Pharmacology Biochemistry & Behavior, Vol 26, pp 539–542 Copyright © Pergamon Journals Ltd , 1987 Printed in the U S A 0091-3057/87 $3 00 + 00

Short-Term and Delayed Behavioral Effects of Pre- and Post-Weaning Morphine in Mice[1]

ENRICO ALLEVA AND GIOVANNI LAVIOLA

Section of Neurobehavioral Pathophysiology, Laboratorio di Fisiopatologia di Organo e di Sistema
Istituto Superiore di Sanità, V.le Regina Elena, 299, I-00161 Roma, Italy

Received 15 April 1986

ALLEVA, E AND G LAVIOLA *Short-term and delayed behavioral effects of pre- and post-weaning morphine in mice* PHARMACOL BIOCHEM BEHAV **26**(3) 539–542, 1987 —Ninety mouse pups of the CD-1 outbred strain were used to assess activity (Varimex Activity Meter, Columbus Instr , OH) and analgesia (hot plate) after morphine hydrochloride given IP either on days 14–16 (preweanlings) or on days 21–23 (postweanlings) In preweanlings morphine depressed activity already at the lowest dose tested (0 5 mg/kg), and higher doses (1, 5, and 10 mg/kg) did not produce a significantly larger effect Activity of postweanlings was not depressed until a very high dose (20 mg/kg) By contrast, morphine produced clear analgesic effects at all doses in both preweanlings (day 14) and postweanlings (day 21) Around day 70, activity and hot-plate tests in the no-drug state showed no differences due to prior treatment, except for the fact that hot-plate latencies of mice previously injected with saline as preweanlings were higher than those of all other groups Twenty-four hr later the tests were repeated after morphine injection (10 mg/kg), and showed a significantly greater depression of activity in mice previously exposed as preweanlings On the other hand, all groups previously exposed to morphine at either the pre- or the post-weanling stage showed tolerance to the analgesic effect of the drug These developmental profiles confirm that opioid systems contribute to the modulation of activity by mechanisms which are at least in part separate from those mediating analgesia

Mouse behavioral development Morphine analgesia Hot plate Locomotor activity Adult tolerance Environmentally induced analgesia (EIA)

SYSTEMIC administration of morphine to altricial rodents at different postnatal stages results in quite different physiological and behavioral changes [6, 14, 15, 17, 19, 20] Specifically, the analgesic effect of the drug in rats increases from early postnatal period until about day 15 [6,14] in parallel to a rapid rise in the number of μ-receptors [19] In the subsequent period, drug sensitivity declines [6,15] in parallel to the reduction of general drug toxicity [17], and these changes are apparently related to the maturation of the blood/brain barrier [6,17]

Locomotor activity is also differentially affected by morphine as a function of postnatal age. One study showed a reduction of activity in rats of ten days, and either no effects or activity enhancements at later ages [9] In another study, inbred mice of the C57Bl/6 strain showed mainly hyperactivity during ontogeny, except for a brief period (days 20–22) in which the drug produced a marked catatonia [11]

More recently, attention has been given to the proactive effects of postnatal morphine exposure on subsequent responding to the drug For example, extended morphine administration in rats from birth to weaning produced tolerance to the analgesic and hypoactivating effects of the drug at 22 days without, however, any appreciable effect on μ-receptors proliferation [7]. The same investigators also showed that a single dose of morphine given to newborn rats sufficed to produce a similar change in sensitivity 26 days later. This change failed to appear in pups injected only a few days later, that is, either on day 5, 9, or 13 [8].

The present work was designed to assess the effects of different doses of morphine on pain sensitivity and locomotor activity of mice before and after weaning, and the consequences of such exposure on adult sensitivity to the same drug. Animals were treated and tested for three consecutive days either during the last part of the pre-visual stage (preweanlings, days 14–16, eye opening is completed on day 17 in the strain used [5]), or starting on the day after weaning (postweanlings, days 21–23) These treatment periods were chosen also on the basis of a previous study which showed that mouse pups of these two ages have quite different patterns of activity, habituation, and response to model drugs such as amphetamine and scopolamine [2]. All animals were retested at about 70 days of age for activity, pain reactivity, and morphine effects thereon

[1]Part of the data on activity and analgesia in immature mice were presented at the IX European Neuroscience Association congress (Oxford, U K , 8–12 September, 1985) [4]

