EFFECT OF ISOLATION ON PAIN THRESHOLD AND ON DIFFERENT EFFECTS OF MORPHINE

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Abstract


1. The effect of three periods of isolation (8, 15 and 30 days) were studied in mice on the pain threshold and the sensitivity to morphine.

2. The pain threshold was unchanged after 8 and 15 days of isolation but increased after 30 days of isolation.

3. The analgesic effect of morphine was unchanged after 8 and 15 days of isolation but increased after 30 days of isolation.

4. The tolerance to morphine analgesia was unchanged after 8 and 15 days of isolation but increased after 30 days of isolation (morphine-induced analgesia was reduced).
5. The physical dependence on morphine induced by precipitated withdrawal was unchanged after 8 and 15 days of isolation but decreased after 30 days of isolation.

6. It is suggested that isolation may modify the metabolism the metabolism/absorption of morphine in a different way according as the treatment is unique or chronic.

Keywords: analgesia, dependence, mice, morphine, nociception, social isolation, tolerance.

Introduction

Isolation in rodents can induce a great number of behavioural modifications, such as increased aggression, decreased learning, decreased conditioning, enhancement of the locomotor activity, alteration of the reactivity to external stimuli (Valzelli, 1974, Morgan et al., 1975; Einon et al., 1978; Schenk et al., 1983, 1985; Coudereau et al., 1996).

A number of experiments were focused on the neurophysiological and neurochemical mechanisms underlying isolation; in particular, the role of catecholaminergic (Valzelli, 1978; Francès et al., 1980; Oehler et al., 1985, 1987), serotonergic (Valzelli and Bernasconi, 1979, Yanai and Sze, 1983; Coudereau et al., 1995) and GABAergic systems (Simler et al., 1982; Oehler et al., 1985).

Modifications of the opioid system are also induced by isolation. It was observed in mice (Bonnet et al., 1976) and in rats (Schenk et al., 1982) a significant reduction of opioid receptors in the brain as a result of isolation. The hot-plate latencies were increased in mice following 48 or 72 hours of isolation (Konecka and Sroezynska, 1990). A single day of isolation increased tail-shock induced vocalizations in young rats (Panksepp, 1980) and a short-term (5 min) isolation from mother, siblings and nest caused a significant analgesic response to heat (48°C) relative to non isolated siblings (Kehoe and Blass, 1986a).

The pharmacological effect of drugs, particularly that of opiates, may also be altered by isolation which is coherent with an alteration of the opioid system. Indeed, Katz and Steinberg (1972), De Feudis et al. (1976), Kostowski et al. (1977), Kehoe and Blass (1986b) showed that, in isolated rats, the analgesic effect of morphine was greater than in non isolated rats, whereas Adler et al. (1975a), Puglisi-Allegra
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and Olivierio (1983) showed that no difference occurs between isolated and non-isolated rats regarding the analgesic effect of morphine. In opposition to these results, Pankseep (1980) observed that, in young rats isolated 3-4 days, morphine was less effective in reducing tail-shock induced vocalizations than in socially housed animals. These results led to different conclusions perhaps due to the different methods used to assess morphine analgesia.

Another point seems important relative to opiates: the effect of chronic treatments. The tolerance to morphine analgesia and the withdrawal symptoms induced by naloxone may be altered following isolation since Adler et al. (1975b) and Marks-Kaufman and Lewis (1984) reported that isolated rats show less severe withdrawal symptoms following naloxone administration than grouped rats.

The aim of this work was to study, in mice, the effect of isolation on the pain threshold and on different effects of morphine. The duration of isolation was a main factor: 8, 15 and 30 days of isolation were compared. In the same study, the authors compared the effect of every duration of isolation on an effect observed after an acute administration: (1) analgesia and on effects observed after chronic treatment: (2) tolerance to morphine analgesia and dependence to morphine.

Methods

Animals

Male Swiss NMRI mice (20-24 g at the beginning of the experiment) were obtained from CERJ, Genest St Isle 53940 (France). The maintenance and experimental rooms were thermostatically maintained at 21 ± 1°C with 12 hours light/dark schedule (light on from 8 a.m. to 8 p.m.). Food and water were freely available.
Morphine sulfate (Francopia, France) and naloxone hydrochloride (5 mg/kg) (Endo Laboratories, New-York, USA) were dissolved in demineralized water. The volume of injection was 0.2 ml/20g body weight. Administration were performed by the intraperitoneal route (i.p.). The doses of morphine were expressed in base. The different doses used are reported in the experimental procedure for each test.

**Experimental Procedure**

**Housing Conditions**: Mice were housed in groups of 6 in home cages of 30x20x10 cm or isolated in home cages of 24x10x8 cm. The mice were 4-5 weeks old at the beginning of the isolation. There were three periods of social isolation: 8, 15 and 30 days. The animals were isolated from others with respect only to tactile stimuli.

