Pharmacology Biochemistry & Behavior, Vol. 30, pp. 905–910. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Opposite Effects of Restraint on Morphine Analgesia and Naloxone-Induced Jumping

JULIA DYMSHITZ AND SHIMON AMIR

Department of Neurobiology, the Weizmann Institute of Science, Rehovot, 76100, Israel

Received 17 February 1987

DYMSHITZ, J. AND S. AMIR. *Opposite effects of restraint on morphine analgesia and naloxone-induced jumping.* PHARMACOL BIOCHEM BEHAV **30**(4) 905–910, 1988.—It has been demonstrated that the effects of exogenous opiates like morphine could be modified by exposure of an organism to stress, but it is uncertain whether this modification is due to the action of endogenous opioid peptides released by stressful stimuli. The stress of restraint produced an antinociceptive response in mice measured by a latency to escape from a hot plate and, in addition, markedly potentiated analgesia induced by low doses of morphine. Both effects were antagonized by naloxone in a dose-dependent manner. On the other hand, restraint reduced the naxolone-precipitated jumping after single morphine injection. Morphine analgesia and a jumping response were not correlated when tested in two different strains of mice. It is suggested that the enhancement of morphine analgesia by restraint and the reduction in naloxone-induced jumping are mediated via independent mechanisms.

Stress-induced analgesia Morphine analgesia Potentiation Naloxone-induced jumping Opioid peptides Mice

IN the light of multiplicity of opiate receptor subtypes [8,30] and of their endogenous ligands [1] it is likely that the physiological actions of opioid peptides include complex interactions between different components of the opiate system. An indication that such interactions occur is found in the studies concerned with modifications of morphine effects by endogenous opioids. It has been demonstrated that the administration of leucine-enkephalin prior to or a short time after injection of morphine produced powerful potentiation of morphine analgesia [17,25]. This effect was naloxone-reversible and occurred following central as well as peripheral drug administration [25,26]. Morphine analgesia can also be potentiated by certain stressful stimuli [3, 23, 24] which may induce antinociceptive effects by themselves via the release of endogenous opioid peptides [18]. Thus it seems plausible that the potentiation phenomenon could be a result of interaction between morphine and the endogenous opioids, in particular leucine-enkephalin, which was shown in addition to potentiate acute tolerance and dependence induced by a single dose of morphine [25].

The enhancement of morphine analgesia by the stress of restraint was investigated systematically by Appelbaum and Holtzman who found that this effect was centrally mediated [5], not dependent on activation of the pituitary-adrenocortical axis [3] and not reversible by naloxone [4]. In fact, in the latter study, the ED_{50} values for morphine analgesia upon naloxone administration were lower in restrained animals than in unstressed group. One possible interpretation of this finding would be that some nonopioid mechanisms are involved in the potentiation phenomenon. This view is compatible with a recent suggestion that the enhancement of morphine analgesia by stress was a result of an augmented central serotonin release due to the effect of restraint on free tryptophan levels in blood [12,16].

In the experiments by Appelbaum and Holtzman [3–5], as in other studies concerned with potentiation of morphine analgesia by stress [12,16], the analgesic reaction was measured by a tail flick test. The tail flick response has been reported to be relatively insensitive to naloxone and to treatment with enkephalinase inhibitors [11]. Furthermore, restraint by itself does not result consistently in the elevation in tail flick latency ([3], but see [7]). Thus it could be that the combination of this particular form of stress and analgesic measure was not optimal for detection of naloxone antagonism.

In the present study we used another testing procedure—hot plate test—to investigate the effects of naloxone on enhancement of morphine analgesia by restraint. It has been demonstrated that in rats this type of stress increased the latencies to escape from the hot plate, but did not affect the paw lick latencies [2]. The effect on escape was reduced by a high dose of naloxone (10 mg/kg)—the only dose used in this study. Thus, as first step, we sought to characterize the restraint-induced changes in different antinociceptive measures obtainable with a hot plate. We also used an additional test—water escape—to discard the possibility of some gross motor artifact due to the employment of a particular combination of restraint stress and escape response. Then we demonstrated a potentiation of morphine analgesia, assessed by escape from the hot plate, and tested its sensitivity to naloxone.

