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Time-Dependent Inhibition by Naloxone, an Opiate Receptor Antagonist, of the Sensitization to Morphine-Induced Ambulatory Stimulation

Hisashi KURIBARA

Junior College, Tokyo University of Social Welfare (Isesaki Campus), 2020-1 San'o-cho, Isesaki-city, Gunma 372-0831, Japan

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Abstract:A significant sensitization to the ambulation-stimulating effect of morphine (10 mg/kg s.c.), a narcotic analgesic drug with agonistic action on opiate receptors, was produced when mice were repeatedly administered morphine at intervals of 3 days, and their ambulation was measured for 3 hr in an activity cage of 20 cm in diameter after each administration. Naloxone (0.01-1 mg/kg s.c.), an opiate receptor antagonist, blocked the morphine-induced ambulation in a dose-dependent manner. The mice treated with naloxone at post-morphine period of 3/4 hr and later showed a strong ambulatory sensitization as high as that in the mice repeatedly given morphine alone. The treatment with naloxone at post-morphine period of 1/2 hr caused a partial sensitization, and at 0-1/3 hr no significant sensitization. The repeated administrations of naloxone alone or saline caused no significant change in the sensitivity to morphine. These results suggest that experience of morphine effect and the resultant ambulation for 1/2 hr prior to the peak effect is important for induction of the sensitization to the ambulation-increasing effect of morphine in mice. This finding also indicates that the reward effect of morphine appears in the early post-morphine period prior to the peak effect, which is the essential factor for induction and maintenance of the abuse and dependence of narcotic analgesic drugs such as morphine and heroin.
(Reprint request should be sent to Hisashi Kuribara)

Key words: Morphine, Naloxone, Opiate receptors, Ambulatory activity, Sensitization, Mice.

Introduction

Morphine, a prototype of narcotic analgesic drug, facilitates the dopamine release through an agonistic action on opiate receptors in the brain (Buxbaum, 1973; Joyce and Iversen, 1979; Teitelbaum et al., 1979). Mesolimbic dopaminergic systems (Van der Heuval and Pasterkamp, 2008) play significant roles in the behavioral and psychological activities, particularly motivation (Matsumoto and Hikosaka, 2009), learning and memory (Arias-Carrion and Poppel, 2007; Ikemoto, 2007), and substance abuse (Piercem and Kumaresan 2006; Berridge, 2007; Ikemoto, 2007).

In the drug abuse and dependence, which are intimately related to the stimulant effect on the mesolimbic dopaminergic systems in the brain (Valdman, 1986), the drugs having dependence liability are repeatedly administered, indicating the importance to study the change in the drug effects following repeated administrations.

The behavioral study in terms of ambulatory activity in mice demonstrated that the repeated administrations of morphine at intervals of shorter than 12 hr resulted in tolerance (Kuribara, 1996c), whereas significant sensitization at intervals of 12 hr and longer (Kuribara and Tadokoro, 1989; Kuribara, 1996c). It has been considered that changes in the opiate and/or dopaminergic neurotransmission are involved in the induction of behavioral sensitization to morphine because of the inhibitory action of naloxone, an opiate receptor antagonist, or the dopamine D-2 receptor antagonist, nemonapride (Kuribara, 1995a).

Furthermore, it has been demonstrated that, to induce the sensitization to the ambulatory stimulant effect of methamphetamine (Kuribara, 1995b, 1996a,b) and cocaine (Kuribara, 1996a,2009), a free ambulation for 1/2 hr and 1/4 hr, respectively, during the post-drug period of 0-1 hr is important.

The aim of this study was to assess the time-dependent modification by naloxone of sensitization to the ambulatory stimulant effect of morphine in mice.

Materials and Methods

Animals

Male mice of ddY strain (SLC Japan, Hamamatsu) were used at 6 weeks of age and a weight of 25-28 g. Groups of 10 mice each had been housed in Polycarbonate cages (20W × 25L × 15H cm), and they were given a solid diet (MF: Oriental Yeast, Tokyo) and tap water except during the behavioral tests. The conditions of the breeding room were controlled (temperature; 23 ± 1℃, relative humidity; 55 ± 3%, and a 14:10-hr light-dark cycle; lights on between 05:00-19:00 hr).

