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Stereospecific, Dose-Dependent Antagonism by Naloxone of Non-Opiate Behavior in Mice

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JACQUET, Y. F. *Stereospecific, dose-dependent antagonism by naloxone of non-opiate behavior in mice.* PHARMAC. BIOCHEM. BEHAV. 13(4) 585–587, 1980.—When mice were placed in a novel environment, they exhibited behavioral activation, characterized by a high frequency of jumps, rearings, groomings, digging, etc. Naloxone exerted a dose-dependent antagonism of this behavior. The antagonism was stereospecific, with the enantiomer, (+)-naloxone failing to antagonize this behavior. Morphine-injected mice showed a different behavioral syndrome, i.e., Straub tail and a compulsive, robot-like ambulation around the perimeter of the bin, with a total absence of jumps, rearings, etc. The morphine behavioral syndrome was antagonized by naloxone at 1 mg/kg, while higher naloxone doses were required to antagonize the behavioral activation in a novel environment. These results suggest that stereospecific antagonism by naloxone is a necessary but not sufficient condition for defining opiate-like action.

Naloxone antagonism Morphine Behavioral activation Mice

ALTHOUGH naloxone was once considered to be a "pure" opiate antagonist, (i.e., antagonizing opiate action only, while exerting no agonist action of its own), recent findings reporting that naloxone exerts other actions, e.g., antagonism of nitrous oxide analgesia [1], GABA action [2], and hypovolemic shock [3], have cast some doubt on this view (see also [6] for latest review), thus weakening the central role played by naloxone antagonism as the defining criterion for opiate action. However, with the recent availability of the enantiomer, (+)-naloxone, the stereospecificity of naloxone antagonism has become an additional criterion for defining opiate action. The present study examined behavior in mice which appeared to be non-opiate in origin, but which is antagonized dose-dependently by naloxone in a stereospecific manner.

METHOD

Subjects and Procedure

Forty-eight adult male Swiss Webster mice were randomly assigned to one of 4 groups and given intraperitoneal (IP) injections of either the vehicle (physiological saline) or naloxone hydrochloride at 1, 10 or 100 mg/kg (in a volume of 0.005 ml/g body weight) over 7 trials (1 trial in the p.m. on the first day, a.m. and p.m. for the next 3 days). Following each injection, the mice were placed, 4 cagemates together, in a glass jar (30.5 cm in diameter, 61 cm in height) which represented a novel environment on the first day, and their behavior observed for 30 min. The observer, "blind" as to the drug treatment of each mouse, rated the subject on general activity (on a 6-point rating scale) as well as counted the number of jumps by each subject.

To ascertain the stereospecificity of the naloxone effects, an additional 3 subjects were injected with (+)-naloxone at 100 mg/kg (the small *n* being due to the limited availability of this enantiomer).

An additional 8 mice were injected with morphine sulphate at 25 or 50 mg/kg. Another 4 were given a "dry" injection (needle puncture alone) and another 4 given no injection. These last 2 controls were to see if saline alone, or needle puncture alone, exerted an action.

RESULTS AND DISCUSSION

An analysis of variance for each measure showed that the groups differed significantly on general activity level, $F(3,44)=69.35$; $p<0.001$, and number of jumps, $F(3,44)=6.07$; $p<0.002$. (These two scores essentially measure the same variable of behavioral activation. This overlap in measurement was for the purpose of having one measure corroborate the other.) As seen in Figs. 1 and 2, the saline-injected control group showed a high level of behavioral activation, characterized by a continuous stream of behaviors such as jumps, rearings, grooming, running, digging, etc. Naloxone reduced this behavior in a dose-dependent manner. At the highest dose, 100 mg/kg, there was an almost total lack of behavior, with the subject lying quietly, and appearing to be asleep within 2–3 min, and remaining asleep for the remainder of the 30-min session.

The 3 mice injected with 100 mg/kg of (+)-naloxone showed the same behavioral activation, i.e., jumps, rearings, grooming, digging, etc., as the saline-injected mice. Thus, naloxone antagonism of the behavioral activation was stereospecific. The additional controls given a "dry" IP injection, or no injection, also showed the same behavioral activation as the saline-treated mice, thus indicating that the behavioral activation was due to being placed in a novel environment, and not due to the saline or the injection *per se*. There was little habituation to the novel environment, as indicated by no consistent decrement in the behavioral activation, probably due to the short exposure (30 min) during each of the 7 trials.

