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Effect of Central Administration of β-Endorphin on Lung Ornithine Decarboxylase Activity in Developing Rats

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ABSTRACT. Results from a number of studies suggest a role for endogenous opioids in the regulation of lung development and function. Although it is not known which opioid peptides are involved in these processes, accumulated evidence suggests a prominent role for β-endorphin (BE). Our study examines the effect of BE on lung ornithine decarboxylase (ODC) activity in preweanling rats. ODC catalyzes the rate-limiting step in the synthesis of the polyamines spermidine and spermine, key regulators of cell growth, multiplication, and differentiation. Central (but not peripheral) administration of BE reduced lung ODC activity by as much as 80% in the 6-d-old rat. Significant decreases in ODC activity were seen at doses of BE as low as 0.5 μg/g brain wt. In contrast to the reductions in ODC activity, plasma levels of corticosterone in animals administered BE were approximately five times higher than those seen in control animals. BE's actions on ODC activity and plasma corticosterone levels were prevented by naloxone or naltrexone, indicating that both responses are mediated by opioid receptors. Studies of ODC kinetics showed a profound reduction in V_{max} (70% below control values), but no change in Km. The effect was observed only during the first 2 wk of postnatal age, a period of time in lung maturation that is characterized by active alveolarization. Because changes in ODC levels during early postnatal life are associated with perturbations in tissue growth and/or function, the data suggest that CNS BE may influence lung maturation through an indirect action that may involve glucocorticoids. (*Pediatr Res* 29: 182–186, 1991)

Abbreviations

BE, β-endorphin
ODC, ornithine decarboxylase
i.c., intracisternal

Narcotic addiction during pregnancy retards general fetal growth and increases the incidence of prematurity (1–3). However, it is clinically well recognized that lung function in these low-birth-weight babies is similar to that found in full-term newborn infants, as evidenced by a high lecithin/sphingomyelin ratio and, consequently, by a low incidence of respiratory distress syndrome (4, 5). Precocious fetal lung development is also found in animals treated with opioids during pregnancy (6, 7), whereas administration of naloxone (a potent opioid antagonist) has an opposite effect (7). The latter finding is important because it indicates that endogenous opioid systems (*i.e.* opioids and opioid receptors) tonically modulate fetal lung maturation.

Accumulated knowledge suggests that BE may influence pulmonary development. Pituitary and hypothalamic levels of BE are elevated in fetal and neonatal rats after maternal morphine treatment (8). Plasma BE levels in normal newborn babies are three to four times higher than the concentrations found in their mothers, and decline to adult levels by the 5th day of life (9, 10). In infants of heroin-addicted mothers, plasma BE levels show even larger increases after birth (up to 1000 times adult levels) and remain elevated at 40 d of age (9). These and other observations support the hypothesis that BE may have a prominent role in the modulation of lung maturation (11–14).

Studies of the consequences of opioid exposure on lung function have predominantly addressed prenatal effects, primarily due to their relevance to respiratory distress syndrome. However, it is equally important to investigate opioid actions on the postnatal lung. Both the newborn human and rat lungs are extremely immature and undergo vast morphologic restructuring after birth; the majority of alveoli are formed postnatally, increasing from approximately 20 million at birth to 300 million in the mature lung (15–18). The neonatal lung is highly responsive to trophic hormones (19–21). Finally, as the early postnatal lung development (saccular stage) is fundamentally a continuation of the late fetal maturation, the findings obtained during the first days of age essentially reflect prenatal mechanisms.

