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Dissociation of Naloxone-Sensitive and Naloxone-Insensitive Effects of U-50,488H[1]

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YOUNG, G. A. *Dissociation of naloxone-sensitive and naloxone-insensitive effects of U-50,488H.* PHARMACOL BIOCHEM BEHAV **33**(1) 215–217, 1989.—This study was designed to compare the dose-related effects of U-50,488H on the cortical electroencephalogram (EEG) and behavior, following either saline or naloxone pretreatment. Adult female Sprague-Dawley rats were implanted with chronic cortical EEG and temporalis muscle electromyographic (EMG) recording electrodes and with permanent cannulae in the external jugular vein. U-50,488H injection produced initial "psychotomimetic-like" EEG and behavioral effects for about 10–20 min, followed by a predominance of behavioral stupor and associated EEG slow-wave bursts for about 20 min. Naloxone pretreatment completely antagonized the behavioral stupor and associated EEG slow-wave bursts. However, the "psychotomimetic-like" effects were not antagonized by naloxone pretreatment. Thus, dissociation between naloxone-sensitive and naloxone-insensitive effects of U-50,488H was demonstrated.

U-50,488H Naloxone-sensitive Naloxone-insensitive Dissociation

AN original proposal, based upon in vivo findings of acute and chronic opioid effects on neurophysiological and behavioral parameters in the chronic spinal dog preparation, suggested that selective opioid agonists activate different receptor populations (3,7). Thus, morphine was described as a prototypic opioid acting on mu receptors producing miosis, bradycardia, hypothermia, depressed nociception, and indifference to environmental stimuli; ketocyclazocine acting on kappa receptors producing miosis, flexor reflex depression, sedation, and no change in pupil size; and SKF 10,047 (N-allylnormetazocine) acting on sigma receptors producing mydriasis, tachycardia, tachypnea, and mania, and producing abstinence in morphine-dependent dogs.

U-50,488 is a structurally novel opioid agonist that produced analgesia, sedation, diuresis and corticosteroid elevations in mice and rats (9). In a receptor binding study, U-50,488 appeared to have a 1,300-fold selectivity at kappa sites compared to mu sites (4). U-50,488 also appeared to produce naloxone-insensitive effects. That is, motor activity of rats decreased as a function of increasing doses of U-50,488 (9). However, combined doses of the opioid antagonist naloxone and U-50,488 produced increases in motor activity. Apparently, the antagonism of U-50,488-induced effects by naloxone unmasked naloxone-insensitive properties.

Thus, in the present study, dose-related effects of U-50,488H on EEG and behavior were assessed in rats that were pretreated with either saline or naloxone injection.

METHOD

Animals

Five female Sprague-Dawley rats (250–300 g) were implanted with bipolar epidural frontoparietal EEG and temporalis muscle electromyographic (EMG) recording electrodes (5) and with chronic cannulae in the jugular vein (10,11).

During the experiments, rats were housed in individual cages, $12 \times 12 \times 24$ in. To permit free movement of the rats, each cage was equipped with a swivel connector having concentric mercury pools which served as noise-free sliding contacts (6). These freely-moving rats were allowed to acclimatize to the experimental cages for 2–3 days before experimentation. Lighting conditions consisted of a timer-regulated lights-off period from 10 p.m. to 6 a.m.

For each rat, direct EEG activity was filtered to pass frequencies between 1–75 Hz. The EEG and integrated EMG activities were continuously recorded on a Grass polygraph. Behavioral states of nonrapid eye movement (non-REM) sleep, REM sleep and wakefulness were identified by the corresponding changes in

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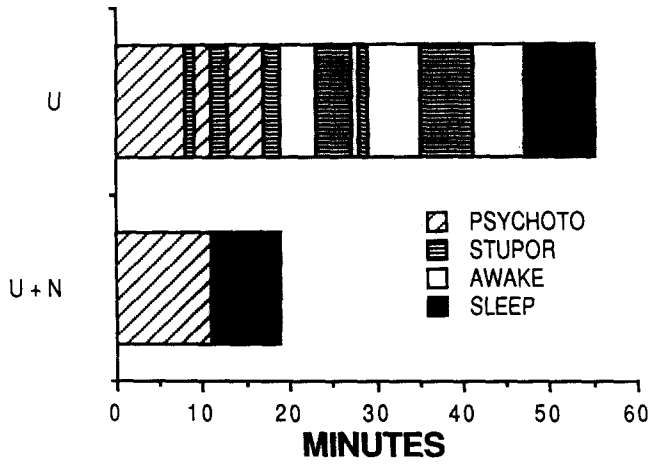

FIG. 1. The EEG and behavioral responses of an individual rat to a 2.5 mg/kg (IV) dose of U-50,488H (U), and U-50,488H + 1.0 mg/kg (IV) of naloxone (U + N).