Different groups of mice were used for every duration of isolation and, also, for every social group. As an example, the mice used for 30 days isolation and their grouped controls were introduced in the animal house at the same date; then, half of the mice were isolated and the other half was maintained in collective cages in the same animal house, for the 30 days before the performance of the test. The experiments relative to one test and one duration of isolation were performed during the same days for the isolated mice and also for the corresponding non isolated mice.

**Assessment of Pain Threshold and Analgesic Effect of Morphine**: Pain threshold and painful responses were determined using a 55 ± 1°C hot-plate as the nociceptive stimulus and the latency to paw-lick or an attempt to escape by jumping as the painful response. Pain threshold was tested just before the injection of morphine (control latency). Analgesia was tested 20, 40, 60, 100, 150, 200 and 250 minutes after the injection of morphine (8mg/kg) (test latency), between 10.00 a.m. and 4.00 p.m. The percentage of analgesic effect at each time was calculated according to the following formula: % analgesic effect = 100 x (test latency - control latency) / (cut-off time - control latency). To prevent tissue damage, the mice that showed no response within 60 seconds (cut-off time) were removed from the hot-plate.
Assessment of the Tolerance to Morphine Analgesia. The analgesic effect of morphine was assessed using the same procedure described previously. The assessment of the analgesic effect of morphine was studied by the same procedure than previously. Mice were treated with morphine (10 mg/kg) daily for 5 days. For isolated-mice and their corresponding grouped mice, morphine was administered during the latter part of isolation.

The analgesic effect was measured 30 minutes after the injection of morphine. The test trials were performed between 10.00 a.m and 1.00 pm. The first day, the pain threshold was measured before morphine injection (t₀ day 1).

The analgesic effect obtained every day was compared to the analgesic effect of the first day. The percentage of analgesic effect was calculated according to the following formula: % analgesic effect = 100 x (latency at day d - latency at t₀ day 1 / (cut off time - latency at t₀ (day 1).

A diminution of the percentage of analgesic effect indicated a development of tolerance to morphine analgesia.

Assessment of the Physical Dependence to Morphine. Mice received 50 mg/kg at 10 a.m. and 4 p.m. on day 1, 50 mg/kg at 10 a.m. and 2 p.m. and 100 mg/kg at 6 p.m. on day 2, 50 mg/kg at 10 a.m. on day 3. The withdrawal syndrome was precipitated at 4 p.m. on day 3 with an injection of naloxone (5 mg/kg). For isolated-mice and their corresponding controls morphine was administered during the latter part of isolation.

Each mouse was placed in a glass cylinder (30 cm high, 15 cm diameter) and observed during 15 minutes after naloxone injection.

The severity of the withdrawal syndrome was quantified by the number of repetitive vertical jumps.

All mice were used only once. Mice were killed using CO₂ in a special container.

Data Analysis

Results were expressed as mean ± standard error to the mean (S.E.M.). The pain threshold, the dependence and the area under the curve were analyzed using the Student's t-test. For the morphine analgesia and the tolerance to morphine
analgesia, the results were analyzed using a one-way analysis of variance for repeated measures followed by the Bonferroni’s tests for each duration of isolation. There were 10-15 animals in each group.

**Results**

**Threshold of Nociception**

Figure 1 indicates that isolated mice (8 days (t=0.28) or 15 days (t=1.41) of isolation) did not present a pain threshold different from that of non isolated mice. On the other hand, after 30 days of isolation, the pain threshold was higher than in grouped animals (t=4.20).

**Morphine Induced Analgesia**

Morphine induced an analgesia in isolated and in non isolated mice. There was no significant difference with 8 and 15 days of isolation (Fig 2A and 2B). In animals isolated during 30 days, the analgesic effect of morphine was significantly higher (Bonferronni test) at 20 and 40 minutes (Fig 2C). The analgesic effect of morphine increased considerably for 30 days isolated animals at 20, 40 and 60 minutes (increase from 10 to 30 % of analgesic effect).

Analgesia was still present in isolated and non isolated animals at 250 minutes (5 to 12 % of analgesic effect).

**Development of the Tolerance to Morphine Analgesia**

Whatever the conditions of rearing (isolation or non isolation), there was a tolerance to morphine analgesia developed over the 5 days, as reflected by the progressive decrease in percentage of analgesia following repeated administration of the same dose of morphine (Fig 3). An isolation of 8 or 15 days didn’t modify the analgesic tolerance to morphine (Fig 4A and 4B). However, following 30 days of
isolation, the tolerance to morphine analgesia was extremely increased. Indeed, in isolated mice, the area under the curve was half that observed in non isolated mice (Fig 4C).