Our study examines the effect of central administration of BE on lung ODC activity (EC 4.1.1.17) in developing rats. ODC catalyzes the formation of putrescine from ornithine, the rate-limiting step in the synthesis of the polyamines spermidine and spermine, thought to be essential for normal tissue maturation (22–24). Due to its extremely short t½ (10 to 20 min), ODC activity is susceptible to rapid and profound change. ODC levels are highest during accelerated cellular growth, multiplication, and differentiation, and decrease quickly as these processes decline. Each tissue has a characteristic ontogenetic pattern of ODC activity (22), and perturbations of these patterns are associated with subsequent alterations in tissue growth and function (25–28). The rat lung exhibits a parallel increase in ODC activity and alveolarization during the 1st week of postnatal age (16, 17, 29), and both events are reduced in animals administered α-difluoromethylornithine (a specific inhibitor of ODC activity), thus confirming a critical role for this enzyme in pulmonary development (29).

MATERIALS AND METHODS

Animals and drug treatments. Timed-pregnant Sprague-Dawley rats (Zivic-Miller Laboratories, Allison Park, PA) were housed individually in breeding cages in a vivarium maintained at 22°C on a 12-h light-dark cycle (0700–1900 h), with food (Purina Lab Chow, Ralston-Purina, St. Louis, MO) and water available *ad libitum*. The day after birth, litters were randomized and culled

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to 10 or 11 pups/dam to maintain a uniform nutritional and maternal status. Animals were transported to the laboratory the day before experimentation and randomized among dams. The day of the experiment, pups were again randomized and, in addition, various treatment groups were distributed among several litters to minimize nutritional or maternal differences. Animal care at Duke University is governed by the Duke University Institutional Animal Care and Use Committee and the Guide for the Care and Use of Laboratory Animals, published by the Public Health Service. Animal facilities are fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Two, 6-, 10-, 14-, or 18-d-old rat pups were injected i.c. with 0.25 to 1.5 μg BE/g brain wt or saline and killed by decapitation 4 h later. Whole lungs were quickly dissected and weighed before analysis. In another set of studies, 6-d-old rats were injected s.c. with BE or saline and killed 4 h later. Injection volumes were 5 μL for 2-d-old pups and 10 μL for 6-, 10-, 14-, and 18-d-old pups for i.c. injections and 1 μL/g body wt for s.c. injections. In 2- and 6-d-old pups, i.c. injections were given through the bregma, whereas older animals were injected via the foramen magna because the bregma landmark becomes less apparent with age (due to the appearance of hair and thickening of the skull). Identical drug distribution patterns throughout the cerebral ventricular systems were verified in rats injected with methylene blue. Mean brain weights from previous studies were used to calculate the doses of BE (μg/g brain wt) for the i.c. injections. Actual individual values measured *post-mortem* fell within 5% of the mean calculated dose. Intracisternal injections were performed under light ether anesthesia.

ODC responses to hypoxia were examined by separating 6-d-old pups from their dams and placing them in a covered 6-L plastic pan kept at 37°C in an incubation bath. The container was then ventilated for 1, 2, or 4 h with warmed, humidified room air (controls) or warmed humidified 7% oxygen + 93% nitrogen at a rate of 15 L/min (30). Under these conditions, the Po_2 of source and exhaust gases were equal. Because studies in our laboratory have demonstrated that even a short-term separation of developing rat pups from their dams evokes a decrease of ODC activity in most tissues (31), simultaneous measurements were conducted in animals that were left with their dams and not gassed. The data was then compared with that obtained in littermates that went through the separation procedure and were exposed to room air in the apparatus.

Synthesis of proteins *in vivo* was evaluated by measuring incorporation of [^{3}H]leucine into proteins as described in detail in an earlier publication (32).

ODC activity. Whole lung was homogenized (Polytron) in 19 volumes of chilled 10 mM Tris buffer (pH 7.2), centrifuged at 26 000 × *g* for 20 min, and ODC activity measured in the supernatant by a modification of the method of Russell and Snyder (33). The incubation medium contained 0.9 mL of supernatant, and final concentrations of 4.8 μM L-[1-^{14}C]ornithine, 1.8 mM DTT, and 50 μM pyridoxal-5′-phosphate in a total volume of 1 mL. Incubation vials were capped with serum stoppers into which center wells containing paper filter wicks had been fitted. After a 30-min incubation at 37°C, 0.5 mL of 10% trichloroacetic acid was added to stop the reaction and then 0.2 mL of hyamine hydroxide was added to the paper wicks to trap the liberated $^{14}CO_2$ during a second 30-min incubation. Center wells were placed in scintillation fluid and counted for radioactivity. ODC activity is expressed as nmol $^{14}CO_2$ evolved/g of lung/h.