Apparatus

The ambulatory activities of 10 mice were individually and simultaneously measured with a tilting-type ambulometer which had 10 bucket-like Plexiglas activity cages of 20 cm in diameter and 15 cm in height (SMA-10: O' hara & Co., Tokyo). The apparatus selectively detected horizontal, but not vertical, movements (ambulation) of the mouse.

Drugs

The drugs used were morphine HCl (Takeda Chemicals, Osaka) and naloxone (Sigma, Chicago, IL). Morphine and naloxone were dissolved in physiological saline, and subcutaneously (s.c.) administered at a constant volume of 0.1 ml/10 g body weight of the mouse.

Experimental schedules

All the experimental treatments, i.e., the administration of morphine and naloxone, and measurement of ambulation of the mouse, were carried out between 09:00-16:00 hr.

All drug administrations to the mice were conducted after a habituation period for 10 min to the activity cage.

Experiment 1. Single administration of morphine, and co-administration of morphine with naloxone

Four groups of 10 mice each were administered saline, or morphine (2.5, 5, 10 or 20 mg/kg), and the ambulatory activity of each mouse was measured for 3 hr. Six groups of 10 mice each were s.c. co-administered morphine (10 mg/kg) with naloxone (0: morphine alone, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg), and the activity of each mouse was measured for 3 hr. Other 6 groups of 10 mice each were s.c. administered naloxone (0: saline, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg) alone.

Experiment 2. Evaluation of time course of changes in morphine-induced ambulatory stimulation after the repeated administrations

Following the results of the previous studies (Iizuka and Hirabayashi, 1983; Kuribara and Tadokoro, 1989) and Experiment 1, the dose of morphine (10 mg/kg) was considered to be optimum for induction of sensitization to the ambulatory stimulant effect in mice. Ten mice were repeatedly given morphine 6 times at 3-day intervals, and they were allowed to freely move in the activity cage for 3 hr after each administration. The activity counts were recorded every 30 min epoch.

Experiment 3. Repeated administrations of morphine followed by the treatment with naloxone

Following the results of the previous study (Kuribara, 1995a) and Experiment 1, the dose of naloxone (1 mg/kg) was selected to be optimum for complete blockade of morphine-induced ambulatory activity.

Nine groups of mice (10 mice each) were given morphine (10 mg/kg) 3 times at intervals of 3 days, and their ambulatory activities were individually measured for 3 hr. Furthermore, naloxone (1 mg/kg) was administered at the post-morphine period of either 0 (simultaneous administration), 1/6, 1/3, 3/4, 1/2, 1, 3/2, 2 or 3 hr. As the control administration for morphine, other 9 groups of mice were given saline, and followed by administration of naloxone.

Three days after the 3rd treatment, all mice were challenge-administered morphine (10 mg/kg s.c.) alone.

Ethical condition for experimental animals

All the experimental procedures mentioned above were carried out according to the" *Guiding Principles for the Care and Use of Laboratory Animals"* approved by The Japanese Pharmacological Society.

Statistical analysis

The mean activity counts for 3 hr in each group were analyzed by analysis of variance (ANOVA). Post-hoc analyses were carried out by Bonferroni test. Values of p less than 0.05 were considered significant.

Results

1. Single administration of morphine, and co-administration of morphine with naloxone (Experiment 1)

As shown in Table 1, morphine dose-dependently accelerated the ambulatory activity of mice, and the activity counts following 5, 10 and 20 mg/kg were significantly higher than the saline control.

The increased morphine-induced ambulatory activity was observed for about 3/2 hr after 5 mg/kg, 3 hr after 10 mg/kg, and longer than 4 hr after 20 mg/kg.

As shown in Table 2, naloxone, at 0.1 mg/kg and higher doses, significantly inhibited the morphine (10 mg/kg)-induced ambulatory stimulation, though the administration of naloxone alone did not change the activity of mice at any doses.

