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Naloxone Modulates Body and Organ Growth of Rats: Dependency on the Duration of Opioid Receptor Blockade and Stereospecificity

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ZAGON, I. S. AND P. J. McLAUGHLIN. *Naloxone modulates body and organ growth of rats: Dependency on the duration of opioid receptor blockade and stereospecificity.* PHARMACOL BIOCHEM BEHAV 33(2) 325–328, 1989.—Endogenous opioid systems (i.e., endogenous opioids and opioid receptors) act in the trophic regulation of biological development. Opioid antagonist paradigms have served to elucidate the nature of this relationship. If growth is mediated at the level of the opioid receptor, one would expect that this interaction would be stereospecific. This study shows that daily injections of 20–60 mg/kg (−) naloxone, given chronically throughout the preweaning period, depress body weight when monitored at Day 21. Opioid challenge experiments using nociceptive measures show that these dosages of (−) naloxone invoked an opioid receptor blockade for no more than 10–12 hr/day. A dosage of 100 mg/kg (−) naloxone, which blocked the opioid receptor for 12–16 hr/day, did not alter body weight in comparison to control levels. In subsequent experiments, 40 mg/kg (−) naloxone depressed body weight of 21-day-old rats, and the wet weights of the liver, spleen, thymus, heart, and triceps surae muscle from these animals were subnormal. A dosage of 40 mg/kg (+) naloxone did not alter growth. These results show that opioid action in regard to growth is stereospecific and dependent on the duration of opioid receptor blockade, providing additional evidence that endogenous opioid systems play an important role in developmental events.

Opioids Naloxone Somatic Development Growth Embryology Endorphins Enkephalins Rodent Opioid receptor

ENDOGENOUS opioid systems (i.e., endogenous opioids and opioid receptors) appear to play an important role in the regulation of development (6, 12–16, 18). Endogenous opioids serve as inhibitory trophic growth factors that regulate cell proliferation (18) and influence cell differentiation (6,12) and neurobehavioral ontogeny (13,15).

If development is mediated by opioids at the level of the opioid receptor, one would expect that this interaction would be stereospecific. In earlier experiments (6, 12–16, 18) we have employed an opioid antagonist paradigm to block the opioid receptor from endogenous opioids. In these experiments which utilized naltrexone, the duration of opioid receptor blockade was found to determine the course of biological development (14). Thus, concentrations or schedules of drug administration capable of blocking the opioid receptor for the entire injection period were found to stimulate growth (e.g., body weights were greater than controls in 21-day-old rats). Dosages that blocked the receptor for less than 12 hr/injection period were inhibitory to growth. If growth is mediated at the level of the opioid receptor, one would expect that this interaction would be stereospecific. In the present study we have found that the opioid antagonist, naloxone, depressed body weights of 21-day-old rats given drug daily for the first 3 weeks of development. This effect was dependent on the duration of opioid receptor blockade, and stereospecific in regard to body and organ growth.

METHOD

Animals

The offspring of female and male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) were utilized in this study. Animals were housed in solid-bottom cages containing Bed-o-Cobs litter (Landsdale, PA) in an environment of 21 ± 0.5°C with a relative humidity of 50 ± 10%. The room had a complete exchange of air 12–15 times per hour and a 12-hour light-dark cycle with no twilight; water and Wayne Lab Blox were continuously available.

Females were mated (1 male to 1 female) and placed in individual solid-bottom cages to deliver their young. Within 24 hr of parturition (Day 0), litters were culled to 8 pups per mother; each litter contained an equal number of males and females.

Drug Treatment and Growth

Beginning on Day 1 and continuing until Day 21 (weaning),

animals received a daily subcutaneous (SC) injection of 20, 40, 50, 60, or 100 mg/kg (−) naloxone hydrochloride (Sigma, St. Louis, MO) or sterile water (=controls). Body weights were recorded on Day 21.

In a second experiment, animals were given daily SC injections of 40 mg/kg (−) naloxone or (+) naloxone (NIDA), or sterile water beginning on Day 1 and continuing until Day 21. A dosage of 40 mg/kg was selected because results from the initial dose-response experiment indicated that this was capable of altering preweaning growth. At weaning, animals were weighed, anesthetized, and killed by decapitation. The following organs were removed and weighed: liver, heart, spleen, thymus, and the triceps surae muscle.