Determination of enzyme kinetics of ODC. Four h after i.c. administration of 1.5 μg BE/g brain wt or saline to 6-d-old rats, lung ODC activity was measured (in triplicate) in pooled supernatants from each treatment group, using a range of L-ornithine concentrations from 2.43 to 155.2 μM as previously reported by us and other investigators (34–36). ODC activity at the different concentrations of ornithine was then analyzed by linear regression using a double reciprocal plot to obtain the Km and V_{max} for each treatment. To ensure that the measured enzyme activity did not result from nonspecific decarboxylation (which might be found when high concentrations of ornithine are used), lung ODC activity was also determined in a different group of rats given a maximally effective dose of α-difluoromethylornithine, a specific, potent, and irreversible inhibitor of ODC activity (37). The residual enzyme activity after α-difluoromethylornithine treatment was then subtracted from the total ODC values at each ornithine concentration. Nonspecific activity ranged from 5 to 20% of the total ODC values.

Corticosterone concentrations. Blood was collected into polystyrene tubes, allowed to clot at 4°C for 10 min, and then centrifuged at 1000 × *g* for 10 min. Serum was stored at −40°C until assayed. Corticosterone was assayed by RIA after extraction from serum with ethylacetate using antiserum and corticosterone standard supplied by Radioassay Systems Laboratories, Inc. (Carson City, CA) and [^{3}H]corticosterone from New England Nuclear (Boston, MA). Sensitivity of the assay was 0.1 ng/mL.

Materials. BE [human β-lipotropin (61–91)] and metenkephalin were obtained from Peninsula Laboratories, Inc. (Belmont, CA), naloxone HCl from Endo Laboratories, Inc. (Garden City, NY), and L-[1-^{14}C]ornithine monohydrochloride (51.6 mCi/mmol) and L-[3,4,5-^{3}H(N)]leucine (143.0 Ci/mmol) from New England Nuclear Corp. (Boston, MA). DL-α-difluoromethylornithine (MDL 71 782 A) was kindly provided by Dr. P. McCann, Merrell Dow Research Institute (Cincinnatti, OH). Pyridoxal-5′-phosphate and L-ornithine hydrochloride were obtained from Sigma Chemical Co. (St. Louis, MO), DTT from Bachem Inc. (Torrance, CA), and hyamine hydroxide (scintillation grade) from Research Products International Corp. (Mount Prospect, IL).

Statistics. Data are reported as means ± SEM. Statistical analysis used two- or three-way analysis of variance as indicated, followed post hoc by either Duncan's multiple range test or *t* test (two-tailed, unpaired) where appropriate. Significance was tested at $p < 0.05$.

RESULTS

Effect of BE on lung ODC activity in 6-d-old rats. Administration of BE i.c. produced a dose-related decrease in lung ODC activity (Fig. 1). Enzyme activity in animals given the highest dose (1.5 μg/g brain wt) was reduced to approximately 20% of control values.

