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Detection of Antagonist Activity for Narcotic Analgesics in Mouse Hot-Plate Test

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JANICKI, P AND J LIBICH *Detection of antagonist activity for narcotic analgesics in mouse hot-plate test* PHARMAC BIOCHEM BEHAV 10(4) 623–626, 1979 —On the assumption that by the use of the hot-plate procedure the antagonist properties of narcotic analgesics could be detected, the effect of morphine, pentazocine, nalorphine and naloxone were investigated The latency of paw-licking and jumping-off were determined and compared. The agonist, morphine, at doses of 0 025, 0 05 and 0 1 mmole/kg injected IP significantly increased paw-lick and jump-off latency above that seen in saline controls The mixed agonist-antagonist, pentazocine, at doses of 0 048, 0.096 and 0 192 mmole/kg and nalorphine, an antagonist with some agonist activity, at doses of 0 032, 0 064 and 0 128 mmole/kg significantly increased the latency of paw-licking, but did not significantly change the jump-off latency At a dose of 0 016 mmole/kg naloxone treated mice jumped from the hot-plate significantly sooner than controls but no effects of naloxone on paw-licking latency were observed These results suggest that agonist properties are involved in the paw-lick response and that antagonistic properties determine jumping-off behavior

Hot-plate behavior Opiate agonist Opiate antagonist Endorphins

ALL the narcotic drugs are proposed to possess dual agonist and antagonist actions, whether they are clinically used as "agonist" or "antagonist" [15]. Agonistic properties have been examined on the longitudinal muscle of guinea pig ileum, on mouse vas defferens preparations [15], by the tail pinch test or by the electrical tail shock method [25].

In investigation of agonistic properties of opiate drugs the hot-plate method is commonly used However, the mixed agonist-antagonists such as pentazocine, or the antagonists with some agonist activity can not be checked by use of this test because their agonistic activity presumably balances their agonistic ones [4, 7, 13] Although naloxone does not modify the paw-licking latency in the hot-plate method, other changes in behavioral responses have been observed [11,16] In fact, naloxone significantly decreased the latency of jumping-off behavior in the hot-plate procedure Since naloxone possesses only antagonist activity it might be suggested that the difference between latency for paw-licking and jumping-off are mediated by endogenous opioids [11].

Based on the above mentioned considerations it was interesting to compare the effects of naloxone with the action of pentazocine and nalorphine using the modified hot-plate method, as well as to check the action of the relatively pure agonist, morphine, under these experimental conditions.

The following experiments were conducted to determine whether paw-licking and jumping-off behavior are similar for drugs showing the antagonistic action and whether the hot-plate test may be a predictive test for determining such antagonistic properties.

METHOD

Experiments were performed on male Swiss mice, 0.017–0 023 kg. The hot-plate method, 55°C ± 0.5, was used. Animals were placed on the surface of the hot-plate and covered by a transparent glass cyldinder, 25 cm high and 12 cm dia. The time for paw-licking and jumping-off was measured [11,25]. Each drug-treated group consisted of 10 mice Each mouse was tested only once The following drugs were used morphine hydrochloride (Polfa), at doses 0.025, 0 05 and 0 1 mmole/kg body weight, pentazocine hydrochloride (Polfa) 0.048, 0 096 and 0 192 mmole/kg; nalorphine hydrobromide (Chinoin) 0 032, 0.064 and 0.128 mmole/kg and naloxone hydrochloride (Narcan from Endo-Lab) at dose 0 016 mmole/kg Only one dose of the naloxone was used since, as reported by Grevert and Goldstein [11] naloxone in all doses, higher than 0.01 mmole/kg significantly decreased the time of jump-off, but not paw-lick Drugs were dissolved in 0 9% saline and injected interperitoneally at volumes of 0.01 ml/g body weight. The injections were made 30 min before testing, except for naloxone which was injected 10 min before testing [11]

The reaction times (RT) were estimated and compared

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with the appropriate control conditions. The non-parametric "series" test was used [9,22] for statistical analysis. The selected alpha level of significance was 0.025. For each drug dose group the mean value and its standard error (SEM) were calculated. Differences in latency between the control group mean and a drug-treated animal does not provide any indication of the relationship between the latency of the drug treated animal and the distribution of the reaction times in the control population. Therefore it was more informative to define the analgesic state not only with respect to the mean of the control population but also with respect to its variance For this reason analgesia scores for experimental animals were standardized with respect to the control group according to the following formula [5]:

$$\text{Standardized Analgesia Score} = \frac{\text{RT (experimental animal)} - \text{RT (control group)}}{\text{one standard deviation (control group)}}$$

RESULTS AND DISCUSSION

In this study the differences between the two responses were measured at 55°C. At higher temperatures (55–60°C) the latency between paw-lick and jump-off was too short for proper measurement. At all doses of morphine, pentazocine and nalorphine administered the latency for hindpaw-licking was prolonged The effect of morphine was markedly stronger than that of pentazocine and nalorphine. No significant effect of naloxone on paw-lick latency was found. These results confirm the findings of Jacob *et al* and Grevert and Goldstein [11,16]. Morphine significantly increased jumping-off latency, whereas no significant differences were found between pentazocine or nalorphine treated mice and saline controls