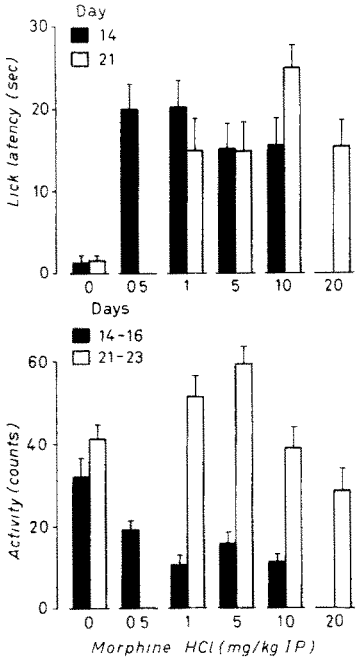

FIG 1 Effects of morphine hydrochloride on hot-plate reactivity (upper graph) and activity (lower graph) of CD-1 mice before and after weaning (data on activity were pooled for days 14–16 and 21–23, respectively, see text) Data are means (S E M) of 9 pups

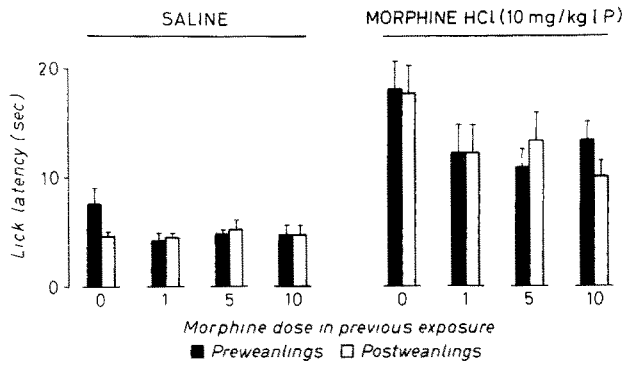

FIG 2 Hot-plate reactivity on first and second day of retesting at about 70 days (after saline and morphine, respectively) of mice previously exposed as preweanlings or postweanlings (see Fig 1) Data are means (S E M) of 9 animals

METHOD

Animals and Breeding

Mice of an outbred albino Swiss-derived strain (CD-1) weighing 25–27 g were purchased from a commercial breeder (Charles River Italia, I-22050 Calco, Italy). Upon arrival at the laboratory the animals were housed in an air conditioned room (temperature 21±1°C, relative humidity 60±10%) with lights on from 9:30 p.m. to 9·30 a m Males and nulliparous females were housed separately in groups of 8–10 in 42×27×15 cm Plexiglas boxes with sawdust as bedding and a metal top. Pellet food (enriched standard diet purchased from Piccioni, I-25100 Brescia, Italy) and water were continuously available. After 2–3 weeks, eighteen breeding pairs were formed and housed in 33×13×14 cm boxes The females were inspected daily at 9:00 a.m. for the presence of vaginal plug and for delivery (postnatal day 1) The stud was removed ten days after the finding of the plug

Apparatus and Procedures

At birth all litters were culled to five males and one female and randomly assigned to one of two groups tested respectively on days 14–16 (preweanlings) and 21–23 (postweanlings). At weaning on day 20, the females were sacrificed, and the five males of each litter transferred to a 42×27×15 cm Plexiglas box where they were housed until the end of the experiment (day 72) On day 14 or 21, the pups were weighed at the nearest 0.01 g on a PK-300 Mettler Balance set for automatic compensation of variations due to movements during weighing One pup from each litter was randomly assigned to one of five treatment conditions, including saline (NaCl 0.8%, i.e , 8 mg/ml) and four doses of morphine hydrochloride (Carlo Erba, I-25100 Milano; injections IP, in a volume of 0.01 ml/g) A preliminary experiment showed a much more marked sensitivity to the depressant effects of the drug in preweanlings than in postweanlings, and a 20 mg/kg dose was sometimes lethal in the former Therefore, the doses were 0 5, 1, 5, or 10 mg/kg for preweanlings and 1, 5, 10, or 20 mg/kg for postweanlings After injection, animals were immediately returned to their respective home boxes

Twenty min later, single pups were introduced in a clean box of the same type as the home box The box was placed on a Varimex Activity Meter apparatus (four units, Columbus Instruments, OH), set at a standard level Only the horizontal sensor systems were used, and the recording session lasted 15 min Immediately after the end of this session, individual pups were placed on a hot plate apparatus (Model-DS37 Socrel, Basile I-21025 Comerio) set at 55±0 5°C, and their latency to lick a forepaw was recorded (cutoff time 60 sec) On each of the following two days each pup was similarly weighed, injected, and tested for activity but not for pain reactivity