Because tissue ODC responses to i.c. BE could result from direct peripheral actions of this neuropeptide after its leakage

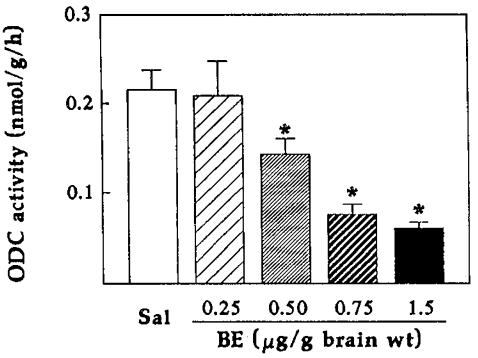

Fig. 1. Dose-response effect of central administration of BE on lung ODC activity in 6-d-old rats. Animals were injected i.c. with BE at the doses indicated above or with saline, and ODC activity was assayed 4 h later. Data represent the means ± SEM of values from two or three experiments using 8 animals/group/experiment. One-way analysis of variance indicates a significant effect of BE ($F_4 = 15.2$; $p < 0.01$). *Asterisks* denote significant differences of individual BE groups *vs* saline as evaluated by Duncan's multiple range test ($p < 0.05$ or less).

into systemic blood, similar studies were conducted in a different group of rats treated s.c. with BE. Peripheral administration of 0.5 or 1 μg of BE, amounts equivalent to those given centrally (0.75 or 1.5 on a μg/g brain wt basis, respectively), or even 10 μg of BE had no overall significant effect on ODC activity (data not shown).

Mechanisms underlying effect of BE on lung ODC activity in 6-d-old rats. To determine whether the effect of CNS BE on lung ODC activity is mediated by classical opioid receptors, similar studies were conducted in animals treated with 3 μg of naloxone/g brain wt (a dose shown to block brain opioid receptors in the rat) (14, 38). Coadministration of naloxone with BE i.c. reversed the decreases in ODC activity normally seen in rat pups given BE (Fig. 2). Naloxone alone did not significantly alter basal lung ODC levels.

Kinetic studies were undertaken to establish whether BE actions on lung ODC activity reflect changes in enzyme affinity or V_{max}. As shown in Figure 3, BE treatment evoked a dramatic reduction in V_{max} (from 10.53 in controls to 3.13 nmol/g/h in BE-treated animals) without a significant change in Km.

Glucocorticoids have been shown to delay the pattern of cellular development in rat lung, in part, by acting on ODC activity (26). Thus, it is possible that the lung ODC responses we observed in rat pups given BE i.c. reflect actions of this neuropeptide on plasma corticosterone levels. As shown in Figure 4, plasma corticosterone was 25 ± 3 ng/mL 1 h after receiving BE, a value approximately five times higher than that seen in control animals. As was the case for ODC, the corticosterone effect was totally reversed by naltrexone (a long-lasting opioid antagonist) (Fig. 4).

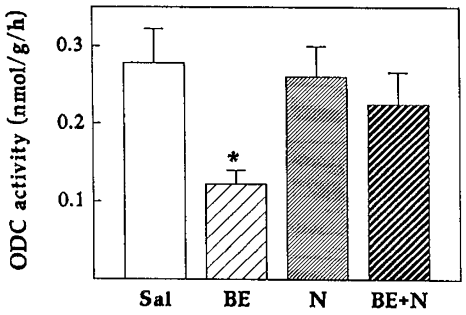

Fig. 2. Effect of central administration of naloxone on the decreases in ODC activity evoked by BE in 6-d-old rats. Animals were injected i.c. either with 1.5 μg BE/g brain wt, 3 μg naloxone/g brain wt, BE plus naloxone, or saline, and ODC activity was measured 4 h later. Data represent the means ± SEM of values from 14 or 15 animals/group. The *t* test indicates a significant difference only for BE *vs* saline ($p < 0.005$).