An additional issue investigated in the present study was

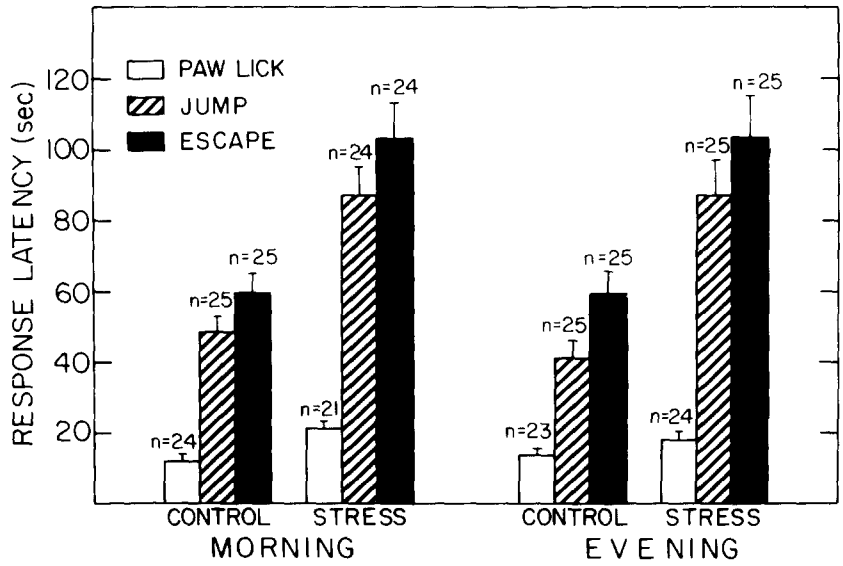

FIG. 1. Effect of restraint on latencies to three nociceptive responses: paw lick, jump and escape. The animals were tested in the morning or in the evening. Each value represents mean±SEM. n=number of mice.

the influence of restraint on naloxone-precipitated jumping. While administration of leucine-enkephalin before morphine increased the naloxone potency in eliciting withdrawal jumping [27], the stress of restraint was shown to have an opposite effect [29]. Thus animals that were injected with morphine, restrained for an hour and tested for withdrawal jumping two hours later exhibited less sensitivity to naloxone than unstressed animals. Pretreatment with corticosterone mimicked the effect of stress [29]. Since a relatively long time elapsed between the termination of restraint and the jumping test, it might be that the opioid component of the reaction to stress was too short-lived to manifest itself in increasing the jumping response. An absence of potentiation of morphine analgesia by restraint, reported in the same study [20], supports this possibility.

To further explore the effects of restraint on naloxone-induced jumping we tested the animals for a jumping response immediately after stress termination, using different doses of morphine and naloxone. In addition, two strains of mice, C57Bl/6J and DBA/1J, were used to demonstrate a lack of correlation between morphine analgesia and intensity of withdrawal jumping after pretreatment with morphine.

METHOD

Animals

Male ICR mice (5–7 weeks of age) were used in all experiments except the last one, where male DBA/1J and C57Bl/6J (8–12 weeks of age) were used. The animals were housed in standard laboratory conditions for at least two weeks prior to experimentation with water and food supplied ad lib. The experiments were conducted between 12:30 and 18:30, if not stated otherwise, during the light part of a 12:12 dark-light cycle (06:30–18:30 lights on). Room temperature was maintained at 23±1°C.

Restraint Stress

The animals were placed in plastic tubes (50 ml volume) sealed at one end and closed at the other end with a plug. The tubes were ventilated through multiple holes in their walls.

Analgesia Assessment

To measure a nociceptive reaction a hot-plate apparatus was used with a water temperature in the bath kept at 58°C. Each animal was placed on the heated copper plate within a transparent restraining cylinder (15 cm height, 11 cm diameter) and latencies to all or some of the following responses were determined: (a) lick of a hind paw, (b) jump with both hind paws in the air, (c) escape, which consisted of jumping and climbing up the cylinder wall.

Water Escape

A round container (16 cm diameter, 8.5 cm height) filled with water (3 cm high) at room temperature was used to determine the latency to water escape. The response consisted of reaching the container wall and climbing up.

