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# Methylnalorphinium Fails to Reverse Naloxone-Sensitive Stress-Induced Analgesia in Mice

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RAE, G A , R L. N. SOUZA AND R N TAKAHASHI *Methylnalorphinium fails to reverse naloxone-sensitive stress-induced analgesia in mice* PHARMACOL BIOCHEM BEHAV 24(4) 829–832, 1986 —Exposure of mice to cold-restraint stress markedly decreased the number of abdominal constrictions induced by IP acetic acid Naloxone pretreatment significantly attenuated the antinociceptive effect of cold-restraint stress, suggesting a partial mediation by opioid mechanisms Pretreatment with the quaternary opioid antagonist methylnalorphinium did not reverse analgesia in stressed mice Also, nociception in both stressed and non-stressed mice was not modified by pretreatment with the selective $\alpha_2$-adrenoceptor blocker yohimbine. The results suggest that cold-restraint stress promotes analgesia in mice which is mediated in part by opioid but not $\alpha_2$-adrenoceptor mechanisms Furthermore, the results do not substantiate a peripheral analgesic role for circulating opioids in this model of stress

Cold-restraint stress analgesia Yohimbine in stress analgesia Quaternary opioid antagonist
Opioid mediated stress analgesia Peripheral opioid analgesia

AN extensive body of evidence shows that exposure to stressful conditions promotes analgesia in several species (for review see [8]) including the human [27]. This stress-induced analgesia (SIA) appears to be mediated by activation of opioid and/or non-opioid systems, depending on the characteristics of the procedure employed [8]. A clear example of the diversity of the systems responsible for SIA has been reported using rats submitted to inescapable electric foot-shock [17]. Whereas prolonged-intermittent shocks resulted in naloxone-sensitive SIA, brief-continuous shock produced SIA which was insensitive to the opioid antagonist

The antinociceptive effects of morphine and other opioids have generally been ascribed to an action within the CNS [15,21]. However, in recent years, several studies have demonstrated that opioids can also exert analgesia by means of an action outside the neuroaxis [2, 11, 12]. The most compelling evidence for a peripheral component in opioid-induced analgesia is derived from results obtained with quaternized opioid agonists or antagonists which do not readily permeate the blood-brain barrier [11, 12, 24]. Smith *et al* [25] demonstrated, in rats, that N-methylnalorphine (methylnalorphinium, [20]) antagonizes the antinociceptive effects of methylmorphinium but not that of morphine, whereas naloxone blocks analgesia induced by both opioid agonists

Several stressful stimuli can induce a substantial rise in plasma levels of $\beta$-endorphin of pituitary origin [4,23]. Also, it has been demonstrated that opioids coexist with catecholamines in adrenomedullary cells [16,18] and that stimuli which elicit catecholamine secretion also release large quantities of enkephalins into the circulation [7,26] It could be reasoned, therefore, that activation of opioid systems during certain stressful situations may result in analgesia mediated, at least partially, by a peripheral mechanism. The present study was undertaken to investigate such a possibility. We have analyzed the effects of naloxone and methylnalorphinium pretreatment on analgesia induced by exposure of mice to cold-restraint stress. Since it has been reported that stimulation of $\alpha_2$-adrenoceptors promotes analgesia [3, 6, 13], that stress involves sympathetic activation [1,14] and that analgesia induced by certain forms of stress is mediated via $\alpha_2$-adrenoceptors [9], we have also investigated the influence of pretreatment with the $\alpha_2$-selective antagonist yohimbine upon SIA.

## METHOD

### *Animals*

Male Swiss mice weighing 25 to 30 g were used. The animals were housed in colony cages, 10–15 in each cage

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TABLE 1

INCIDENCE OF ABDOMINAL CONSTRICTIONS IN NON-STRESSED AND COLD-RESTRAINT STRESSED MICE INDUCED BY IP INJECTION OF ACETIC ACID 0 6% (15 ml/kg) FOLLOWING SC PRETREATMENT WITH SALINE, NALOXONE, METHYLNALORPHINIUM OR YOHIMBINE

| Treatment | Dose (mg/kg) | Total Number of Abdominal Constrictions in 20 min§ Non-Stressed | Stressed | % of Analgesia Induced by Cold-Restraint Stress¶ |
|---|---|---|---|---|
| Saline | — | 69 6 ± 5 4 | 3 3 ± 2 3† | 95 3 |
| Naloxone | 10 | 68 8 ± 4 8 | 37 0 ± 11 4*‡ | 46 8 |
| Methylnalorphinium | 3 | 63 4 ± 8 7 | 0 7 ± 0 4† | 98 9 |
| | 10 | 44 8 ± 4 1 | 6 1 ± 5 3† | 91 23 |
| Yohimbine | 1 | 69 4 ± 5 6 | 6 6 ± 3 7† | 90 5 |
| | 8 | 50 9 ± 9 6 | 3 0 ± 3 0† | 94 1 |