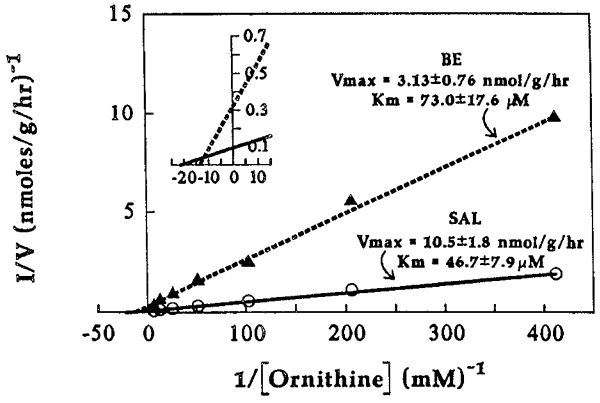

Fig. 3. Pharmacokinetic analysis of ODC activity. Pooled lung preparations from 6-d-old rats treated i.c. with BE (1.5 μg/g brain wt) or saline were analyzed for ODC activity against a range of L-ornithine concentrations from 2.43–155.2 μM. Linear regression analysis (*r* for saline = 0.998; *r* for BE = 0.998) indicates a significant reduction in V_{max} ($p < 0.02$) without a change in Km after BE treatment.

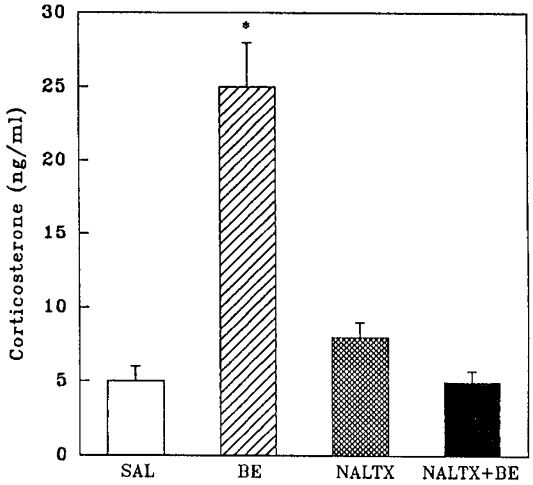

Fig. 4. Effect of central administration of BE and/or naltrexone on plasma corticosterone levels. Animals were injected i.c. either with 1.5 μg BE/g brain wt, 4 μg naltrexone (*NALTX*)/g brain wt, *NALTRX + BE*, or saline, and plasma corticosterone was measured 1 h later. Data represent the means ± SEM of values from five or six determinations (2 animals/determination/group. The *t* test indicates a significant difference only for BE *vs* saline ($p < 0.001$).

Table 1. *Effect of hypoxia (7% O_2) on lung ODC activity in 6-d-old rats**

	ODC activity (nmol/g/h)	
Time (h)	Air	7% O_2
1	0.121 ± 0.019	0.627 ± 0.087†
2	0.186 ± 0.063	2.33 ± 0.45†
4	0.141 ± 0.036	2.13 ± 0.45†

* Animals were removed from the dam and placed in the gassing chamber. The containers were then gassed with air or 7% O_2 for the times indicated above, and animals were immediately killed afterward. Lung ODC activity in rat pups that remained in the home cage with the dam for the duration of the experiment was 0.342 ± 0.087 nmol/g/h. Data represent the means ± SEM of values from 8 to 10 animals/group.

† Significantly different from control group ($p < 0.05$ or less; *t* test).

Central administration of BE has been reported to depress respiration in dogs (39). Similarly, in human studies exogenous opiate alkaloids inhibit respiration as well as ventilatory responses to hypoxia and hypercapnia (40). Thus, it is conceivable that the decreases in lung ODC activity that we have observed in BE-treated pups may be related to a decline in cell metabolism usually associated with ventilatory depression. One way to investigate this possibility is by assessing the effect of hypoxia on lung ODC activity. Studies of neonatal hypoxia require the dams to be absent during the gassing period as they become extremely agitated under the low O_2 conditions. Because even short-term interruption of mother-pup interactions are known to decrease ODC levels in most tissues (31), we also measured enzyme activity in rat pups that were left with their dams for the duration of the experiment. As shown in Table 1, exposure to 7% O_2 for 1, 2, or 4 h markedly increased lung ODC activity, whether compared with that of separated animals gassed with room air or that of control rats (left with their mothers).