Naloxone-Precipitated Jumping

Mice were injected SC with saline or naloxone and placed into transparent cylinders (30 cm high, 12 cm diameter) for 10 minutes. During this time the number of vertical jumps (more than 5 cm high) were counted by an observer. Four mice were tested simultaneously in 4 cylinders, separated from each other by opaque partitions. After each session the test area was thoroughly cleaned from urine and feces.

Experimental Procedure

At the beginning of each experiment the animals were allowed to adapt to the experimental room for two hours.

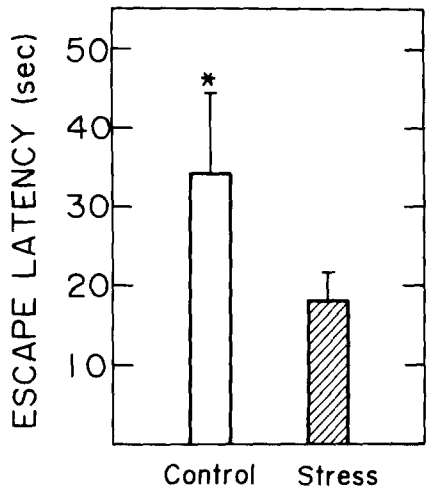

FIG. 2. Effect of restraint on water escape. Stress and control group differed significantly from each other (t-test, $p<0.05$). Each value represents mean±SEM for 10 mice.

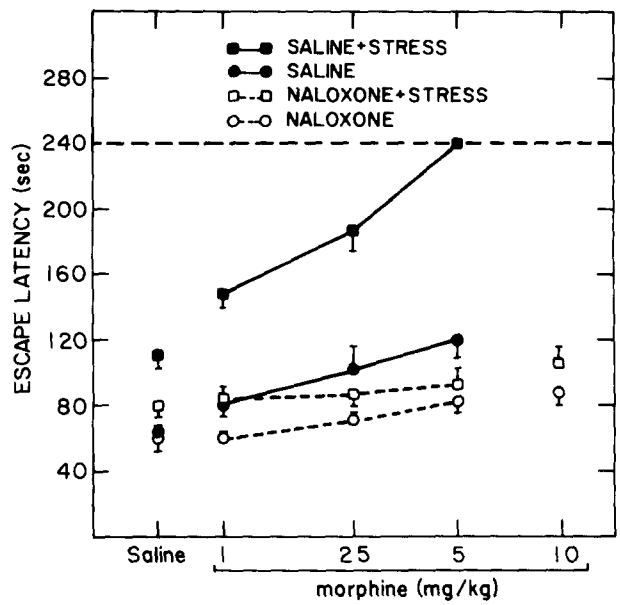

FIG. 3. Influence of restraint on morphine-induced analgesia and its antagonism by naloxone. Animals were injected with morphine (1, 2.5 or 5 mg/kg) or saline and placed into restrainers or returned to a home cage. Fifteen minutes later they were injected with naloxone (0.05 mg/kg) or saline and continued to be treated according to the experimental condition prescribed until the test on a plate. Each point represents mean±SEM for 12 mice.

The order of stress exposure and testing was balanced between different experimental groups. Each animal was used only once and the allocation to different treatments was random.

To evaluate the nociceptive effects of restraint mice were divided into two groups; animals from the stressed group underwent restraint for 30 min and immediately after that were tested on a hot-plate, while the control animals remained in their home cage till the analgesic test. Half of the sample was run during the morning hours(9:00–10:30) and another half during the evening (17:00–18:30). The effect of restraint on water escape was also determined in stressed and unstressed groups.

In the treatment on potentiation of morphine analgesia the procedure was similar, except that the animals were injected with 1, 2.5 or 5 mg/kg morphine or saline before being placed into the restrainers (stressed group) or returned to the home cage (control group). Half of each group, stressed and control, received an injection of naloxone (0.05 mg/kg) 15 min following the first injection, and another half received saline.

For the assessment of naloxone-precipitated jumping in stressed and unstressed animals two experiments were performed. In the first experiment mice received 1, 2.5 or 5 mg/kg morphine or saline and after 30 minutes in a restrainer or in a home cage were injected with 1 mg/kg naloxone and tested for jumping. In the second experiment all mice were injected with only one dose of morphine (5 mg/kg), but the naloxone dose varied (0.1, 1 or 10 mg/kg).