On day 28, all litters were reduced to the four animals treated previously with either saline or 1, 5, or 10 mg/kg of morphine On day 70 (±2 days) the mice were injected IP with saline, and 20 min later their activity was measured as described above, except for a longer duration of the session (30 min). Immediately after, the animals were exposed to the hot plate Twenty-four hr later, activity and hot-plate reactivity were assessed again after morphine (10 mg/kg IP)

All tests were carried out between 9·30 and 12·00 a m , i e , during the initial hours of the dark period. The experimental designs were counterbalanced in order to equate the representation of various groups at different test times, while repeated testing of the same animal took place at about the same time of day In the case of activity tests, the designs were also counterbalanced for assignment of animals to different Varimex units

RESULTS

Treatment effects on weight gain were limited to a slight short-term depression at the highest morphine dose This effect, however, failed to reach statistical significance Adult weights were practically identical in all groups.

The data on hot-plate responding at 14 and 21 days (Fig 1, upper graph) clearly show maximal or near-maximal anal-

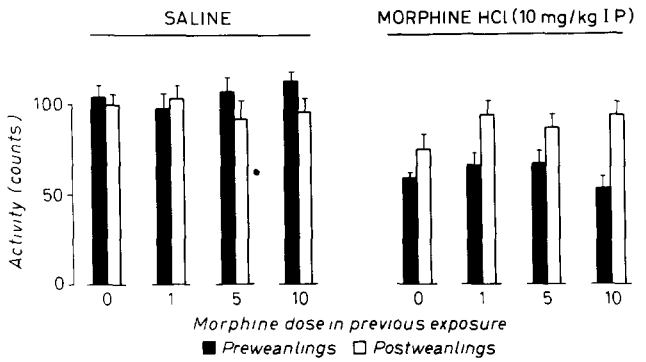

FIG 3 Activity on first and second day of retesting at about 70 days (after saline and morphine, respectively) of mice previously exposed as preweanlings or postweanlings (see Fig 1) Data are means (S E M) of 9 animals

gesic effects of morphine already at the lowest dose used Separate ANOVAs were performed for each age, considering the litter random factor in a randomized blocks design [1,10]. These yielded highly significant treatment effects both in pre- and in postweanlings, respectively, $F(4,32)=8.41$ and $F(4,32)=7.26$, $p<0.001$ Two sets of post-hoc multiple comparisons (Newman-Keuls test) showed significant differences at both ages between saline-injected mice and each of the four morphine groups ($p<0.01$) There were no significant differences between drug doses, which also applies to the apparently more marked analgesia produced at 21 days by 10 mg/kg of the drug, than by other doses

The activity data did not show differences between successive daily sessions, therefore they are presented after pooling of the scores obtained on days 14–16 and 21–23, respectively (Fig 1, lower graph) In preweanlings, morphine depressed activity already at the lowest dose used, $F(4,32)=3\ 75$, $p<0\ 01$, Newman-Keuls tests $p<0\ 05$ for saline group versus each of the four morphine groups which did not differ from each other. In postweanlings, the drug effect was apparently nonmonotonic, with a slight stimulation at the lower doses and depression at the highest dose Only the latter effect, however, was statistically significant, $F(4,32)=5\ 53$, $p<0.001$, Newman-Keuls tests 20 mg/kg different from saline ($p<0\ 05$) and from 1 and 5 mg/kg ($p<0.01$)

The results of the hot-plate measurements performed at the adult stage after saline injection are depicted in Fig 2. The group treated with saline at 14–16 days showed an average latency substantially higher than that of all other groups This was confirmed by appropriate Newman-Keuls tests ($p<0\ 05$) after a mixed design ANOVA with age of previous treatment as grouping factor, litters as nested random variable, and previous treatments as fixed variable within litters [age × type of previous treatment, $F(3,48)=2.97$, $p<0.05$].

On the following day, the effects of morphine (10 mg/kg) on hot-plate latency were clearly less marked in mice which had been previously exposed to the same drug than in mice previously treated with saline This was confirmed by the ANOVA [prior treatment, $F(3,48)=3.00$, $p<0\ 05$] and by an appropriate Sheffe's test comparing all animals previously exposed respectively to saline and to morphine ($p<0.05$)

The adult activity data (Fig 3) failed to show significant differences between the various groups in the first day of testing (saline injection). On the following day, after morphine injection (10 mg/kg), the animals previously exposed to either saline or drug at 21–23 days maintained a higher activity level than those pre-exposed at 14–16 days This was confirmed by the ANOVA, which yielded a highly significant effect of age of prior testing, $F(1,16)=9\ 66$, $p<0.01$, without an effect of type of previous treatment.