EEG and EMG recordings (5,6). Latencies to onset of non-REM sleep following drug administration were also assessed. It is well established that latencies to onset of non-REM and REM sleep after opioid administration provide a useful measurement of opioid effects (13–15). Occurrences of opioid-induced high-voltage bursts in the EEG and associated behavioral stupor were identified from corresponding EEG and EMG recordings and behavioral observations (5,6).

Drugs

U-50,488H (The Upjohn Company, Kalamazoo, MI) was dissolved in physiological saline at a concentration of 6.25 mg/ml. Ketamine HCl (Ketalar, Parke-Davis Division of Warner-Lambert Co., Morris Plains, NJ) was used to induce anesthesia.

Procedure

Five minutes after intravenous saline pretreatment (0.1 ml of isotonic saline solution), doses of U-50,488H (1.25, 2.50 and 5.00 mg/kg) were administered intravenously to five rats in a randomized fashion. Each rat received only one U-50,488H injection per 24 hr, and effects of injection on EEG and behavior were assessed. All doses of U-50,488H were repeated in the same rats five min following naloxone pretreatment (1.00 mg/kg).

Statistics

Data were statistically assessed with two-way analyses of variance with repeated measures on both factors, followed by the Newman-Kuels test (12).

RESULTS

Temporal effects of U-50,488H injection (saline pretreatment) on EEG and behavior are shown for a representative rat in the top of Fig. 1. For the initial eight min after U-50,488H injection (2.5 mg/kg, IV), behavioral occurrences of sniffing and backward movement were seen, along with extensive periods of stillness while backed into a corner with a hunched back. All of these behaviors were associated with desynchronized EEG and are collectively defined as "psychotomimetic-like." Then, alternations between behavioral stupor and associated EEG slow-wave

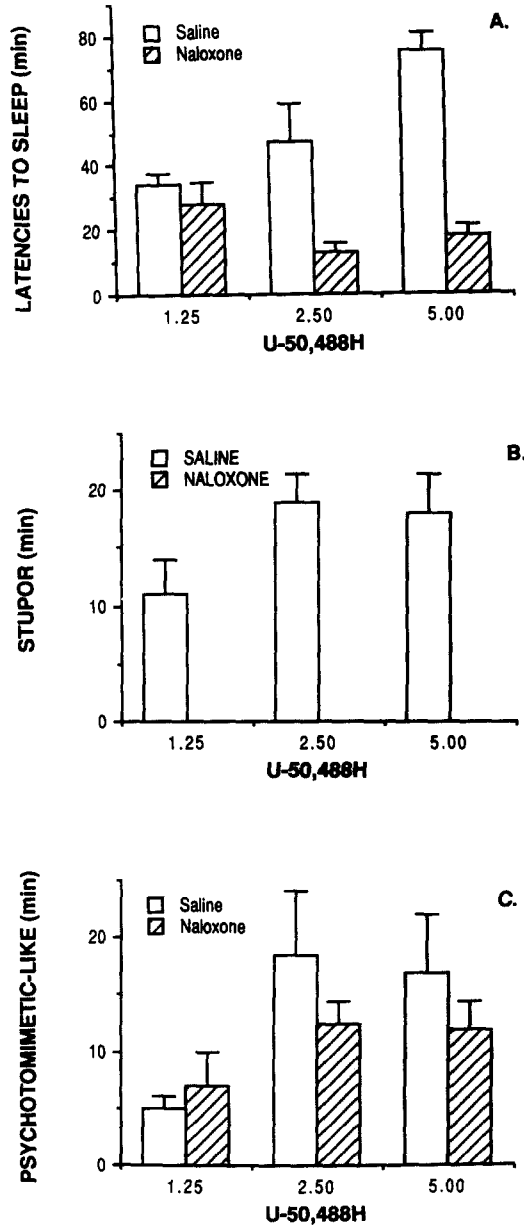

FIG. 2. Mean latencies (min) to non-REM sleep ±SDs, and mean durations (min) ±SDs of stuporous and psychotomimetic-like behaviors are shown as a function of increasing doses of U-50,488H. Data are shown following either saline or naloxone pretreatments.

