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Short communication

ENHANCED RESPONSES TO OPIATES PRODUCED BY A SINGLE GENE SUBSTITUTION IN THE MOUSE

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Adult male mice of the C57Bl/6J strain, or mice differing at a single locus from the above, pallid mice (C57Bl/6J-pa), were treated with morphine or vehicle and two responses associated with opiate receptor stimulation were examined. In comparison with vehicle treatment, morphine produced stereotyped running and reduced core temperature in the parent C57Bl/6J line. These responses were significantly enhanced in the coisogenic pallid line. Thus, a single gene may enhance normal sensitivity to opiates. This suggests the existence of discrete and identified biochemical influences upon sensitivity to opiates through genetic means, and offers a novel model of naturally occurring permanently altered opiate sensitivity.

Activity Hypothermia Morphine Opiate Pallid gene

1. Introduction

Behavioral and physiological responses to opiates are known to vary widely across inbred strains of mice (Brase et al., 1977; Castellano and Olivero, 1975). Such differences establish a genetic basis for sensitivity to opiates, however they address neither the nature of genetic control nor the particular genes involved. The use of co-isogenic lines allows for considerably greater characterization, indeed to the level of a single identified gene (Green, 1966). We report herein a single gene analysis of opiate sensitivity based upon co-isogenic lines. The finding of enhanced opiate sensitivity by one gene offers a novel genetic model of potential interest both to geneticists and opiate researchers.

2. Materials and methods

Adult male pedigreed C57Bl/6J mice (PaPa) (28—34 g/mouse — Jackson Laboratory, Bar Harbor) were group-housed 6 mice/cage with food (Teklad 4.0% fat rodent diet S-0836) and tap water continuously available and 12 h/12 h lighting (lights on = 08:00-20:00 h). Pallid mice (Roberts, 1931; Bodmer, 1961) (C57Bl/6J (papa)) were obtained from the above supplier and bred in our own colony (Univ. Michigan) by back-crossing pedigreed pallid males to heterozygous (i.e., C57Bl/6J Papa) females. Two tests of opiate sensitivity were performed, with testing carried out between 12:00 and 16:00 h using naive age and weight matched mice (n = 6/cell for all data points). In the first test behavioral responses were assessed using stereotyped psychomotor activation to morphine as a dependent variable. Activity was measured upon four tuned oscillators of commercial manufacture (Stoelting, Chicago) matched to within 5% sensitivity of each other as described in greater detail elsewhere (Katz and Schmaltz, 1979). 4 h of habituation to the 36 × 33 × 17 cm testing boxes, containing fresh pine chip bedding and resting directly upon the monitors, preceded drug or vehicle injection. In the second test, hypothermia was measured using

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a Yellow Springs 43-G telethermometer with a 1.0 cm rectal probe. Temperatures were taken after 15—20 sec of probe insertion to allow equilibration. Morphine sulphate (12.5 or 25.0 mg/kg), or vehicle (0.9% sodium chloride) solution were injected 1 ml/0.1 kg intraperitoneally and four measures were recorded per subject for consecutive 15-min blocks post injection. A single mean score was then computed based upon an average across blocks. It should be noted that only pallid mice without overt neurological symptoms were tested. In our hands 20—30% of pallid mice show marked disorders of equilibrium due to reduced otoliths (Lyon, 1953). These mice were screened from the subject pool prior to the start of the experiment. In both experiments statistical analysis was by two-way (i.e., dose by strain) analysis of variance.

3. Results

Results for drug induced behavioral activation are presented in fig. 1, and for hypothermia in fig. 2. It may be seen in fig. 1, that both lines had a similar behavioral response to vehicle, and that activity was increased in a graded manner by morphine. This increase was greater in the pallid line than in the parent line. This proved to be true also of the hypothermic response, presented in fig. 2. While the response was present in both lines, it also was greater for the pallids. Main effects for gene and drug in both models were statistically reliable beyond chance. Respective F ratios for the two experiments (activity, temperature) are: for genetic effects $F = 5.9$, 12.7; $df = 1.30$; for drug dosage effects $F = 4.0$, 4.6; $df = 2.30$ (in all cases $P < 0.05$). In further confirmation of the hypothesis that drugs were differentially effective across lines F ratios for gene by dose were also significant ($F = 5.2$, 4.9 and 3.1, 2.8; df, respectively, 2.30; again $P < 0.05$). It may be concluded