Duration of Opioid Receptor Blockade

Twenty-one-day-old rat pups were given daily SC injections of 20, 40, 60, or 100 mg/kg (−) naloxone, or vehicle (=sterile water), from birth to Day 21 and evaluated for nociceptive response following opioid agonist challenge. Rats were injected with (−) naloxone or vehicle and then given 2 mg/kg levorphanol 30 min prior to testing on an analgesia meter (55°C, Technilabs). Animals were tested only once and they were removed from the analgesia meter after 40 sec if no response was observed; endpoints included licking of paws or rapid removal of hindlimbs from the surface of the analgesia meter. Five animals in each group were examined at each time point.

Statistical Analysis

Body and organ weights, as well as latencies on the analgesia meter, were analyzed using analysis of variance; subsequently planned comparisons were made using the Newman-Keuls test. Analyses were performed with the ANOVA statistical package adapted for the Apple IIe computer.

RESULTS

Postnatally developing rats receiving (−) naloxone exhibited a dose-dependent response in regard to body weight. In comparison to the body weight of control subjects (53.0 ± 0.6 g) at 21 days, animals chronically receiving (−) naloxone at dosages of 20, 40, 50, and 60 mg/kg were subnormal (Fig. 1); the (−) naloxone-treated rats were 14–23% below control levels. However, animals injected daily with 100 mg/kg (−) naloxone were similar in body weight to controls.

Evaluation of the duration of opioid receptor blockade using an opioid challenge test to measure nociception (Fig. 2) revealed that a dosage of 20 or 40 mg/kg (−) naloxone was capable of blocking the effects of an injection of levorphanol for 4–6 hr, whereas a dosage of 60 mg/kg (−) naloxone blocked antinociception induced by levorphanol for 10–12 hr. A dosage of 100 mg/kg (−) naloxone was effective as an opioid antagonist for 12–16 hr.

Based on the above results showing that postnatal administration of either 20, 40, 50, or 60 mg/kg (−) naloxone decreased the body weight of 21-day-old rats, a dosage of 40 mg/kg was selected for evaluation of stereospecificity of opioid antagonist action. Chronic administration of 40 mg/kg (−) naloxone resulted in a body weight decrease of 25% from control levels at 21 days (Table 1), whereas animals given daily injection of (+) naloxone at the same dosage were comparable in body weight to control levels. The wet weights of the liver, thymus, heart, spleen, and triceps surae muscle from rats of the (−) naloxone group were subnormal (reduced 19–33% from control values); organ weights from animals of the (+) naloxone group were similar to control levels (Table 1). When tested 2 hr following administration of 40 mg/kg (+) naloxone or sterile water (control), both groups of rats

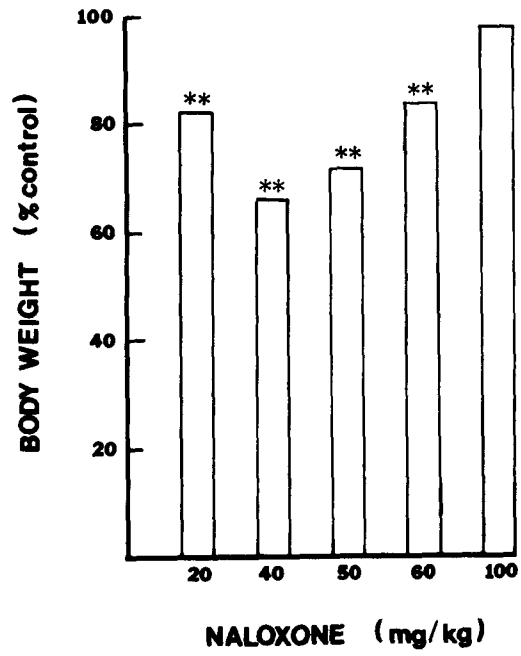

FIG. 1. The body weight (expressed as % of control levels) of 21-day-old rats receiving 20, 40, 50, 60, or 100 mg/kg (−) naloxone daily (SC) from birth to Day 21; n = at least 16 animals per group. **Significantly different from controls at $p<0.01$.

exhibited latencies in response to challenge with levorphanol of 40.0 ± 0.0 sec. In contrast, the (−) naloxone group had a latency of 22.2 ± 1.8 sec when tested 2 hr following (−) naloxone administration; this differed significantly ($p<0.01$) from controls.