To evaluate the analgesic response to morphine and the intensity of naloxone-induced jumping in DBA/1J and C57Bl/6J mice two morphine doses were used, 2.5 mg/kg and 5 mg/kg, respectively. The jumping was precipitated with 1 mg/kg of naloxone.

Drugs

Morphine HCl and naloxone HCl (ENDO) were dissolved in normal saline and administered SC at the back of the neck on a 0.2 ml volume. All doses are expressed as salt.

Statistical Analysis

Data on analgesic responses were subjected to an analysis of variance; the effect of stress on water escape was evaluated by a t-test. Since the distribution of jumping scores was rather skewed, the nonparametric Wilcoxon rank sum test was used for analysing these data.

RESULTS

Analgesic and Motor Effects of Acute Restraint

The stress of restraint consistently elevated the latencies to the nociceptive responses elicited by thermal stimulation (see Fig. 1). A two-way ANOVA revealed a significant main effect of restraint for all three measures of analgesia: paw lick, $F(1,88)=10.31$, $p<0.01$, jump, $F(1,95)=30.05$, $p<0.001$ and escape, $F(1,95)=27.78$, $p<0.001$. The effect of time of the day (morning versus evening) and interaction were not statistically significant for any analgesic measure. Thus the stress of restraint produced an antinociceptive reaction which did not exhibit circadian changes, at least when measured at two time points. Since latencies to jump and to escape were found to be highly correlated ($r=.94$, $p<0.001$), the jump index was abandoned in the subsequent experiments. In contrast to the effect of restraint on escape from hot-plate, the latency to water escape (Fig. 2) was significantly decreased in the stressed group, $t(18)=2.11$, $p<0.05$.

Stress Potentiation of Morphine Analgesia and its Reversibility by Naloxone

As can be seen in Fig. 3, restraint markedly potentiated the response to morphine in saline-treated group and this

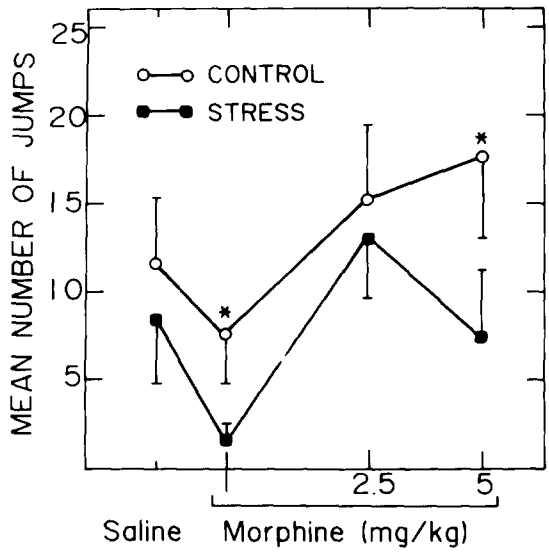

FIG. 4. Effect of restraint on naloxone-precipitated jumping in mice treated with different doses of morphine. The animals were injected with morphine (1, 2.5 or 5 mg/kg) or saline and placed into restrainers or returned to a home cage. Thirty minutes later they were injected with naloxone (1 mg/kg) and tested for a jumping response. Each point represents mean±SEM for 20 mice. Asterisks indicate significant difference from the corresponding unstressed group (Wilcoxon rank sum test, $p<0.05$).

