MODIFICATION OF MORPHINE-INDUCED ANALGESIA, TOLERANCE AND DEPENDENCE BY BROMOCRIPTINE

ADEL A. GOMAA
S.A. MOUSTAFA
L.H. MOHAMED
H.N. AHMED

SUMMARY: The effect of small doses of bromocriptine, a dopamine agonist, on morphine-induced analgesia, tolerance and dependence was investigated in mice. Bromocriptine at dose of 0.04 and 0.08 mg/Kg did not affect the base line of tail flick latency of mice but dose-dependently potentiated the morphine analgesia. Pretreatment of mice with 5 mg/Kg of sulpiride, a D-2 antagonist, not only blocked the effect of 0.08 mg/Kg of bromocriptine but also antagonized the morphine analgesia. Daily injections of 10 mg/Kg of morphine rapidly developed tolerance to the analgesic effect in control animals. Daily combined treatment of bromocriptine with morphine suppressed the development of tolerance to morphine in a dose-dependent manner. However, in the animals daily treated with bromocriptine (0.08 mg/Kg) plus sulpiride (5 mg/Kg), the development of tolerance to the morphine analgesia was not significantly modified. Acute dependence was induced by administration of 100 mg/Kg of morphine. Administration of bromocriptine 30 min before naloxone significantly decreased the naloxone ED50 for inducing jumping in mice. Coadministration of sulpiride with bromocriptine to potentiate the withdrawal syndrome of morphine dependence.

Key Words: Bromocriptine, analgesia, dependence.

INTRODUCTION

Several studies have demonstrated an overlap and interaction of dopaminergic and encephalinergic neurons in several brain regions (24,29). It has been reported that opiates can influence the synthesis (17,32,37), turnover (2,14) and release of dopamine (DA) from dopaminergic neurons (39). Furthermore, there is accumulating evidence that enhancement of dopamine function produces antinociception (4,13,19,25,30).

Recently, Dackis and Gold (11) reported that a small dose of bromocriptine, a dopamine receptor agonist, was effective in the management of cocaine abuse, it could reduce the cocaine abstinence syndrome, craving for cocaine and increased postsynaptic dopamine receptor density. However, no information is available concerning the effect of bromocriptine on the opiate induced analgesia, tolerance and dependence. Therefore, the present study was undertaken to determine the effect of small doses of bromocriptine on the nociceptive sensitivity in mice and morphine analgesia. Also, we aimed to evaluate whether the stimulation of dopamine receptors by small doses of bromocriptine could alter the degree of tolerance and dependence induced by morphine. Since the previous studies have emphasized the major role of neuronal D-2 receptors in the action of bromocriptine (12,22), we used the specific D-2 antagonist, sulpiride (3,33) to verify the role of D-2 receptors in the possible bromocriptine action.

MATERIALS AND METHODS

Male Swiss-Webster mice weighing 20-25 g were used in all experiments, they were assigned to groups randomly, they were kept in a room maintained at 22°C and were given normal laboratory diet and tap water ad libitum.
**Evaluation of analgesic effect**

Animals were tested for antinociception using a modification of the tail immersion technique of Sewell and Spencer (31). Briefly, the mouse was held gently in a mouse holder and the tail was immersed in a hot water bath (47°C). The latency to flick the tail from the water was measured using a stopwatch. The tail was removed from the water both after a cutoff time of 15 sec. Each animal was injected with saline and tested 30 min later on the predrug day for a baseline or control assessment of the tail flick latency (T0). The next day each animal received a randomized drugs treatment and was tested 30 min later for assessment of test tail flick latency (T1). Percentage of analgesia was calculated according to method of Harris and Puisen (15):

\[
100 \times \frac{T1 \times T0}{T0 - T1}
\]

A dose-response curve was plotted on probability paper and the ED50 of morphine and the 95%. Confidence limits (CLs) were estimated by the method of Litchfield and Wilcoxon (21). The antinociceptive activities of morphine was measured in animals pretreated with bromocriptine, bromocriptine and sulpiride or vehicle. In another group of mice, effect of bromocriptine on the tail flick latency was assessed.

**Assessment of tolerance**

The analgesic effect of 10 mg/Kg (S.c.) of morphine was determined daily for 8 days and expressed as percent of the effect obtained in the first day. The development of tolerance to analgesic action of morphine was determined in four groups of mice. In the first and second group, mice were pretreated daily with 0.04 and 0.08 mg/Kg of bromocriptine while the mice in the third group was treated daily by 0.08 mg/Kg of bromocriptine and sulpiride or vehicle. In another group of mice, effect of bromocriptine on the tail flick latency was assessed.