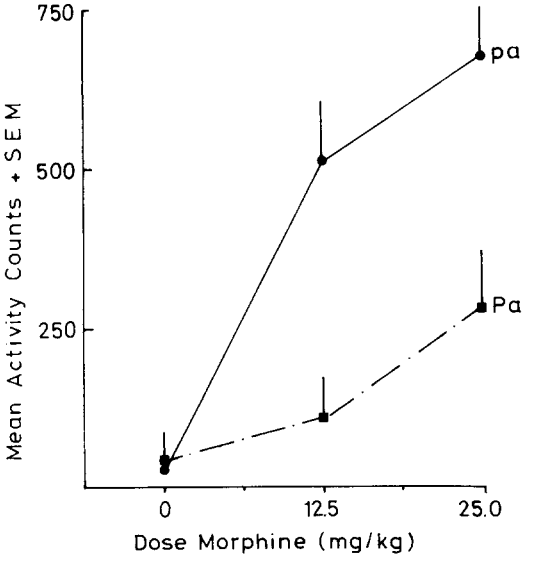

Fig. 1. Influence of morphine upon behavioral activation in C57Bl/6J and C57Bl/6J (PaPa) mice. Behavioral scores based upon remote sensing are presented. Group means and standard errors are presented.

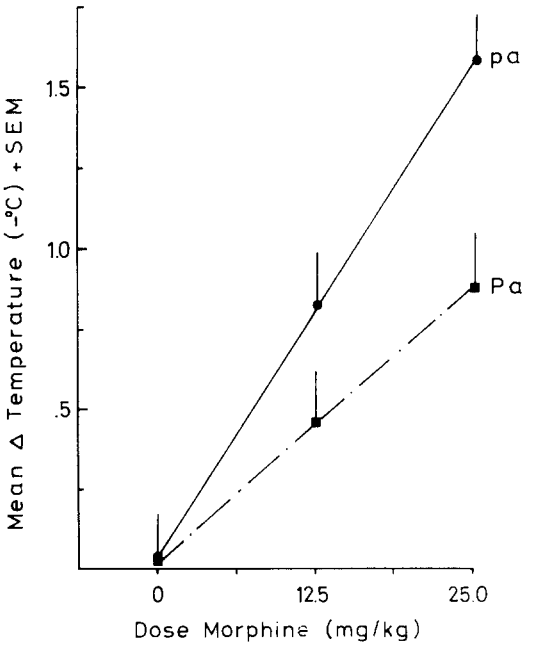

Fig. 2. Influence of morphine upon hypothermia in C57Bl/6J and C57Bl/6J (PaPa) mice. Mean core temperatures plus standard errors of experimentally naive subjects are presented. It should be noted that lower doses of drug may produce hyperthermia. Doses in the present experiment were chosen to maximize hypothermic effects.

that pallid mice are more sensitive to opiates than C57Bl mice.

4. Discussion

These findings are of interest in identifying both (a) novel pharmacological properties for the pallid mutation, and (b) a novel gene affecting behavioral and physiological sensitivity to opiates. This is the first identification, to our knowledge, of a single gene modifying normal responses to opiates. The cellular mechanisms underlying the above effects are not known. One possibility is a direct pleiotropic effect upon endogenous opioid systems either at a biosynthetic or receptor level. Alternately, these effects may be mediated through non-opioid systems which in turn influence the above. One possible mechanism for this effect is reduced manganese metabolism in pallid mice, which in turn might affect a variety of biosynthetic reactions leading to opiate hypersensitivity due to the indirect effects of ion deprivation. This could be tested by dietary supplementation, which has convincingly been shown to be effective for manganese related otolith reductions in related experiments (e.g., Erway et al., 1970). Because pallid mice are no longer routinely maintained by us at this time such an experiment could not be carried out. However, it might be noted that animals with severe neurologic problems indicating otolith reduction due to manganese deficiency were screened from our population prior to the start of testing. This clearly remains an important issue for future investigation, however. Monoamine systems such as dopamine or serotonin also may be considered as candidates for indirect mediation especially given the existence of a genetically induced chronic alteration in precursor transport (Cotzias et al., 1972). In either event the findings are of interest, suggesting the utility of these particular genetic lines for further pharmacological and behavioral analysis.

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