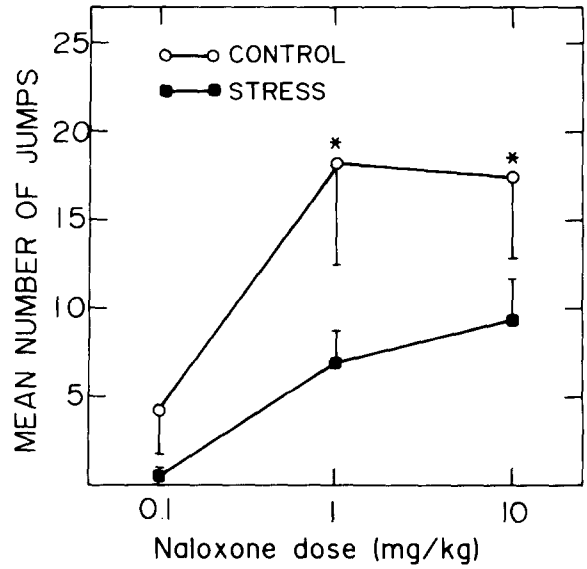

FIG 5. Effect of restraint on naloxone-precipitated jumping in mice treated with different doses of naloxone. The animals were injected with morphine (5 mg/kg) and placed into restrainers or returned to a home cage. Thirty minutes later they were injected with naloxone (0.1, 1 or 10 mg/kg) and tested for a jumping response. Each point represents mean±SEM for 16 mice. Asterisks indicate significant difference from the corresponding unstressed group (Wilcoxon rank sum test, $p<0.05$).

effect was antagonized by naloxone. A three-way ANOVA (stress condition × morphine dose × group) performed on the latencies to escape from a hot-plate revealed that the three main effects and all the interactions were statistically significant ($p<0.05$). The significance of a tertiary interaction implies that stress interacts with morphine dose differently in saline- and in naloxone-treated groups.

To clarify the statistical picture we applied a two-way ANOVA separately to these two groups. The potentiation of morphine analgesia by restraint was evident from an increase in the slope of dose-response curve in stressed as compared to unstressed animals (significant stress × morphine interaction, $F(3,88)=7.43$, $p<0.001$. Naloxone abolished this effect ($F<1$ for stress-morphine interaction). In both groups the main effects of stress and morphine dose were significant [$F(1,88)=198.14$ and $F(3,88)=48.44$, respectively, $p<0.001$ for saline-treated group; $F(3,88)=14.35$ and $F(3,88)=3.01$, $p<0.05$ for naloxone-treated group]. Consequently, while 0.05 mg/kg of naloxone only partially reduced the effects of stress and of morphine, the potentiation effect was cancelled by this dose. To verify the above conclusion we used an additional dose of morphine (10 mg/kg). Although there was still a residual effect of morphine in unstressed animals after naloxone injection [compared to unstressed saline-saline group, t-test, $t(22)=3.65$, $p<0.01$], the exposure to the stress of restraint resulted in just a slight enhancement in an analgesic response, apparently, accounted for by the residual effect of stress (see Fig. 3).

The Effects of Restraint on Naloxone-Precipitated Jumping

The results presented in Fig. 4 demonstrate that the stress of restraint decreased the mean number of jumps precipitated by nalxone. This effect was significant for 1 and 5 mg/kg morphine doses (Wilcoxon rank sum test, $p<0.05$). The control groups, which received morphine and underwent stress, but were injected with saline instead of naloxone, did not exhibit jumping during the test. In view of the fact that naloxone elicited jumping in a control group pretreated with saline and that other unstressed groups did not differ from it significantly, the jumping response could not be considered a manifestation of acute dependence on morphine. The low doses of morphine used in this study just tended to increase the naloxone-induced response. The data from the second experiment, where the dose of naloxone varied, are shown in Fig. 5. As could be seen, the stress of restraint reduced the jumping elicited by two higher doses of naloxone (Wilcoxon rank test, $p<0.05$), while at a dose of 0.1 mg/kg the incidence of jumping was very low in both groups.

Strain Comparison of Morphine Analgesia and Naloxone-Precipitated Jumping

Morphine, 2.5 mg/kg, induced a pronounced analgesic response in both strains [$t(8)=2.82$, $p<0.05$ for DBA/1J and $t(8)=3.18$, $p<0.05$ for C57Bl/6J] relatively to their base line escape latencies (183% and 180%, respectively, as a percent of a base line values). However, the jumping was elicited only in C57Bl mice, and not in the other strain (see Fig. 6). When the dose of morphine was elevated up to 20 mg/kg DBA mice still did not exhibit the jumping response.