Metenkephalin has also been shown to cause respiratory depression (41, 42). Therefore, to further investigate the potential role of hypoxia on the ODC effect of BE, we measured lung ODC activity in rats given metenkephalin i.c. Administration of doses of metenkephalin as high as 1.65 μg/g brain wt (10 times the highest dose of BE used in our studies, on a molecular weight

basis) had no overall significant effect on enzyme activity (data not shown).

The possibility that the decreases in ODC activity reflect nonspecific toxic responses to BE was investigated through measurements of protein synthesis, as assessed by [^{3}H]leucine incorporation into trichloroacetic-precipitable material. Rats were injected i.c. with BE followed 1 h later by a s.c. injection of [^{3}H] leucine (0.5 μCi/g body wt), and protein synthesis was determined 20, 40, and 80 min after the second injection. BE treatment did not alter the percentage of [^{3}H]leucine incorporation into proteins as analyzed by two-way analysis of variance (control groups = 27.7 ± 0.6, 34.4 ± 1.3, and 39.5 ± 0.5; BE groups = 24.9 ± 1.1, 33.0 ± 1.7, and 45.3 ± 1.3 at 20, 40, and 80 min, respectively; data represent the means ± SEM of values from nine to 19 animals/group/time point).

Effect of BE on lung ODC activity in rats of different ages. In agreement with previous reports, control animals showed a peak in basal lung ODC activity at 6 d of age followed by a decrease to very low values at postnatal d 14 and a substantial late rise in enzyme activity from postnatal d 18–30 (26, 29) (Fig. 5). BE administration inhibited tissue ODC activity during the first 2 wk of age, but had no effect on 18- or 30-d-old animals.

DISCUSSION

The results from these studies demonstrate that ODC activity in the neonatal rat lung is highly sensitive to changes in CNS BE levels. We found that i.c. doses of BE as low as 0.5 μg/g brain wt significantly decreased ODC activity. Subcutaneous administration of even 10 times the highest amount of BE given i.c. did not alter lung ODC activity, indicating that the effect results indirectly from CNS actions of BE. Centrally mediated effects of CNS BE on other peripheral tissues have been recently reported by our laboratory (14, 43, 44).

Coadministration of naloxone with BE i.c. prevented the decreases in lung ODC activity evoked by BE alone, indicating an opioid-receptor-mediated phenomenon. This finding contrasts with our previous observations showing that the same dose of naloxone i.c. did not block the inhibitory effect of BE on liver ODC activity (14). Concurrent determinations of ODC activity in the lung and liver confirmed these tissue differences.

The postnatal rat lung progresses through three distinct developmental stages: saccular (up to d 4), proliferative (postnatal d 4–13), and terminal differentiation (postnatal d 14–21) (15). The opioid effect was clearly seen on postnatal d 2–14 but was no longer apparent by postnatal d 18. The insensitivity to BE during the terminal differentiation period could not be explained by its typically low basal ODC levels inasmuch as 30-d-old pups (whose basal lung ODC activity is very high) also did not respond to BE, even when twice the dose of BE administered to younger pups was used.