DISCUSSION

The present results clearly demonstrate that disruption of endogenous opioid systems by the opioid antagonist, (−) naloxone, has a significant impact on the course of preweaning development, supporting earlier conclusions that endogenous opioid-opioid receptor interactions play a pivotal role in growth (6, 12–16, 18). We show that daily administration of (−) naloxone at dosages that block the opioid receptor for less than 12 hr/day depresses the body weight gain of postnatally developing rats. Dosages of (−) naloxone ranging from 20 to 60 mg/kg all had a similar growth-inhibiting effect, with no differences between dosage groups detected. These results are consistent with earlier findings using naltrexone where the duration of opioid receptor blockade was found to determine the course of development (14). In that study, dosages of naltrexone (i.e., 0.1, 1, or 10 mg/kg) that persisted no longer than 12 hr/day in developing rats suppressed growth (12–14). Endogenous opioids such as [Met^5]-enkephalin are inhibitory to growth processes (18), and opioid antagonist administration has been found to produce an increase in endogenous opioids, opioid receptors, and supersensitivity to opioid agonists (10, 11, 20). Thus, the depressed growth by (−) naloxone may be the result of an increased quantity of opioids interacting with developing cells having an increased number of growth-related opioid receptors. This would lead to an enhanced inhibitory response to opioid-receptor interaction during the interval when naloxone is no longer present. Our study also shows that a dosage of 100 mg/kg (−) naloxone, which blocked the opioid receptor for 12–16 hr/day as determined in opioid challenge/nociceptive tests, did not alter the body weights of 21-

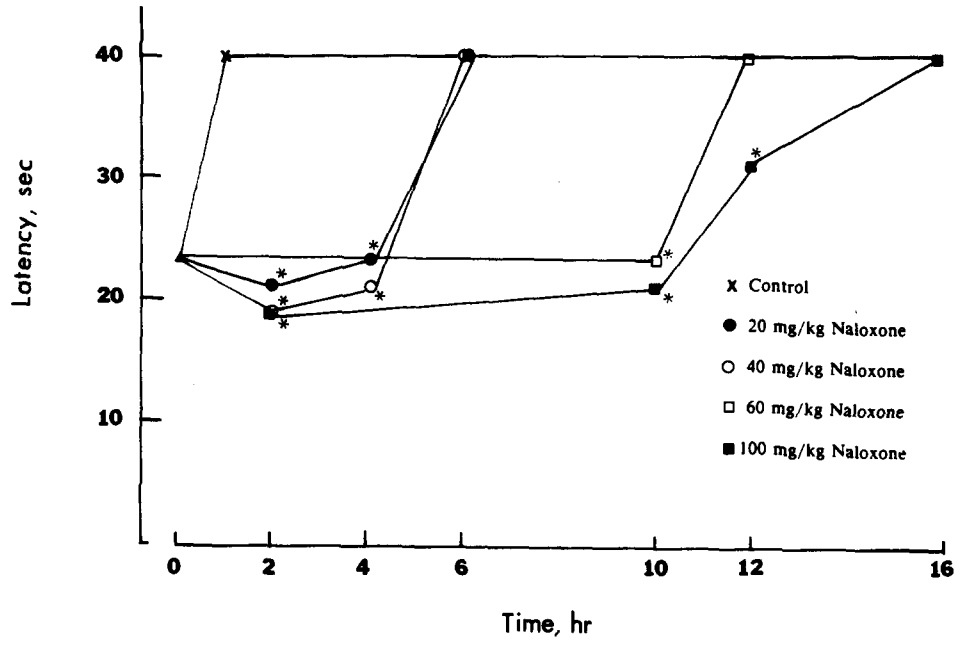

FIG. 2. The latency of response in 21-day-old rats using an analgesia meter (55°C). Animals received (−) naloxone (20, 40, 60, 100 mg/kg) or sterile water (= control) daily from birth to Day 21. Rats were injected with (−) naloxone or sterile water and then given 2 mg/kg levorphanol 30 min prior to testing on an analgesia meter (55°C); n = 5 animals at each time point/group. *Significantly different from controls at $p<0.05$.

day-old rat pups. Given the results of previous experiments (14) in which the duration of opioid receptor blockade was demonstrated to determine developmental response, it could be conjectured that the interval of enhanced opioid-opioid receptor interaction was too short, not allowing enough time to produce growth inhibition.