bursts, and the above aforementioned "psychotomimetic-like" effects, occurred for about 10 min. Next, alternations between behavioral stupor and associated EEG slow-wave bursts, and wakefulness occurred for about 30 min. Finally, a non-REM sleep episode emerged at about 45 min after U-50,488H injection. After naloxone pretreatment (1 mg/kg, IV), U-50,488H injection (2.5 mg/kg, IV) produced the aforementioned "psychotomimetic-like" effects for about 11 min, followed by the emergence of a non-REM sleep episode. Occurrences of behavioral stupor and associated EEG slow-wave bursts were completely antagonized.

Grouped data of mean latencies to non-REM sleep (min) following U-50,488H injection, and mean total durations (min) of stuporous behavior and "psychotomimetic-like" effects are shown in Fig. 2. Data are shown following either saline or naloxone

pretreatment. Mean latencies to non-REM sleep following U-50,488H injection increased as a function of U-50,488H dose, $F(2,8)=28.37$, $p<0.05$ (Fig. 2A). Mean latency to onset of non-REM sleep was significantly greater following saline pretreatment than naloxone pretreatment, $F(1,4)=160.43$, $p<0.05$. The interaction factor was also significant, $F(2,8)=40.11$, $p<0.05$. An examination of the data suggests that the interaction occurred because mean latencies to non-REM sleep following saline pretreatment increased as a function of U-50,488H dose, while mean latencies to non-REM sleep following naloxone pretreatment did not change or possibly decreased as a function of U-50,488H dose. Subsequent multiple comparisons between means were made with the Newman-Keuls test. Mean latency to non-REM sleep was significantly greater at the 5.00 mg/kg dose of U-50,488H than the 1.25 or 2.50 mg/kg doses ($p<0.05$). Mean latencies to non-REM sleep were significantly greater following saline pretreatment than naloxone pretreatment at the 2.50 and 5.00 mg/kg doses of U-50,488H ($p<0.05$).

Mean total duration of behavioral stupor following U-50,488H injection was significantly longer following saline pretreatment than naloxone pretreatment, $F(1,4)=121.50$, $p<0.05$ (Fig. 2B).

Mean total durations of "psychotomimetic-like" effects increased as a function of U-50,488H injection, $F(2,8)=18.28$, $p<0.05$ (Fig. 2C).

DISCUSSION

Naloxone-sensitive and naloxone-insensitive effects of U-50,488H were dissociated by studying EEG and behavioral correlates. U-50,488H injection produced initial behavioral occurrences of sniffing and backward movement, along with extensive periods of stillness while backed into a corner with a hunched back. All of these "psychotomimetic-like" effects were associated with EEG desynchrony and were of relatively short duration. These "psychotomimetic-like" effects were naloxone-insensitive and were followed by occurrences of naloxone-sensitive EEG slow-wave bursts and associated behavioral stupor. The predominant initial "psychotomimetic-like" effects most likely masked early opioid effects. Previous dissociations of naloxone-sensitive and naloxone-insensitive effects on EEG and behavior have been demonstrated with the benzomorphans SKF 10,047 (15) and cyclazocine (1).

Opioid-receptor-mediated effects are generally defined as those effects that are blocked by opioid antagonists. Occurrences of behavioral stupor and associated EEG slow-wave bursts were completely antagonized by naloxone pretreatment. The "psychotomimetic-like" EEG and behavioral effects that were not antagonized by naloxone pretreatment may reflect sigma receptor activation. U-50,488 inhibits the binding of (+)-[^{3}H]-SKF-10,047 in rat whole brain approximately as well as several benzomorphans and PCP, but poorly inhibits the binding of [^{3}H]-PCP (2). Thus, U-50,488 appears to selectively bind to sigma sites, in comparison to PCP sites. One must not rule out the possibility, however, that activation of dopaminergic or serotonergic systems by U-50,488H may account for at least a portion of U-50,488H-induced "psychotomimetic-like" responses (8).

In summary, the present results demonstrate a time-related dissociation between naloxone-sensitive and naloxone-insensitive effects of U-50,488H. U-50,488H produced early "psychotomimetic-like" EEG and behavioral effects which were naloxone-insensitive. U-50,488H also produced later emerging EEG and behavioral effects that were completely antagonized by naloxone pretreatment.

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