DISCUSSION

The data obtained in the immature mice in the present experiment confirm and extend previous rat data showing marked changes of morphine effects during development. Specifically, the sensitivity to the depressant action of the drug on activity was markedly attenuated between postnatal days 14–16 and 21–23. It has also to be noticed that activity levels were substantially the same in successive daily tests, pointing to an absence of morphine tolerance with these combinations of testing ages and treatment schedules By contrast, the differences in analgesic effects of morphine at the two ages, if any, were minimal. Therefore, the overall profile cannot be explained either by the increase of opioid receptors during the third week [24,25] or by an increased efficiency of the blood/brain barrier [6,17] As concerns the latter, however, one cannot exclude differential maturation phenomena involving separate brain areas responsible for effects on activity and pain reactivity, respectively

On the other hand, it must be emphasized that major changes in activity patterns and response to model drugs such as amphetamine and scopolamine occur during the third week of postnatal development (for mouse data see [2]). This suggests that the differences in response to morphine in preweanlings and postweanlings may not be due to differences in the drug effect per se, but to a modified profile of interactions between opioid modulatory mechanisms and other regulatory systems

In fact, morphine effects on activity during development appear to be markedly affected by organismic and test variables which are known to influence activity patterns and responses to neurotransmitter agonists and antagonists Specifically, Filibeck and coworkers [11], using an inbred (C57Bl/6) mouse strain and a different (unfamiliar) test environment, found an increase of baseline activity during the third postnatal week much larger than that observed in the present experiments Moreover, these investigators found morphine effects which were almost constantly in the direction of a marked hyperactivity in spite of different developmental ages and activity baselines.

As concerns long-term consequences of early morphine and test exposure, the results of the present experiment further support the view that the drug affects activity and pain reactivity by different mechanisms [3,18] Prior morphine exposure did not influence activity and response to morphine at the young adult stage. But, the animals with a history of testing at the pre-weanling stage were more sensitive than those pretested at a later stage to the depressant effect of a 10 mg/kg drug dose By contrast, morphine analgesia was attenuated by prior drug exposure, independently of age of previous testing

This picture is further complicated by the finding that age of early exposure and type of early treatment interacted to determine the level of adult pain sensitivity in the no-drug state. The lack of controls left undisturbed at the time of early testing, however, does not allow one to discriminate between two main possibilities The first is that all combinations of early treatment and time of early exposure produced hyperalgesia, except exposure in the no-drug state at the

pre-weanling stage The second is that of a hypoalgesia which was produced only by exposure at the pre-weanling stage and was prevented if such exposure took place in the morphine state.

It appears difficult to suggest any mechanism that might account for the former type of effect. (For example, a conditioned hyperalgesia [16] could explain the profiles of the groups previously exposed to morphine; but, a host of additional assumptions would be necessary to account for a hyperalgesia in the group treated with saline at the postweanling stage.) On the other hand, some indications are available which indirectly support the alternative explanation outlined above Specifically, the reduced sensitivity to pain of animals exposed at the pre-weanling stage resembles the well-known phenomenon of environmentally induced analgesia (E.I A : [13,22]), which can follow a wide variety of stressful experiences The fact that a similar long-term effect did not occur in the case of early exposure in the morphine state could be ascribed to an attenuation of the pain experience and/or to a state-dependence phenomenon. At this point, one can speculate about the difference between the groups exposed in the no-drug state at the pre-weanling and the post-weanling stage, respectively. Some critical differences between the two developmental ages are well known, for example, cholinergic (muscarinic) mechanisms which contribute to the control of the organism's activity become functional during the third postnatal week [2] On the other hand, opioid and non-opioid mechanisms, including muscarinic ones, are known to interact in a complex fashion to produce various components of E I A. [12, 21–23]. Therefore, one should test the hypothesis that changes in the interactions between different systems, particularly opioid and cholinergic ones, may make the organism progressively more selective with respect to long-term repercussions of early environmental stimulation

ACKNOWLEDGEMENTS

We acknowledge Dr G Bignami for critical reading of the manuscript, Dr Flavia Chiarotti for expert statistical advice, and Dr U Avico for kindly providing the morphine used in the present study

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