Seven animals were tested for each group
*$p<0$ 05 when compared to non-stressed saline group
†$p<0$ 01 when compared to non-stressed saline group
‡$p<0$ 05 when compared to cold-restraint stressed saline group
§Mean ± S E M
¶Relative to the non-stressed saline group

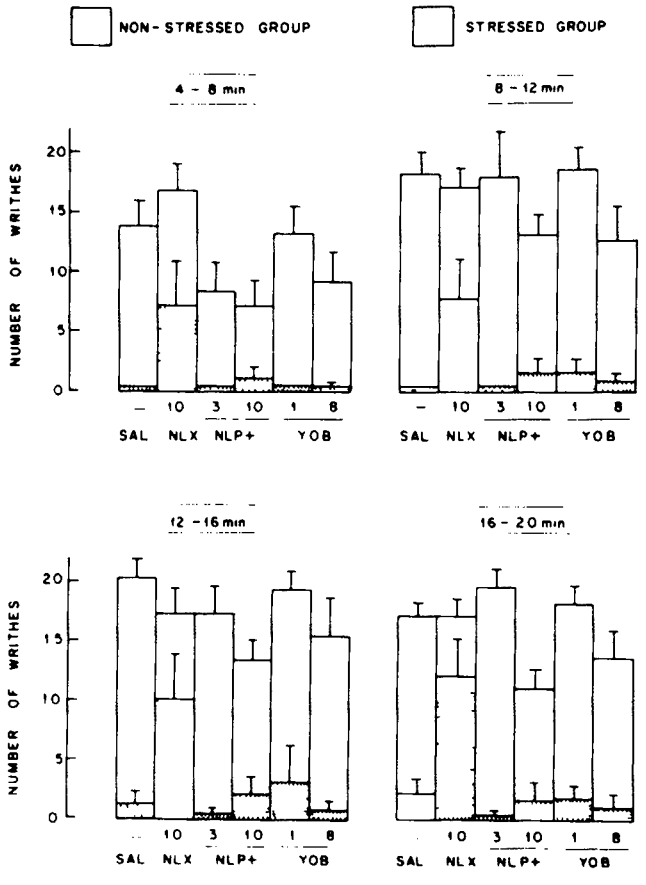


FIG 1 Effects of SC pretreatment with saline (SAL), naloxone (NLX), methylnalorphinium (NLP+) or yohimbine (YOB) on acetic acid-induced abdominal constrictions in non-stressed or cold-restraint stressed mice Values were obtained at 4 min intervals during 20 min The doses (mg/kg) are indicated in the figure below each pretreatment Each value is the mean of 7 observations and vertical lines represent the standard error of the means

Ambient room temperature was 22±1°C and the room was kept on a 12 hr light/12 hr dark cycle. All animals had free access to Purina lab chow and tap water

*Procedure*

Fifteen min after subcutaneous administration of either saline, naloxone (10 mg/kg), methylnalorphinium (3 or 10 mg/kg) or yohimbine (1 or 8 mg/kg), half of the animals were restrained in ventilated plastic tubes (6.5 cm length and 3 2 cm diameter) and placed in a refrigerator (8°C) for 30 min The second half remained in their home cages for the same period, after which all animals received an intraperitoneal injection of 0 6% acetic acid in 0 9% saline (15 ml/kg) Nociception was evaluated using the abdominal constriction test [3] by counting the number of writhes at 4 min intervals throughout the 20 min period following acetic acid injection. The values obtained during the first 4 min after acetic acid were discarded because only after this time did writhing attain stable levels in non-stressed saline-pretreated mice

*Drugs*

The following drugs were used methylnalorphinium free-base (Wellcome foundation, kindly donated by Prof S H Ferreira), naloxone hydrochloride (a gift from Endo Labs) and yohimbine hydrochloride (Sigma Chemical Co ) Drugs were dissolved in 0.9% saline except yohimbine which was dissolved in distilled water. The doses indicated refer to the salts.