DISCUSSION

The stress of restraint increased both paw lick and escape latencies measured in a hot plate test, but decreased the latency to water escape. The latter supports the notion that the changes in response to hot plate are indeed related to the perception of pain and are not due to some artifactual influences of restraint on a motor reaction. Furthermore, in line

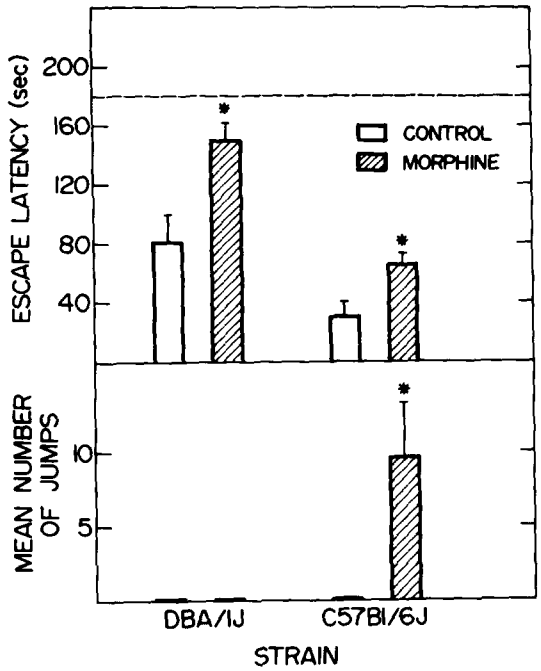

FIG. 6. Analgesic effect of morphine (top panel) and intensity of naloxone-precipitated jumping (bottom panel) in DBA/2J and C57Bl/6J mice. Latencies to escape from a hot plate were measured 30 min after morphine administration. Each value (top panel) represents mean±SEM for 5 mice. Asterisks indicate significant difference from a saline-treated group (t-test, $p<0.05$). Jumping (bottom panel) was precipitated with 1 mg/kg of naloxone 30 min after morphine (5 mg/kg of naloxone 30 min after morphine (5 mg/kg) injection. Each value represents mean±SEM for 10 mice. Asterisk indicates significant difference from a saline-treated group (Wilcoxon rank sum test, $p<0.05$).

with the existent experimental evidence [3–5] restraint markedly potentiated the analgesic response induced by morphine. The potentiation did not result from the mere summation of stress and morphine effects, but was supra-additive, indicating that interaction between the two factors took place.

As opposed to the conclusion in a recent study [4] that the enhancement of morphine analgesia by stress was resistant to naloxone antagonism, we found that it was completely abolished by 0.05 mg/kg of naloxone. The effect of stress alone was only partially reduced by this dose, but it was cancelled by higher doses of the antagonist (results not shown). It should be emphasized that the low dose of naloxone used in the present study did not antagonize completely the response induced by the highest morphine dose (10 mg/kg), the latter being roughly equivalent to the effect of 1 mg/kg of morphine in saline-injected animals. Consequently, under these conditions both residual effects of morphine and of stress were present, yet the enhancement of analgesia was not observed. Thus, the potentiation of morphine analgesia by stress, as revealed by the present experimental paradigm, appears to be dependent on a continuous occupation of opiate receptors by their exogenous and endogenous ligands. However, it is yet to be determined what endogenous ligands and what opiate receptor subtypes are involved in this process.

It has been proposed that the enhancement of morphine analgesia by leucine-enkephalin resulted from the presence of enkephalin at delta binding sites and, as a consequence, from the increase in the efficiency of mu receptor coupling to the effector [28]. Also, the possibility has been raised that the stress of restraint potentiated the antinociceptive effects of morphine in such a manner [3], but later it was discarded, since this effect was shown to be naloxone-insensitive and the affinity of mu receptors in the stressed animals was found to be unchanged [4]. The results of our experiment suggest that the involvement of the opioid peptides in the potentiation of morphine analgesia by stress cannot be excluded and that the use of different analgesimetric procedures might possibly lead to different conclusions.

In agreement with the findings of Wong and Bentley [29], the stress of restraint consistently reduced the naloxone-precipitated jumping in animals injected with low doses of morphine or saline. The latter effect is incompatible with a "simple" model of interaction between morphine and leucine-enkephalin released by stress, since exogenous leucine-enkephalin was shown to potentiate both morphine analgesia and acute dependence [25]. Thus, if such a kind of interaction actually takes place it is probably limited to specific neuronal populations, e.g., endogenous pain inhibitory system. On the other hand, naloxone-induced jumping might be affected via some nonopioid mechanisms activated by stress or, alternatively, the stress of restraint might produce a change in functional receptor reserve in certain brain areas, thus reducing the sensitivity to naloxone.