**Assessment of physical dependence**

Acute dependence was induced by administration of 100 mg/Kg of morphine. Six hr later the mice were administered naloxone which induces a withdrawal jumping in dependent mice. This jumping syndrome has proven to be a reliable indicator of precipitated withdrawal in mice (16). After naloxone injection each mouse was placed in a clean, I-gallon glass jar with perforated metal lid. The percentages of mice displaying five or more jumps during the next 20 min of naloxone injections were used to calculate the ED50 of naloxone and 95% confidence limits, (CL). The ED50 of naloxone-induced withdrawal jumping were assessed in animals pretreated with bromocriptine, bromocriptine and sulpiride or vehicle. All the drugs or vehicle administered 30 min before naloxone.

**Drugs**

Sulpiride (Delagrange, Paris, France) and Bromocriptine (Sandoz, Basal, Switzerland) were dissolved in 0.02 M acetic acid to the desired final concentration. Morphine sulphate (Mirs, Cairo, Egypt) and naloxone HCl (Endo Pharmaceuticals Inc., NJ, USA) were dissolved in saline to the desired final concentration. All drugs solutions were made up immediately before injection. They were administered so that the appropriate dose was contained in a volume of 0.1 ml/10g of body weight of animal. In control experiments, 0.02M acetic acid was given to the animals.

**Statistical analysis**

The results were expressed as the mean ± S.E. Significance of the difference was examined by Student’s t-test.

**RESULTS**

**Effect of bromocriptine on the tail flick latency**

Neither 0.04 nor 0.08 mg/Kg of bromocriptine had significant effects on the threshold of pain as compared with that of controls injected with vehicle. The base line response latencies was 2.9 ± 0.2 (mean ±S.E) seconds. Bromocriptine failed to alter base line latency at 0.04 mg/Kg i.p. However, there was a statistically insignificant increase in tail flick latency (3.4 ± 0.2 seconds) following bromocriptine at 0.08 mg/Kg i.p.

**Effect of bromocriptine on morphine analgesia**

Bromocriptine had no significant influence on the analgesic effect of morphine at the dose of 0.04 mg/Kg, but, it effectively potentiated the antinociceptive effects of morphine at the dose of 0.08 mg/Kg (Table 1).

When vehicle was injected i.p., the ED50 value for antinociceptive effect of morphine was 3.9 (3.3-4.6) mg/Kg. When 0.04 mg/Kg of bromocriptine was injected i.p., the ED50 for morphine analgesia was 3.3 (2.7-4)

**Table 1:** Effect of bromocriptine and bromocriptine with sulpiride on ED50 value for morphine induced antinociception.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED50 of morphine (95% CI) mg/Kg S.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.9 (3.3 - 4.6)</td>
</tr>
<tr>
<td>Bromocriptine 0.04 mg/Kg (i.p.)</td>
<td>3.3 (2.7 - 4.0)</td>
</tr>
<tr>
<td>Bromocriptine 0.08 mg/Kg (i.p.)</td>
<td>2.2 (1.9 - 2.6)*</td>
</tr>
<tr>
<td>Sulpiride 5 mg/Kg + Bromocriptine 0.08 mg/Kg (i.p.)</td>
<td>5.6 (4.3 - 7.28)*</td>
</tr>
</tbody>
</table>

- At least 24 animals were used to construct each dose-response curve from which morphine ED50 value were determined.
- All the drugs administered 5 min before morphine.
- Test latencies were determined 30 min after the administration of various test doses of morphine.

* Significantly different from vehicle treated mice (P <0.05).
mg/Kg. However, the intraperitoneal administration of 0.08 mg/Kg of bromocriptine caused a great left shift of morphine dose-response curve and the ED50 of morphine was 2.2 (1.9-2.6) mg/Kg.

Influence of bromocriptine and sulpiride on morphine analgesia

Coadministration of sulpiride (5mg/Kg) with bromocriptine not only blocked the effect of bromocriptine on the morphine analgesia but also significantly antagonized the antinociceptive action of morphine.

Simultaneous administration of bromocriptine (0.08 mg/Kg) and sulpiride (5mg/Kg) intraperitoneal resulted in a significant shift of morphine (S.C.) dose-response curve to the right. The ED50 of morphine was 5.6 (4.3-7.28) mg/Kg.

Effect of bromocriptine on the development of tolerance to morphine

Daily injection of 10 mg/Kg of morphine rapidly developed tolerance to the analgesic effect and the effect was almost lost by the 7th injection. Daily combined treatment of bromocriptine with morphine suppressed the development of tolerance to morphine analgesia in a dose-dependent manner. In the animals daily treated with morphine plus 0.04 or 0.08 mg/Kg of bromocriptine, the analgesic effect of morphine on the 4th day was reduced only by 25 or 10% respectively, of value on the 1st day. However, in the animals daily treated with morphine plus vehicle, the analgesic effect of morphine on the 4th day was reduced by 45% (Figure 1).