*Statistics*

The results were analyzed using two-way analysis of variance (ANOVA) and Tukey *post hoc* comparisons where appropriate Differences with $p<0$ 05 were considered statistically significant

## RESULTS

Figure 1 depicts the incidence of writhes exhibited by the various groups throughout the 20 min observation period

Statistical analysis of the pooled data, by means of 2-way ANOVA, revealed significant differences in writhing incidence between the stressed and non-stressed conditions, $F(1,72)=211.96$, $p<0.001$, and drug treatments, $F(5,72)=5.24$, $p<0.01$. The interactions between stress factor and drug treatments was also significant, $F(5,72)=2.76$, $p<0.05$. *Post-hoc* Tukey comparisons did not reveal any significant differences in writhing incidence between the various non-stressed groups ($p>0.05$). However, it should be mentioned that treatment with 10 mg/kg of methylnalorphinium only just failed to induce significant antinociception.

Exposure of saline treated mice to cold-restraint stress resulted in pronounced analgesia ($p<0.01$, Table 1), which probably outlasted the observation period (Fig. 1). These animals exhibited a mean total number of writhes which was only about 5% of that observed in non-stressed mice. The stressed group with naloxone (10 mg/kg) showed significantly more abdominal constrictions than stressed animals which received saline ($p<0.05$). However, this reversal of SIA by naloxone was only partial, since these animals still presented significant analgesia when compared to the saline treated non-stressed group ($p<0.05$). In contrast, injection of methylnalorphinium (3 or 10 mg/kg) prior to cold-restraint stress failed to influence SIA ($p>0.05$). Also, treatment with yohimbine (1 or 8 mg/kg) did not modify the antinociceptive effect of cold-restraint stress ($p>0.05$).

## DISCUSSION

Mice exposed to cold-restraint stress for 30 min developed analgesia which was partially reversed by pretreatment with naloxone. This result clearly shows that the antinociceptive effect is mediated in part by activation of opioid systems. Interestingly, it has been reported that rats submitted to cold-water swim stress also exhibit SIA which is only partially reversed by naloxone treatment [5]. Although opioid-induced analgesia has long been attributed to an exclusive action within the CNS, recent evidence strongly supports an additional peripheral mechanism [12, 19, 25]. Therefore, it seemed appropriate to investigate whether this peripheral mechanism is physiologically activated by the increase in circulating opioid levels which is known to occur in situations of stress [4].

Since methylnalorphinium antagonizes the peripheral analgesia induced by methylmorphinium [19,25] and blocks morphine-induced constipation [12,22], we attempted to reverse SIA using this quaternary opioid antagonist. The results of these experiments, included in Fig. 1 and Table 1, show that pretreatment with either dose of methylnalorphinium did not antagonize SIA. These results therefore do not lend support for the participation of a peripheral component in opioid-mediated analgesia in response to cold-restraint stress.

However, non-stressed animals treated with 10 mg/kg of methylnalorphinium showed a trend towards antinociception in comparison with the saline treated non-stressed group ($0.1>p>0.05$, Tukey test). This result precluded an attempt to reverse SIA with higher doses of methylnalorphinium since it has been reported that, in the carragenin-induced hyperalgesia test, low doses of methylnalorphinium antagonize analgesia induced by injection of morphine or methylmorphinium to the inflammed paw of the rat but that higher doses exert an antinociceptive action of their own [19].

The SIA observed in the present study probably involves other non-opioid mechanisms since naloxone only partially reversed the phenomenon. A participation of $\alpha_2$-adrenoceptors in certain forms of SIA has been suggested [9]. However, pretreatment with yohimbine, a selective $\alpha_2$-adrenoceptor antagonist, failed to affect SIA. Therefore, the system(s) responsible for the non-opioid component of analgesia induced by cold-restraint stress remains to be elucidated.

It may be concluded that, in mice, cold-restraint stress results in significant analgesia which is mediated in part by activation of opioid receptors but not $\alpha_2$-adrenoceptors. Furthermore, the opioid component of this form of SIA does not appear to involve a peripheral mechanism.

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