2. Morphine-induced ambulatory stimulation after the repeated administrations (Experiment 2)

Fig. 1 shows the time course of changes in morphine-induced ambulatory stimulation following 6 repeated administrations at 3-day intervals. Morphine-induced ambulatory

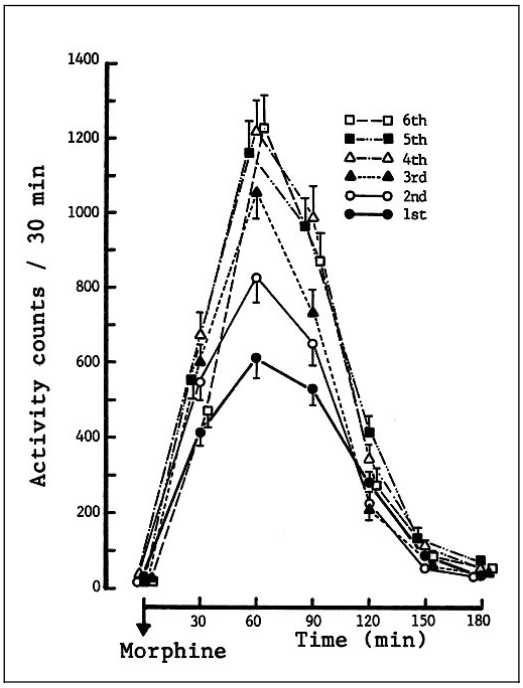

Fig. 1. Time course of changes in the ambulatory stimulant effect of morphine (10 mg/kg s.c.) after 6 repeated administrations at 3-day intervals. Each point represents the mean ambulatory activity count in every 30 min epoch. N=10.

Table 1. Mean activity counts after the s.c. administration of morphine (0: saline, 2.5, 5, 10 and 20 mg/kg).

Doses of morphine	Activity counts	Peak effect	Duration of activity
0 (saline)	38 ± 9	ND	ND
2.5 mg/kg	21 ± 4	ND	ND
5	267 ± 23*	1/2-1 hr	2 hr
10	1395 ± 201*	1/2-1 hr	3 hr
20	3738 ± 550*	1-3/2 hr	<4 hr

Each activity count is mean ± SEM of 10 mice for 3 hr. ND: not determined.
*: significantly different (p<0.05) vs. the activity count after saline administration.

Table 2. Mean activity counts after the combined s.c. administration of morphine (10 mg/kg) and naloxone (0.01-1 mg/kg).

Doses of naloxone	Morphine + Naloxone	Naloxone alone
0 (saline)	1452 ± 283	32 ± 9
0.01 mg/kg	1194 ± 208	39 ± 10
0.03	706 ± 113*	40 ± 12
0.1	175 ± 41*	36 ± 8
0.3	73 ± 33*	51 ± 6
1.0	47 ± 19*	44 ± 3

Each value is mean activity count ± SEM of 10 mice for 3 hr.
*: significantly different (p<0.05) vs. the activity count after morphine alone (naloxone dose=0).

stimulation attained to the peak during the post-morphine period of 1/2-1 hr, and almost disappeared by 3 hr after the administration. The sensitization to the ambulatory stimulant effect was much marked during the post-morphine period of 1/2-1 hr, and the sensitization attained plateau state by the 4th administration. However, no prolongation of the effect was demonstrated even after induction of the sensitization.

3. Repeated administrations of morphine followed by the treatment with naloxone (Experiment 3)

Table 3 shows the activity counts after 3 repeated administrations of morphine followed by naloxone at various post-morphine periods. The repeated administration of morphine followed by naloxone at 3/4, 1, 3/2, 2 and 3 hr resulted in a significant enhancement of the ambulatory stimulation. However, the treatment with naloxone at post-morphine period of 1/2 hr caused a partial sensitization, and at 0, 1/6 or 1/3 hr no sensitization.

The treatment with saline followed by naloxone neither stimulated the ambulatory activity (counts being 43-79), nor changed the sensitivity to morphine.

Table 4 shows mean 3-hr activity counts following the challenge administration of morphine to the groups of mice that had been pretreated with morphine or saline followed by naloxone. The groups of mice pretreated with 3 repeated administrations of saline followed by naloxone did not show significant change in the sensitivity to morphine.