An important finding of the present study was that opioid action was stereospecific, with the (−) but not the (+) isomer of naloxone exhibiting an inhibitory effect on growth. Opioid challenge tests using nociception as a measure confirmed that 40 mg/kg of the (−) isomer of naloxone, but not the (+) isomer, was capable of blocking opioid receptors. Evaluation of the wet weights of the liver, thymus, heart, spleen, and triceps surae muscle revealed that organ weights of the (−) naloxone-treated animals were subnormal, in contrast to the organ weights of (+) naloxone rats which were comparable to controls. This decrease in organ weights of 40 mg/kg (−) naloxone is consistent with earlier results using 1 mg/kg naltrexone (a dosage that blocked the opioid receptor for less than 4–6 hr/day) (14,16).

TABLE 1

BODY AND ORGAN WEIGHTS OF 21-DAY-OLD RATS RECEIVING DAILY SC INJECTIONS OF 40 mg/kg (−) OR (+) NALOXONE, OR STERILE WATER (=CONTROLS) POSTNATALLY FROM BIRTH TO DAY 21

	Control	(+) Naloxone	(−) Naloxone
Body Weight	52.9 ± 0.8	52.8 ± 0.6	39.6 ± 1.3*
Liver	2.87 ± 0.11	3.19 ± 0.11	1.93 ± 0.08*
Thymus	0.26 ± 0.01	0.26 ± 0.01	0.19 ± 0.01*
Heart	0.36 ± 0.01	0.37 ± 0.01	0.24 ± 0.01*
Spleen	0.29 ± 0.02	0.34 ± 0.01	0.14 ± 0.01*
Triceps surae	0.21 ± 0.01	0.22 ± 0.01	0.17 ± 0.01*

All values are given as means (g) ± S.E.; n = 16 for body weights and n = 8 for organ weights. Significantly different from controls at *$p<0.01$.

Opioid antagonist paradigms have been essential in documenting that endogenous opioid-opioid receptor interactions contribute to the regulation of growth (6, 12–16, 18). However, opioid antagonists such as naloxone, often regarded as "pure" antagonists to the many biological actions of opioid substances and devoid of intrinsic activity (2,9), may have a variety of effects unrelated to opioid receptors (9). Thus, the actions of opioid antagonists must be carefully evaluated if opioid receptor mechanisms are suspected. It is known that opioid interactions at the receptor level are stereospecific (8), with isomeric forms showing markedly different affinities for opioid receptors. For example, the (−) isomer of naloxone is known to be 3 or 4 orders of magnitude more active than the (+) isomer in its ability to bind opioid receptors in rat brain preparations as well as in antagonizing physiological responses to opioids (1, 3–5, 7, 17). In fact, the demonstration of stereospecificity of opioid antagonist action has been recommended as a criterion for exclusion of pharmacological effects not due to interactions with opioid receptors. The present results showing that the (−) isomer of naloxone, but not the (+) isomer of naloxone, alters growth, are consistent with the enantiomeric specificity of opioids.

Data from this investigation provide further evidence which implicates endogenous opioid systems as trophic regulators of biological development, functioning to control growth through inhibitory channels. [Met^5]-enkephalin, a pentapeptide derived from proenkephalin A, has been found to depress the proliferation of brain cells during neural ontogeny (18). Moreover, this effect on cell replication can be blocked by concomitant administration of naloxone (18). Results from in vitro studies using neuroblastoma cells in culture reveal that endogenous opioids, particularly [Met^5]-enkephalin, have an inhibitory effect on DNA synthesis and mitosis, with drug concentrations as low as 10^{-10} M altering cell replication; naloxone blocks the antimitotic effects of [Met^5]-enkephalin (19). Given the present experiments with naloxone, as

well as earlier studies with naltrexone (12–14), it now appears that at least 2 opioid antagonists, naloxone and naltrexone, influence developmental events. Studies demonstrating that the duration of opioid receptor blockade, not drug dosage, determines the course of ontogenesis (14) are also consonant with this hypothesis. Finally, the present study provides even further support for this line of reasoning by showing that opioid receptors are stereospecifically involved in developmental events.

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