Naloxone shortened the latency for jump-off effect (Fig 1). Thus, it may be that the paw-lick effect is involved with agonist activity and the jumping-off effect with the antagonistic one. This conclusion is supported by the finding that naloxone, a pure antagonist without agonist activity [3], decreased jump-off latency and did not change paw-lick latency

On the other hand morphine with strong agonist activity but with very little antagonist activity increased significantly the latency of both effects. The lack of effect of nalorphine and pentazocine on the jump-off latency is probably due to their mixed agonistic-antagonistic properties The responses of the analgesics could be divided into three groups: (a) strong, or pure agonists—these drugs increased paw-lick and jump-off latencies (b) mixed agonist-antagonists—these drugs increased paw-lick latency but not jump-off latency. (c) the group of pure antagonists which decreased only the time of jump-off latency and did not modify paw-lick latency (Fig. 2). Some studies have failed to detect the agonistic properties of pentazocine using the hot-plate test [4, 7, 13]. However, in these cases only one effect, for example jump-off, or the mean value of all observed behavioral responses were measured

A number of explanations for these findings can be suggested. Recently the existence of endogenous opiate ligands, endorphins, have been determined. Endorphins, whether a single active peptide or a family of peptide fragments, may serve as neurotransmitters or, more likely, neuromodulators in the brain [2, 8, 10, 12, 24]

Endorphin receptors may be sites at which the morphine-like drugs exert their pharmacological actions If

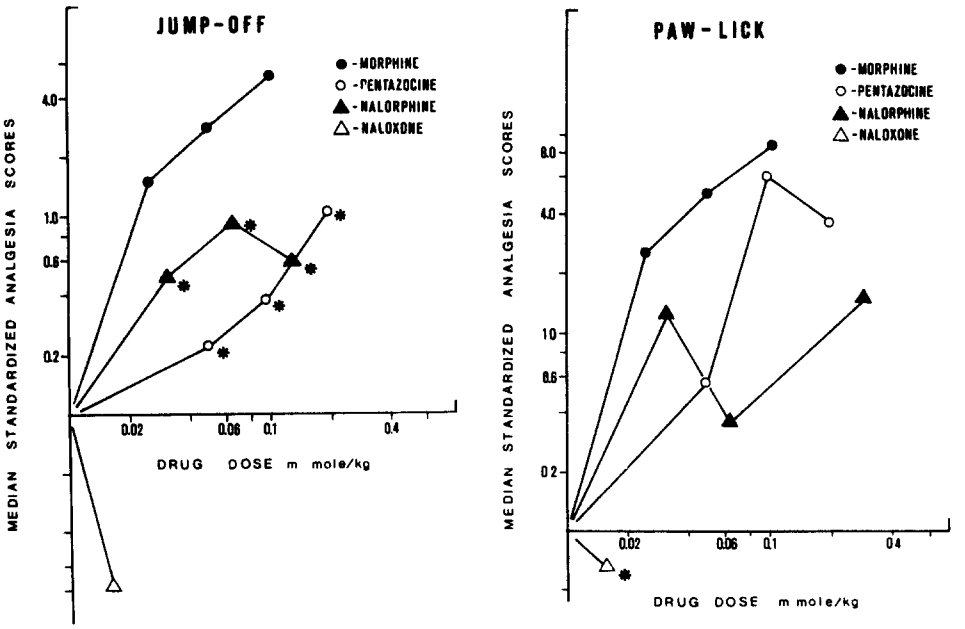

FIG 1 Effect of morphine, pentazocine, nalorphine and naloxone on paw-lick latency (panel on the right) and jump-off latency (panel on the left) represented as analgesia scores (see text) Each point represents the mean of 10 mice The points under abscissa represent the decrease of the reaction time versus control Asterisks denote lack of significant difference (using non-parametric series test, $p=0$ 025) with control values