Development of the Physical Dependence to Morphine

In mice chronically receiving water, the administration of naloxone, 5 mg/kg, induced jumping neither in mice isolated 8 days (n=5), 15 days (n=5) or 30 days (n=5) nor in their corresponding non isolated controls of 8 days (n=5), 15 days (n=5) or 30 days (n=5).

Naloxone (5 mg/kg) precipitated a withdrawal syndrome in mice chronically receiving morphine. The withdrawal syndrome precipitated by naloxone was observed in isolated and non isolated animals. A period of isolation of 8 or 15 days showed a tendency to decrease the withdrawal syndrome (Fig 5A and 5B). After 30 days of isolation, the withdrawal syndrome was significantly less important (Fig 5C).

Discussion

Threshold of Nociception

The threshold of nociception was higher in 30 days isolated mice (+62%) than in non isolated mice. Different types of acute stressors (like an immobilization) produce an increase of threshold of nociception resulting from an increased release of opioids (Amir et al, 1980; Bodnar et al, 1980). Unlike acute stress, which reduces pain responsiveness, prolonged exposure to stress leads to enhanced reactivity to pain (Amir et al, 1980; Bodnar et al, 1980). The role of social isolation in terms of prolonged stress has been debated (Anisman, 1978).

The present findings are in accordance with those of Puglisi-Allegra and Oliverio (1983) who reported that isolated mice showed higher pain thresholds than non isolated mice.
Fig 1: Effect of isolation on pain threshold measured on the hot-plate test. The durations of isolation were: A: 8 days (ns), B: 15 days (ns), C: 30 days. *** p<0.001 (Student's t test)
Fig 2: Effect of isolation on analgesia induced by morphine (8 mg/kg)
The durations of isolation were: A: 8 days; ANOVA: F(13-182) = 2.015 p<0.05, B: 15 days; ANOVA: F(13-182) = 3.03 p<0.001, C: 30 days; ANOVA: F(13-196) = 4.109 p<0.001. *p<0.05 **p<0.01 (Bonferroni test)
Fig 3: Effect of isolation on the tolerance to morphine analgesia: temporal development. The durations of isolation were: A: 8 days; ANOVA: F(9-140) = 9.10 p<0.001, B: 15 days; ANOVA: F(9-135) = 6.86 p<0.001, C: 30 days; ANOVA: F(9-130) = 8.6 p<0.001. *p<0.05 (Bonferroni test)
Fig 4: Effect of isolation on tolerance to morphine analgesia: global estimation (area under the curve of the Fig 3). The durations of isolation were: A: 8 days, B: 15 days, C: 30 days. ***p<0.001 (Student's t test)
Fig 5: Effect of isolation on naloxone-precipitated withdrawal in morphine-dependent mice. *p<0.05 (Student's t test). The durations of isolations were: A: 8 days, B: 15 days, C: 30 days.
The increase of nociception threshold in 30 days isolated mice can be explained by an increased release of endogenous opioids or by an increase of sensitivity to endogenous opioids.

**Analgesia Induced by Morphine**

Morphine induced an analgesia in both isolated and non isolated mice. Action mechanisms of the morphine's analgesic effect involved a complex interaction of peripheral and central nervous systems. Spatial transections markedly reduce the antinociceptive activity of morphine (Ling and Pasternak, 1983). Anatomical and physiological studies have confirmed the presence of descending pathways from the brainstem to the dorsal horn of the spinal cord that are strongly influenced by morphine (Besson and Chaouch, 1987). Within the brainstem, detailed studies established the importance of a number of specific regions, including the periaqueductal gray, nucleus raphe magnus and locus ceruleus (Jacquet and Lajtha, 1973; Bodnar et al, 1988).

The analgesic effect of morphine in 30 days isolated animals was greater than in non isolated animals, at 20 and 40 minutes after injection of morphine (passage from 10 to 30% of the analgesic effect). These differences in results were observed only with a period of isolation of 30 days (and not with 8 and 15 days of isolation).

The serotonin seems to be involved in the analgesic effect of morphine. Samanin et al (1970) have observed a diminution of 5-hydroxytryptamine in midbrain raphe lesioned rats. This decrease in the level of serotonin was associated with reduced analgesic effect of morphine. Garattini et al (1967) have shown a decrease of serotonin's turn-over in isolated mice. Our findings cannot explain these results since the analgesic effect is greater in our isolated animals.

The present results agree with those of Katz and Steinberg (1972), Defeudis et al (1976), Kostowski et al (1977). These authors showed that in isolated animals (rats or mice) the analgesic effect of morphine was greater than in non isolated animals.