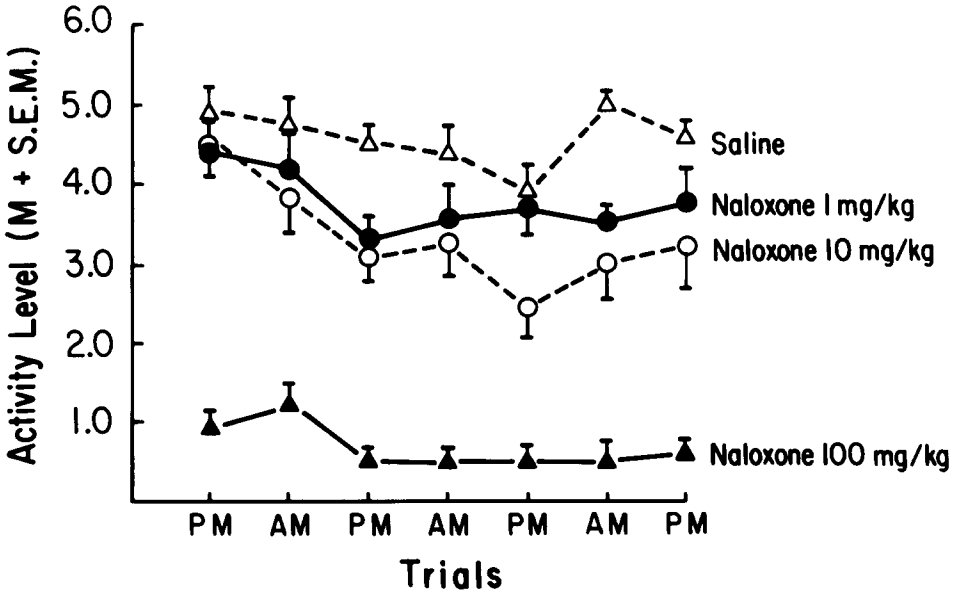

FIG. 1. Means (+SEM) of rating scores for behavioral activation of 4 groups of mice (n=12 ea) given an IP injection of either vehicle (saline) or naloxone at 1, 10 or 100 mg/kg immediately prior to being placed in a novel environment for 30 min twice daily over 4 days. The groups differed significantly, $F(3,44)=69.35$, $p<0.001$.

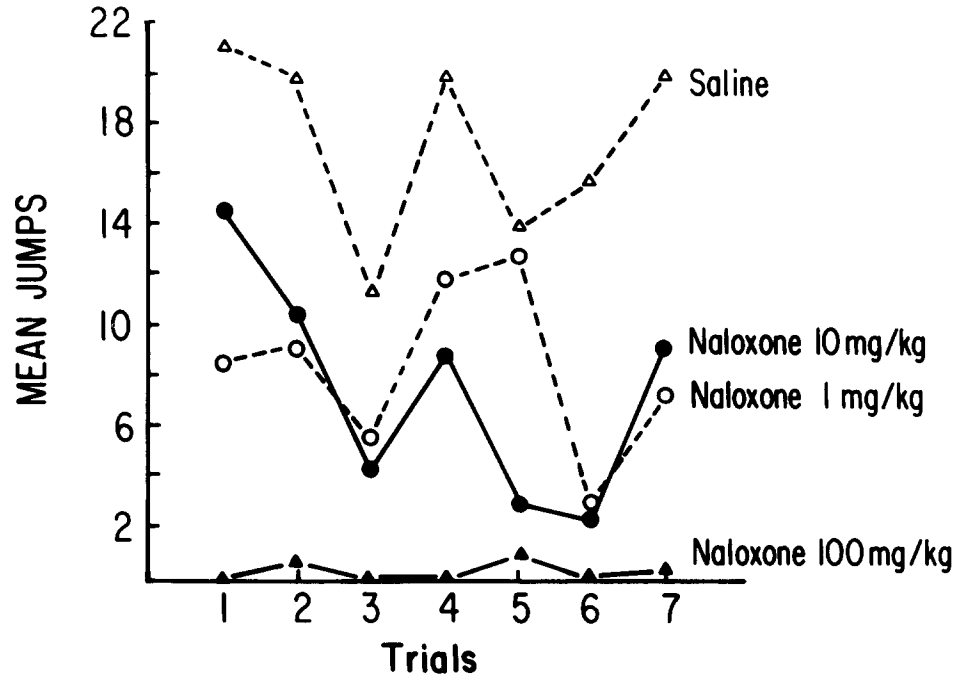

FIG. 2. Mean number of jumps of the 4 groups of mice (same as in Fig. 1) (SEM not presented because of considerable overlap between groups.) Statistical analysis of non-transformed scores revealed a significant difference between groups, $F(3,44)=6.07$; $p<0.002$, overriding the large variability within groups.

The morphine-injected mice (at a dose of 25- or 50 mg/kg) uniformly showed a distinctly different behavioral syndrome, i.e., Straub tail and a rapid and compulsive, robot-like ambulation around the perimeter of the bin. This incessant ambulation occurred to the exclusion of all other behaviors such as rearing, jumping, digging, grooming, etc. A low dose of naloxone (1 mg/kg) eliminated the robot-like ambulation with Straub tail, returning the animal to a state of normal activity (e.g., a moderate level of jumps, rearings, digging, etc.).

Enkephalin analogs have been reported to produce a "stereotyped running syndrome which closely resembles morphine running" [5]. Thus, the present behavioral activation in a novel environment is probably not due to the release of endogenous opioids. Conceivably, the activation may be due to the endogenous release of a non-opiate substance which is displaced stereospecifically from its receptor sites by naloxone. These receptors, however, appear to possess only a moderate degree of affinity for naloxone (showing only partial antagonism at the lower doses).

These results suggest that stereospecific antagonism by naloxone may be a necessary but not sufficient condition for characterizing opiate action.

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