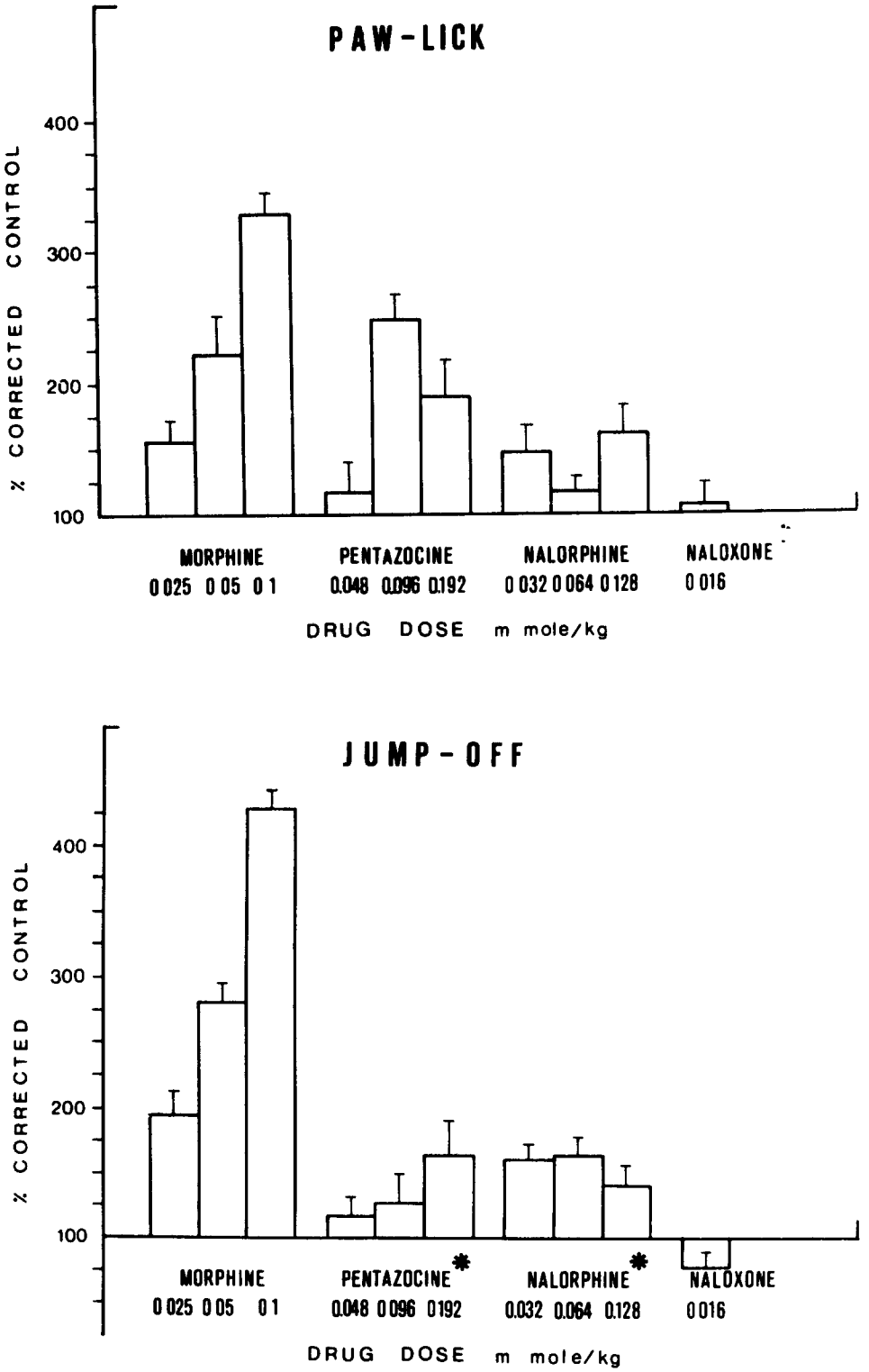

FIG 2 Mean values of the latency time for paw-licking (upper panel) and jumping-off response (lower panel) after morphine, pentazocine, nalorphine and naloxone injected IP ordinate. percent of corrected control Negative (under abscissa) column represents the decrease of the jump-off latency Note that vertical bars (+SEM) indicate the dispersion of the results only but are not important for the significance calculated according to non-parametric statistical test Asterisks denote lack of significant difference (using non-parametric series test, $p=0$ 025) with control values

endorphins exert intermittent or varying tonic influences on neuronal activity in the brain, pharmacological blockade of these receptors by naloxone, would be expected to alter behavioral responses in special testing conditions Naloxone was found to interfere with analgesia produced by stimulation of the periaqueductal gray matter [1], jumping-off latency in the hot-plate test [11], amplitude of evoked auditory potentials in rats [6], escape behavior in rats and mice exposed for the first time to naloxone [16], electrically induced ACh release in the cerebral cortex of rats [17], and thermoregulatory changes induced by olfactory nerve stimulation [18]

It has been suggested that under hot-plate testing conditions, prolonged exposure to the noxious stimulus may be necessary to activate the endorphin system [11] Naloxone apparently interferes with this adaptation because naloxone-treated mice tolerated this stimulus for shorter durations than control. Drugs with partial antagonistic activity like pentazocine or nalorphine probably also interfere with this adaptation but their agonistic activity partially balances their antagonistic activity and the sum of these actions results in unchanged jumping-off latency In light of these observations the comparisons between the paw-lick and jump-off latencies could give more information about agonistic and/or antagonistic properties of various drugs Such information might be useful because of the simplicity of these procedures as new pharmacological tools for testing both properties of drugs. Using these methods the mixed agonistic-antagonistic properties of new drugs might be also estimated However this modified hot-plate method must be considered only as a possible preliminary screening procedure The subsequent measuring of the binding of these drugs to the opiate receptors and the electrically stimulated guinea-pig ileum or mouse vas deferens preparations as well as other methods are required

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