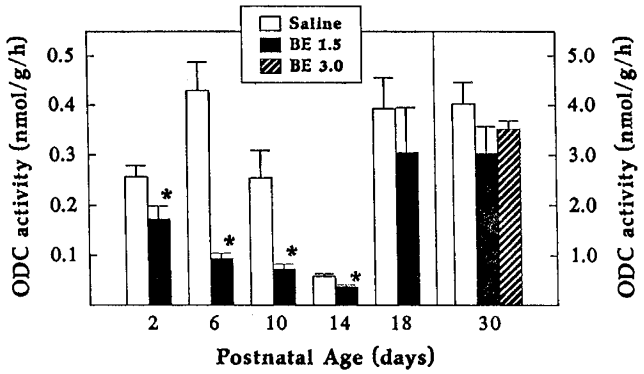

Fig. 5. Effect of central administration of BE on lung ODC activity in rats of different ages. At the ages indicated above, animals were injected i.c. with 1.5 μg of BE/g brain wt or saline, and ODC activity was measured 4 h later. Data represent the means ± SEM of values from 7–17 animals/group at each age. Because of heterogeneity of variance, values were log-transformed before statistical evaluation. This manipulation gives equal weight to equivalent proportional changes, which is appropriate where data span a large range. Two-way log analysis of variance indicates significant effects of BE ($F_1 = 47.9$; $p < 0.01$) and age ($F_5 = 115.2$; $p < 0.01$), as well as an interaction of BE and age ($F_5 = 3.40$; $p < 0.01$). *Asterisks* denote significant differences of individual BE groups *vs* saline as evaluated by *t* test ($p < 0.05$ or less).

Enzyme kinetic studies eliminated the possible participation of a posttranslational inhibitor, inasmuch as no significant changes in Km were obtained. This would be expected if changes in enzyme affinity for ornithine had occurred (35, 45). In contrast, ODC V_{max} in BE-treated rats was approximately 70% lower than control values, a decrease similar to that of ODC activity. It is then clear that BE reduces lung ODC activity by inhibiting its *de novo* synthesis rather than by a posttranslational mechanism.

BE has been reported to cause respiratory depression in dogs (39), suggesting that the decreases in lung ODC activity we have observed in BE-treated rat pups may result from actions of this neuropeptide on ventilation. The following observations, however, make this possibility unlikely. *1*) We found that 6-d-old rats exposed to hypoxia (7% O_2 for 1 to 4 h) actually showed profound increases in lung ODC activity. *2*) Metenkephalin, which has also been shown to cause respiratory depression (41, 42), did not alter lung ODC activity even when five times the highest dose of BE used in these studies was administered. *3*) The respiratory actions of BE were naloxone reversible (39). Accordingly, this opioid antagonist should also block the ODC effect in all tissues if both phenomena are causally related. Although naloxone reversed BE's action on lung ODC (in this study), it did not prevent the effect on liver ODC (14). *4*) Furthermore, rat pups given 0.15 μg BE/g brain wt, a dose that did not significantly alter rectal temperature (an index of metabolic activity), showed significant decreases in brain and liver DNA synthesis (44). *5*) Finally, the ODC effect could not be attributed to a generalized toxic response as protein synthesis was not altered.

At first, BE's inhibition of ODC activity appears to contradict the postulated acceleration of lung maturation by endogenous opioids, but this might not be the case. Although pulmonary function in premature offspring born to opiate-addicted mothers is similar to that found in full-term offspring born to normal mothers (4–7), body weights are markedly subnormal (1). Glucocorticoids are also known to evoke dichotomous developmental effects. For example, dexamethasone accelerates the development of lung function by enhancing surfactant synthesis (19), but simultaneously slows down proliferation of lung cells as indicated by reduced ODC activity and DNA/lung ratios (26, 46). In fact, because we found increased plasma corticosterone concentrations after BE administration, it is possible that CNS BE may influence lung maturation through an indirect action on glucocorticoids/stress mechanisms.

Because changes in ODC levels during early postnatal life are indicative of perturbations in cell development, the results from these studies suggest that activation of endogenous CNS BE systems may have a significant impact on lung maturation. Indeed, results from recent studies conducted in our laboratory show that i.c. administration of doses of BE that significantly reduce ODC activity also profoundly decrease lung DNA synthesis (unpublished experiments). Because changes in DNA biosynthetic rates reflect alterations in cell replication, increases in CNS BE levels during early postnatal life could retard lung growth. It can be speculated that, in a manner similar to that of glucocorticoids, the natural release of BE at birth (9, 10, 12) may favor critical events necessary for early adaptation to extrauterine life (such as surfactant production) by restricting the amount of cellular energy directed toward growth-related processes, including ODC activity and DNA synthesis.

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