Also, they suggest that endogenous CNS opioid systems may exert a tonic inhibitory influence on ODC activity and do so by stimulating μ- as well as δ-opioid receptors. The existence of a fine-tuned regulation of tissue ODC activity by endogenous opioid systems adds further support to our hypothesis that CNS β-endorphin, and possibly other related opioid peptides, play an important role in controlling postnatal development.

Acknowledgements

This research was supported by U.S. Public Health Service Grant RO1 NS 25738. We wish to thank Dr. Norman Kirshner for critically reading the manuscript.

References

Akil, H., E. Young and S.J. Watson, 1981, Opiate binding properties of naturally-occurring N- and C-terminus modified β-endorphins, Peptides 2, 289.

Bals-Kubik, R., T.S. Shippenberg and A. Herz, 1990, Involvement of central μ and δ opioid receptors in mediating the reinforcing effects of β-endorphin in the rat, Eur. J. Pharmacol. 175, 63.

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

Bartolome, J.V., J.G. Johnston and S.M. Schanberg, 1994, The inhibition of liver ornithine decarboxylase expression in neonatal rats by maternal separation or CNS β-endorphin is independent of the pituitary, Life Sci. 54, 679.

Chang, K.-J., 1984, Opioid receptors: multiplicity and sequelae of ligand-receptor interactions, in: The Receptors, Vol. 1, ed. P.M. Conn (Academic Press, Orlando, FL) p. 1.

Chang, K.-J., E.T. Wei, A. Killian and J.-K. Chang, 1983, Potent morphiceptin analogs: structure activity relationships and morphine-like activities, J. Pharmacol. Exp. Ther. 227, 403.

Chang, K.-J., S.G. Blanchard and P. Cuatrecasas, 1984, Benzomorphan sites are ligand recognition sites of putative ϵ-receptors, Mol. Pharmacol. 26, 484.

Cotton, R., M.G. Giles, L. Miller, J.S. Shaw and D. Timms, 1984, ICI-174,864; a highly selective antagonist for the opioid δ-receptor, Eur. J. Pharmacol. 97, 331.

Cowan, A., X.Z. Zhu and F. Porreca, 1985, Studies in vivo with ICI-174,864 and [D-Pen^2,D-Pen^5]enkephalin, Neuropeptides 5, 311.

Garzón, J., R. Schulz and A. Herz, 1985, Evidence for the ϵ-type of opioid receptor in the rat vas deferens, Mol. Pharmacol. 28, 1.

Goldstein, A. and A. Naidu, 1989, Multiple opioid receptors: ligand selectivity profiles and binding site signatures, Mol. Pharmacol. 36, 265.

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

Gulya, K., J.T. Pelton, V.J. Hruby and H.I. Yamamura, 1986, Cyclic somatostatin octapeptide analogues with high affinity and selectivity toward mu opioid receptors, Life Sci. 38, 2221.

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

Hammonds Jr., R.G., P. Nicolas and C.H. Li, 1984, β-Endorphin-(1–27) is an antagonist of β-endorphin analgesia, Proc. Natl. Acad. Sci. USA 81, 1389.

Hauser, K.F., P.J. McLaughlin and I.S. Zagon, 1987, Endogenous opioids regulate dendritic growth and spine formation in the developing rat brain, Brain Res. 416, 157.

Hawkins, K.N., R.J. Knapp, G.K. Lui, K. Gulya, W. Kazmierski, Y.-P. Wan, J.T. Pelton, V.J. Hruby and H.I. Yamamura, 1989, [^{3}H]-[H-D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2] ([^{3}H]-CTOP), a potent and highly selective peptide for mu opioid receptors in rat brain, J. Pharmacol. Exp. Ther. 248, 73.

Haynes, L.W., 1985, Endorphins –natural selection for non-opiate actions, Trends Pharmacol. Sci. 6, 149.

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

Illes, P., R. Bettermann, I. Brod and B. Bucher, 1987, β-Endorphin-sensitive opioid receptors in the rat tail artery, Naunyn-Schmied. Arch. Pharmacol. 335, 420.