Our results do not agree with those of Adler et al (1975a), Puglisi-Allegra and Olivero (1983) who showed that no difference exists between isolated and non isolated rats regarding the analgesic response to morphine.
Development of the Tolerance to Morphine Analgesia

Chronic administrations of morphine lead to a decreased response to the analgesic effect of morphine. The tolerance may be due to changes in receptors number and alterations in second messenger (Blanchard and Chang, 1988). Some evidence suggests a role of antagonistic systems (and/or down-regulation). For example, cholecystokinin (CKK) antagonists, such as proglumide (Dourish et al, 1988; Watkins et al, 1984) and antidepressants such as nefazodone and amitriptyline (Pick et al, 1992; Botney and Fields, 1983) restore morphine's analgesic potency in tolerant animals. However, these facilitatory actions are also seen in naive animals, implying that they are not directed specifically against the mechanisms of tolerance.

Thirty days of isolation increased the tolerance to morphine analgesia (+200%). These increases in the tolerance to morphine analgesia may be explained by a different metabolism (or elimination) of morphine between isolated and non-isolated animals.

Morphine is well-known as the prototype of μ agonist. However, morphine has an affinity not only for μ but also for δ and κ receptors (Wood et al, 1981). Suzuki et al (1992) have shown that inactivation of the κ opioid system by a highly selective κ antagonist, nor-binaltor-phinine (nor-BNI) increased the development of tolerance to morphine analgesia in mice.

Considering these results, it may be suggested that social isolation can induce a decrease in the sensitivity of kappa opioid system which would result in an increase in the tolerance to morphine analgesia.

Dependence

The degree of physical dependence can be assessed by the quantification of the withdrawal syndrome (Way et al, 1969). The quantification of the withdrawal syndrome can be estimated by a multitude of signs and symptoms. There are a great number of methods to estimate this syndrome (Way et al, 1968; Marshall and Weinstock, 1971; Adler et al, 1975b). The most pertinent sign of the withdrawal syndrome precipitated by a receptor opioid antagonist was repetitive jumping (Frances et al, 1992; Ohta and Kaneto, 1992).

The naloxone precipitated withdrawal syndrome was observed in both isolated and non-isolated animals. An isolation of 8 and 15 days is not sufficient to induce a
significant difference in the severity of the withdrawal syndrome between isolated and non isolated animals. After 30 days of isolation the severity of the withdrawal syndrome was decreased compared to non isolated animals. These results showed that a long period of isolation (30 days) can alter the withdrawal syndrome and probably induce some changes in the mechanisms implicated in the dependence and/or abstinence.

Our results agree with those of Adler et al (1975b), Marks-Kaufman and Lewis (1984) who showed that in rats isolated during a long period (12 weeks) the naloxone precipitated withdrawal syndrome showed a smaller number of jumps. Katz and Steinberg (1972) reported that the long term isolation of immature rats did not result in any greater weight loss than in grouped rats following abrupt withdrawal of morphine after a production dependence. However, in Katz and Steinberg's (1972) study, the other signs of the withdrawal syndrome have not been noted.

It has been reported that drugs that interfere with biogenic amines in the brain can affect some signs of withdrawal, particularly jumping in both mice and rats (Way et al, 1968; Collier et al, 1972). It has also been reported that in these two species, prolonged isolation can induce some biochemical changes, particularly in the turnover of brain amines (Valzelli and Garattini, 1972; Modigh, 1973; Weinstock et al, 1978; Anisman and Sklar, 1981; Lasley and Thurmond, 1985). A long period of isolation (30 days) can induce in animals some metabolic changes (synthesis, release, turnover) of the biogenic amines and these modifications can produce a decrease in the severity of the withdrawal syndrome in isolated animals.

Regarding tolerance and dependence, all things look as if mice isolated 30 days had received less morphine than social mice: their analgesic response was smaller (the tolerance is greater); their withdrawal syndrome was smaller. Although the development of physical dependence on and tolerance to morphine did not depend on the same mechanisms (Johnson and Duggan, 1984; Kaneto et al., 1985; Ohta and Kaneto, 1992), it may be suggested that the modifications in the same way of the tolerance to and the dependence on morphine following 30 days of isolation indicates a common mechanism for isolation; it is suggested that isolation may accelerate the catabolism of chronic morphine (or reduce its absorption). This likely explanation is only speculative but deserves to be verified.
Conclusion

The present results show that a long period of isolation (30 days) is necessary to observe modifications of the pain threshold and of the sensitivity to morphine. After an acute treatment an effect of morphine (analgesia) was increased. On the contrary, after chronic treatments things appear as if the dose of morphine was lessened: the tolerance is increased and the dependence is decreased. These results suggest that isolation may modify the metabolism/absorption of morphine in a different way according as the treatment is unique or chronic.

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behavioural models involving serotonergic 5-HT2 and 5-HT1a receptors.


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