The comparison of two strains of mice further supports the possibility that the opiate analgesia and naloxone-induced jumping are mediated through independent processes. It is well documented [13, 21, 22] that C57Bl/6 and DBA/2 mice differ in their sensitivity to antinociceptive and motor effects of morphine, C57Bl strain being less responsive in analgesic tests and more responsive in locomotion. With regard to escape latency (in terms of percentage relatively to a baseline) such a difference was not observed, possibly due to the use of another subline of a DBA strain (DBA/1 instead of DBA/2). While both strains exhibited analgesia after morphine administration, the naloxone-precipitated jumping was observed in C57Bl mice only, which indicated that the two responses were genetically uncorrelated.

The effects of stress on a naloxone-induced jumping, which is a widely used measure of physical dependence, are interesting also from another point of view. It has been suggested in a recent review by Collier [10] that dependence on exogenous opiates like morphine was an exaggerated copy of a natural process occurring under specific environmental conditions. Since different types of stress can serve as powerful activators of endogenous opioid system, it could be expected that certain stressful situations could result in a dependence on the opioid peptides released endogenously. In the studies that used a chronic stress paradigm [9,19] some withdrawal signs were indeed observed after administration of naloxone but, to our knowledge, no such data have been reported with regard to acute stress. While naloxone-precipitated jumping can be elicited by a single injection of beta-endorphin [14], the stress of restraint which has been shown to induce endorphin release from the pituitary gland [6, 15, 20] not only had no such effect, but also decreased the jumping response. On the other hand, unstressed animals, which were used in the present study as a control group, exhibited a relatively high level of jumping after naloxone administration (but not after injection of saline). It might be speculated that an exposure to stress initiates multiple regulatory processes which could normally prevent the occurrence of a physiolog-

ical dependence. However, under certain conditions such as intensive chronic stress, some degree of morphine-like dependence can be manifested through naloxone-precipitated withdrawal signs.