Johnson, N., R. Houghten and G.W. Pasternak, 1982, Binding of ^{3}H-β-endorphin in rat brain, Life Sci. 31, 1381.

Kamei, J., Y. Iwamoto, M. Misawa, H. Nagase and Y. Kasuya, 1993, Antitussive effect of β-endorphin is mediated by μ-opioid receptors, but not by κ- or ϵ-opioid receptors, Eur. J. Pharmacol. 233, 251.

Lemaire, S., J. Magnan and D. Regoli, 1978, Rat vas deferens: a specific bioassay for endogenous opioid peptides, Br. J. Pharmacol. 64, 327.

Martin, W.R., 1984, Pharmacology of opioids, Pharmacol. Rev. 35, 283.

Metcalf, B.W., P. Bey, C. Danzin, M.J. Jung, P. Casara and J.P. Vevert, 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.

Nock, B., A.L. Giordano, B.W. Moore and T.J. Cicero, 1993, Properties of the putative epsilon opioid receptor: identification in rat, guinea pig, cow, pig and chicken brain, J. Pharmacol. Exp. Ther. 264, 349.

Paterson, S.J., L.E. Robson and H.W. Kosterlitz, 1983, Classification of opioid receptors, Br. Med. Bull. 39, 31.

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

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

Sakellaridis, N. and A. Vernadakis, 1986, An unconventional response of adenylate cyclase to morphine and naloxone in the chicken during early development, Proc. Natl. Acad. Sci. USA 83, 2738.

Schoffelmeer, A.N.M., K.C. Rice, A.E. Jacobson, J.G. Van Gelderen, F. Hogenboom, M.H. Heijna and A.H. Mulder, 1988, μ-, δ- and κ-opioid receptor-mediated inhibition of neurotransmitter release

and adenylate cyclase activity in rat brain slices: studies with fentanyl isothiocyanate, Eur. J. Pharmacol. 154, 169.

Schoffelmeer, A.N.M., G. Wardeh, F. Hogenboom and A.H. Mulder, 1991, β-Endorphin: a highly selective endogenous opioid agonist for presynaptic mu opioid receptors, J. Pharmacol. Exp. Ther. 258, 237.

Schulz, R., M. Wüster and A. Hertz, 1981, Pharmacological characterization of the ε-opiate receptor, J. Pharmacol. Exp. Ther. 216, 604.

Shahabi, N.A., K.M. Linner and B.M. Sharp, 1990, Murine splenocytes express a naloxone-insensitive binding site for β-endorphin, Endocrinology 126, 1442.

Shook, J.E., W. Kazmierski, W.S. Wire, P.K. Lemcke, V.J. Hruby and T.F. Burks, 1988, Opioid receptor selectivity of β-endorphin in vitro and in vivo: mu, delta and epsilon receptors, J. Pharmacol. Exp. Ther. 246, 1018.

Simon, E.J., 1987, Subunit structure and purification of opioid receptors, J. Recept. Res. 7, 105.

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

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

Smith, A.P., N.M. Lee and H.H. Loh, 1983, The multiple site β-endorphin receptor, Trends Pharmacol. Sci. 4, 163.

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

Spanagel, R., A. Herz and T.S. Shippenberg, 1990, Identification of the opioid receptor types mediating β-endorphin-induced alterations in dopamine release in the nucleus accumbens, Eur. J. Pharmacol. 190, 177.

Suh, H.H. and L.-F. Tseng, 1990, Different types of opioid receptors mediating analgesia induced by morphine, DAMGO, DPDPE, DADLE and β-endorphin in mice, Naunyn-Schmied. Arch. Pharmacol. 342, 67.

Suh, H.H., L.-F. Tseng and C.H. Li, 1988, β-Endorphin-(1–27) antagonizes β-endorphin-but not morphine-, D-Pen^2-D-Pen^5-enkephalin- and U50,488H-induced analgesia in mice, Neuropharmacology 27, 957.

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