After the challenge administration of morphine, the activity counts in the groups of mice pretreated with morphine and naloxone at post-morphine period of 3/4 hr and later were as high as that in the group of mice given morphine alone. Whereas, following the challenge administration of morphine, the groups of mice pretreated with morphine and naloxone at post-morphine period of 1/2 hr showed a partial sensitization, and at 1/3 hr and earlier no sensitization.

Discussion

Morphine, at 5mg/kg and higher doses, showed a significant ambulatory stimulant effect in mice. The onset, attainment to the peak, and the duration of the increased ambulatory activity were optimum when 10mg/kg of morphine was administered. In addition, naloxone inhibited the morphine (10mg/kg)-induced ambulatory stimulation in a dose-dependent manner, and 1 mg/kg of naloxone was effective for complete inhibition. According to these basic results, 10 mg/kg of morphine and 1 mg/kg of naloxone were selected in Experiments 2 and 3.

As demonstrated in Experiment 2, the repeated administrations of morphine, 6 times at 3-day intervals, induced a significant sensitization to the ambulatory stimulant effect,

Table 3. Mean activity counts following 3 repeated administrations of morphine (10 mg/kg s.c.) followed by naloxone (1 mg/kg s.c.).

Naloxone at post-morphine	Repeated administration		
	1st	2nd	3rd
0 hr	39 ± 7	50 ± 12	44 ± 10
1/6 hr	27 ± 3	31 ± 7	24 ± 9
1/3 hr	53 ± 17	71 ± 26	59 ± 25
1/2 hr	288 ± 64	354 ± 72	453 ± 78*
3/4 hr	558 ± 96	941 ± 126	1335 ± 207*
1 hr	758 ± 141	1158 ± 193	1593 ± 276*
3/2 hr	1228 ± 281	2168 ± 339*	2893 ± 271*
2 hr	1397 ± 310	2574 ± 352*	3379 ± 489*
3 hr	1401 ± 253	2606 ± 392*	3401 ± 420*

Drug administrations were carried out 3 times at 3-day intervals. Each value is mean activity count ± SEM of 10 mice for 3 hr.

*: significantly different (p<0.05) vs. the activity count at the 1st administration within each group of mice.

Table 4. Mean activity counts for 3 hr after the administration of morphine (10 mg/kg s.c.) to the mice pretreated with morphine or saline followed by naloxone (1 mg/kg s.c.).

Naloxone at Post-morphine	Morphine → Naloxone	Saline → Naloxone
0 hr	1390 ± 115	1522 ± 108
1/6 hr	1377 ± 128	1429 ± 131
1/4 hr	1411 ± 103	1497 ± 179
1/2 hr	2507 ± 248*	1401 ± 198
3/4 hr	3205 ± 292*	1501 ± 202
1 hr	3310 ± 313*	1477 ± 129
3/2 hr	3480 ± 322*	1427 ± 141
2 hr	3399 ± 282*	1357 ± 160
3 hr	3417 ± 227*	1387 ± 143
Drug naive	1355 ± 122	
Morphine X 3	3490 ± 309*	

In the pretreatment, drug administrations were carried out at 3-day intervals, and the challenge administration of morphine was conducted 3 days after the 3rd pretreatment.
Each value is mean activity count ± SEM of 10 mice for 3 hr.
*: significantly different (p<0.05) vs. the activity count of the drug-naïve mice.

and the effect attained plateau by the 4th administration. These results were in agreement with the previous reports (Kuribara and Tadokoro, 1989; Kuribara, 1995a). Even though the sensitization to the ambulatory stimulant effect was induced by the repeated administration of morphine, the time course of changes in the morphine-induced ambulatory stimulation was qualitatively the same, and neither change in the latency to the peak effect nor prolongation of the effect was demonstrated throughout the repeated administrations. These results suggest that the present schedule of repeated morphine administration may not cause significant change in the metabolism of morphine, or accumulation of morphine. In respect to these findings, in Experiment 3, the repeated administrations of morphine were carried out 3 times at 3-day intervals, and the challenge with morphine was conducted 3 days after the 3rd pretreatment.

In some cases, handling of mice including injection of drug or saline acts as stressors, and results in an increased sensitivity to morphine (Leyton and Stewart, 1990; Kalivas and Stewart, 1991; Deroche et al., 1992; Shaham et al., 1995). However, the mice pretreated with saline did not show any significant change in sensitivity to challenge-administered morphine, suggesting that these treatments did not alter neurotransmissions of dopaminergic or opiate system which were related to the sensitivity to morphine.