REFERENCES

1. Akil, H.; Watson, S. J.; Young, E.; Lewis, M. E.; Khachaturian, H.; Walker, J. M. Endogenous opioids: Biology and function. Annu. Rev. Neurosci. 7:223–255; 1984.
2. Amir, S.; Amit, Z. Endogenous opioid ligands may mediate stress-induced changes in the affective properties of pain related behavior in rats. Life Sci. 23:1143–1152; 1978.
3. Appelbaum, B. D.; Holtzman, S. G. Characterization of stress-induced protentiation of opioid effects in the rat. J. Pharmacol. Exp. Ther. 231:555–565; 1984.
4. Appelbaum, B. D.; Holtzman, S. G. Restraint stress enhances morphine-induced analgesia in the rat without changing apparent affinity of receptor. Life Sci. 36:1069–1074; 1985.
5. Appelbaum, B. D.; Holtzman, S. G. Stress-induced changes in the analgesic and thermic effects of morphine administered centrally. Brain Res. 358:303–308; 1985.
6. Baizman, E. R.; Cox, B. M.; Osman, O. H.; Goldstein, A. Experimental alterations of endorphin levels in rat pituitary. Neuroendocrinology 28:402–424; 1979.
7. Bhattacharya, S. K.; Keshary, P. R.; Sanyal, A. K. Immobilisation stress-induced antinociception in rats: possible role of serotonin and prostaglandins. Eur. J. Pharmacol. 50:83–85; 1978.
8. Chang, K. J.; Cooper, B. R.; Hazum, E.; Cuatrecasas, P. Multiple opiate receptors: different regional distribution in the brain and differential binding of opiates and opioid peptides. Mol. Pharmacol. 16:91–104; 1979.
9. Christie, M. J.; Chesher, G. B. Physical dependence on physiologically released endogenous opiates. Life Sci. 30:1173–1177; 1982.
10. Collier, H. O. J. Physiological basis of opiate dependence. Drug Alcohol Depend. 11:15–21; 1983.
11. Constentin, J.; Vlaiculescu, A.; Chaillet, P.; Ben Natan, L.; Aveaux, D.; Schwartz, J. C. Dissociated effects of inhibitors of enkephalin-metabolising peptides or naloxone on various nociceptive responses. Eur. J. Pharmacol. 123:37–44; 1986.
12. Franklin, K. B. J.; Kelly, S. J. Sympathetic control of tryptophan uptake and morphine analgesia in stressed rats. Eur. J. Pharmacol. 126:145–150; 1986.
13. Frigeni, V.; Bruno, F.; Carenzi, A.; Racagni, G.; Santini, V. Analgesia and motor activity elicited by morphine and enkephalins in two inbred strains of mice. J. Pharm. Pharmacol. 30:310–311; 1978.
14. Huidobro-Toro, J. P.; Way, E. L. Single-dose tolerance to antinociception, and physical dependence on beta-endorphin in mice. Eur. J. Pharmacol. 52:179–189; 1978.
15. Kant, G. J.; Mougey, E. H.; Meyerhoff, J. L. Diurnal variation in neuroendocrine response to stress in rats: plasma ACTH, beta-endorphin, beta-LPH, corticosterone, prolactin and pituitary cyclic AMP responses. Neuroendocrinology 43:383–390; 1986.
16. Kelly, S. J.; Franklin, K. B. J. Evidence that stress augments morphine analgesia by increasing brain tryptophan. Neurosci. Lett. 44:305–310; 1984.
17. Lee, N. M.; Leybin, L.; Chang, J. K.; Loh, H. H. Opiate and peptide interaction: effect of enkephalins on morphine analgesia. Eur. J. Pharmacol. 68:181–185; 1980.
18. Mayer, D. J.; Watkins, L. R. Multiple endogenous opiate and nonopiate analgesia systems. In: Kruger, L.; Liebeskind, J. C., eds. Advances in pain research and therapy. vol. 6. New York: Raven Press, 1984, pp. 253–275.
19. Morley, J. E.; Levine, A. S. Stress-induced eating is mediated through endogenous opiates. Science 209:1259–1261; 1980.
20. Mueller, G. P. Beta-endorphin immunoreactivity in rat plasma: variations in response to different physical stimuli. Life Sci. 29:1669–1674; 1981.
21. Oliverio, A.; Castellano, C. Genotype-dependent sensitivity and tolerance to morphine and heroin: dissociation between opiate-induced running and analgesia in the mouse. Psychopharmacologia 39:13–22; 1974.
22. Racagni, G.; Bruno, F.; Iuliana, E.; Paoletti, R. Differential sensitivity to morphine-induced analgesia and motor activity in two inbred strains of mice: behavioral and biochemical correlations. J. Pharmacol. Exp. Ther. 209:111–116; 1979.
23. Sherman, J. E.; Proctor, C.; Strub, H. Prior hot plate exposure enhances morphine analgesia in tolerant and drug-naive rats. Pharmacol. Biochem. Behav. 17:229–232; 1982.
24. Sherman, J. E.; Strub, H.; Lewis, J. W. Morphine analgesia: enhancement by shock-associated cues. Behav. Neurosci. 98: 292–309; 1984.
25. Vaught, J. L.; Takemori, A. E. Differential effects of leucine and methionine and dependence. J. Pharmacol. Exp. Ther. 208:86–90; 1979.
26. Vaught, J. L.; Takemori, A. E. A further characterization of the differential effects of leucine enkephalin, methionine enkephalin and their analogs on morphine-induced analgesia. J. Pharmacol. Exp. Ther. 211: 280–283; 1979.
27. Vaught, J. L.; Kitano, T.; Takemori, A. E. Interaction of leucine enkephalin and narcotics with opioid receptors. Mol. Pharmacol. 19: 236–241; 1980.
28. Vaught, J. L.; Rothman, R. B.; Westfall, T. C. Mu and delta receptors: their role in analgesia and in the differential effects of opioid peptides on analgesia. Life Sci. 30:1443–1455; 1982.
29. Wong, C. L.; Bentley, G. A. The effect of stress and adrenalectomy on morphine analgesia and naloxone potency in mice. Eur. J. Pharmacol. 56:197–205; 1979.
30. Zukin, R. S.; Zukin, S. R. Multiple opiate receptors: emerging concepts. Life Sci. 29: 2681–2690; 1981.