Effect of bromocriptine and sulpiride on the development of tolerance to morphine

In the animals treated daily with bromocriptine 0.08 mg/Kg i.p. and sulpiride 5 mg/Kg, daily injection of morphine rapidly produced tolerance to its analgesic effect and after 6 repetitions, the percentage of analgesia produced by 10 mg/Kg of morphine was 35% of that in naive animals (Figure 1).

Effect of bromocriptine on the development of physical dependence on morphine

Bromocriptine administered 30 min before naloxone resulted in a decrease of naloxone ED50 for inducing withdrawal jumping. The potentiation of naloxone-induced jumping by bromocriptine was dose-dependent. The decrease of naloxone ED50 by 0.08 mg/Kg bromocriptine was statistically significant, however, it was non significant after 0.04 mg/Kg of bromocriptine (Table 2).

Effect of bromocriptine and sulpiride on the development of physical dependence

The ED50 of naloxone induced jumping in mice pretreated with 0.08 mg/Kg of bromocriptine and 5mg/Kg of sulpiride was significantly greater than the ED50 of naloxone in mice pretreated with bromocriptine alone (Table 2). However, the ED50 value of naloxone in animals treated with the two drugs was significantly less than that in ani-

Table 2: Effect of bromocriptine and bromocriptine with sulpiride on physical dependence on morphine measured by naloxone-precipitated jumping activity in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED50 of morphine (95% CL) mg/Kg S.C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1.15 (0.94 - 1.38)</td>
</tr>
<tr>
<td>Bromocriptine 0.04 mg/Kg (i.p.)</td>
<td>0.9 (0.69 - 1.17)</td>
</tr>
<tr>
<td>Bromocriptine 0.08 mg/Kg (i.p.)</td>
<td>0.5 (0.38 - 0.65)*</td>
</tr>
<tr>
<td>Sulpiride 5 mg/Kg + Bromocriptine 0.08 mg/Kg (i.p.)</td>
<td>0.8 (0.66 - 0.96)*</td>
</tr>
</tbody>
</table>

- At least 30 were used to generate each dose-response curve.
* Significantly different from vehicle treated mice.
mals treated with vehicle. Thus, sulpiride attenuated but did not block the ability of bromocriptine to potentiate naloxone-induced jumping.

DISCUSSION

The involvement of dopaminergic system in the mod-
ulation of pain perception was suggested early (1). It has been reported that the nigrostriatal dopamine (DA) system responds to a variety of noxious stimuli e.g. tail pinch, sciatic nerve stimulation or radiant heat (10, 26). The results of this study demonstrate that the dose of 0.08 mg/kg of bromocriptine produced a great shift to left in the morphine dose-response curve, although I.P. injection of bromocriptine by itself had no significant effect on the tail flick response of the animals.

In other studies, it was shown that the spinal application of putative D-2 agonist, apomorphine and Ly 17155 (5,20,34) produced a significant elevation of the tail flick latency in untreated naive rats. However, the small doses of bromocriptine did not show any significant antinociceptive action in our study. This discrepancy may be due to the differences of animal species, doses, method of administration and receptor affinity and selectivity of the dopamine agonists.

In the present work, when D-2 receptors were blocked by sulpiride, bromocriptine antagonized the antinociceptive action of morphine. Thus, it can be anticipated that when D-2 receptors were blocked by sulpiride, bromocriptine stimulated D-1 receptors there by resulting in antagonism of the antinociceptive action of morphine. This interpretation is in agreement with the previous investigations. It has been shown that stimulation of D-2 receptors facilitates antinociception whilst D-1 receptors facilitates antinociception whilst D-1 receptors stimulation evokes hyperalgesic response (4,5,7).

Our observation that the coadministration of dopamine agonist with morphine antagonized the morphine tolerance is in disagreement with previous reports that suggest that the decrease in brain dopamine is responsible for the suppression of morphine tolerance (35,36). It has been reported also that the blockade of the development of supersensitive DA receptor by PLG (9) or cyclo (Leucylglycin) (27,28) decrease the degree of analgesic tolerance. However, most recently, Martin and Takemore (23) have shown that the administration of lithium prevents the development of morphine-induced DA receptor supersensitivity while simultaneously enhancing the degree of analgesic tolerance.

It has been shown that there is an inverse relationship between the ED50 of naloxone required to induce with-
MORPHINE VS BROMOCRIPTINE


GOMAA, MOUSTAFA, MOHAMED, AHMED


Correspondence:
Adel A. Gomaa
Department of Pharmacology
Faculty of Medicine
Assiut university,
Assiut, EGYPT.