Naloxone, a prototype of opiate receptor antagonist, inhibited the morphine-induced ambulatory sensitization dependent on the treatment schedules. It is important to note that the groups of mice given naloxone during the post-morphine period of 0-1/3 hr did not show sensitization. On the other hand, the mice given naloxone at post-morphine period of 1/2 hr demonstrated a partial sensitization, and 3/4 hr and later a significant sensitization. These results suggest that a repeated experience of the pharmacological effect of morphine and resultant ambulatory stimulation for at least 1/2 hr during the period of reaching to the peak effect, are important to induce sensitization to the ambulation-increasing effect of morphine. Similar characteristics have been observed in the induction of sensitization to the ambulatory stimulant effect of methamphetamine (Kuribara, 1995b, 1996a) and cocaine (Kuribara, 1996a, 2009). It is therefore considered that the mechanisms of sensitization to the ambulatory stimulant effect of morphine, methamphetamine and cocaine are basically identical, i.e., stimulation of the mesolimbic dopamine system (Van der Heuval and Pasterkamp, 2008; Matsumoto and Hikosaka, 2009) which is strongly related to the reward effects (Piercem and Kumaresan 2006; Berridge, 2007; Ikemoto, 2007).

It has been reported that the induction of the behavioral sensitization to psychotropic drugs is intimately related to the liability of drug abuse (Wise and Bozarth, 1987). The present results also indicate that the early post-morphine period prior to the peak effect is intimately related to the appearance of the reward effect of morphine, which is essential factor for induction and maintenance of abuse and dependence of narcotic analgesic drugs such as morphine and heroin.

Conclusion

A significant sensitization to the ambulatory stimulating effect of morphine (10 mg/kg s.c.), an opiate receptor agonist, was induced when it was repeatedly administered to mice at intervals of 3 days. The treatment with naloxone (1mg/kg s.c.), an opiate receptor blocker, at post-morphine period of 1/2 hr partially, and at 0-1/3 hr perfectly inhibited the induction of the sensitization to morphine. However, the treatment with naloxone at post-morphine period of 3/4 hr and later could not block the morphine sensitization. These results suggest that the experience of morphine effect and the resultant ambulation for 1/2 hr prior to the peak effect is important for induction of the sensitization to the ambulatory stimulant effect of morphine in mice. It is also indicated that the reward effect of morphine appears in the early post-morphine period prior to the peak effect, which is the essential factor for induction and maintenance of the abuse and dependence of narcotic analgesic drugs such as morphine and heroin.

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マウスの移所運動からみたモルヒネ増感現象のオピエート受容体拮抗薬ナロキソンによる時間依存的抑制

栗原　久

東京福祉大学短期大学部（伊勢崎キャンパス）
〒372-0831 伊勢崎市山王町2020-1

抄録： オピエート受容体アゴニストである麻薬性鎮痛薬モルヒネ（10 mg/kg s.c.）のマウス移所運動促進効果は、3日間隔で反復投与すると増強し、増感現象が引き起こされた。オピエート受容体アンタゴニストのナロキソン（0.01-1 mg/kg s.c.）はモルヒネの移所運動促進効果を用量依存的に阻害した。モルヒネ投与から3/4時間以降にナロキソンを投与すると、非投与群と同程度の増感現象が形成されたが、1/2時間後の投与群では、部分的な増感現象の形成にとどまり、1/3時間以前の投与群では全く形成されなかった。ナロキソンあるいは生理食塩水の反復投与はモルヒネ感受性に著しい影響を及ぼさなかった。これらの結果は、マウスの移所運動でみた場合、モルヒネの効果が最大に達するまでの時間帯における1/2時間以上にわたる運動経験が、増感現象の誘発に重要であることを示している。さらに、モルヒネ乱用・依存の誘発と維持の根源である報酬的効果は、投与直後に発現することも示唆している。
（別刷請求先：栗原　久）

キーワード： モルヒネ、ナロキソン、オピエート受容体、移所運動、増感現象、マウス