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 supperssed the development of tolerance to morphine suppressed the development of tolerance to morphine analgesia 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 cocain 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 mouse 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 verity 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*.

From Departments of Pharmacology, Anaesthesia and Neurology, Faculty of Medicine, Assiut University, Assiut, Egypt.

Evaluation of analgesic effect

Animals were tested for antinociception using a madification 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 base-line or control assessment of the tail flick latency (T_0). The next day each animal received a randomized drugs treatment and was tested 30 min later for assessment of test tail flick latency (T_1). Percentage of analgesia was calculated according to method of Harris and Puison (15):

$$100 \text{ X } \frac{T_1 - T_0}{15 - T_0}$$

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 antinococeptive 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 (5 mg/Kg). Vehicle was daily injected in the fourth group.

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, l-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 ED 50 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 (Misr, Cairo, Egypt) and naloxone HCI (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 ±SE) 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 injecte 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.

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

- 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).

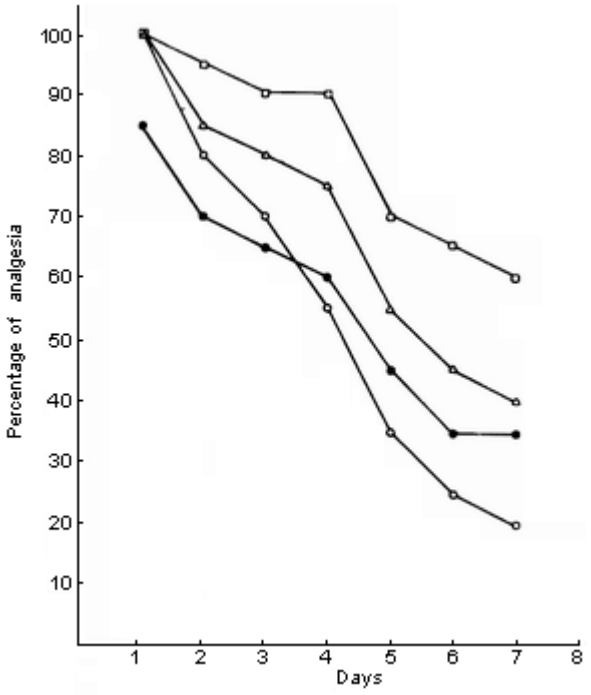

Figure 1: Effect of bromocriptine, bromocriptine with sulpiride and vehicle on the development of tolerance to morphine. Mice were pretreated daily with △ bromocriptine 0.04 mg/Kg, □ bromocriptine 0.08 mg/Kg, ● bromocriptine 0.08 mg/Kg + sulpiride 5 mg/Kg or O vehicle. Each point represents the mean of the respective group of 10 to 15 animals.

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).

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

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

- At least 30 were used to generate each dose-response curve.
* Significantly different from vehicle treated mice.

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-

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 modulation 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 radient 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 withdrawal jumping in mice and the degree of morphine dependence (38). Furthermore, several reports implicate the involvement of dopamine in withdrawal jumping syndrome (18). Also, Ben-Streli *et al.* (8), have described that SKF 38343, D-1 receptor agonist, which was inactive behaviourally, exacertated withdrawal signs in chronically morphine-treated rats. In our study, bromocriptine administration decreased significantly the amount of naloxone required to induce withdrawal jumping, in mice previously administered morphine. This effect of bromocriptine was reduced by sulpiride, however, sulpiride failed to abolish it. Therefore, it seems that bromocriptine enhanced the development of morphine dependence by activating D-2 receptors and others. However, further studies are necessary to characterized the receptors responsible for the actions of bromocriptine reported in this study.

REFERENCES

1. Akil H, Liebeskind JC : Brain Res 94:279C F Gonzalez et al, Br J Pharmac 86:709P, 1985.

2. Alper RH, Demarest KT, Moore KE : Morphine differentially alters synthesis and turnover of dopamine in central neuronal systems. J Neurol Transm 48:157, 1980.

3. Arima T, Samura N, Nomura Y, Segawa T : Comparison of effect of tiapride and sulpiride on D-1, D-2, D-3 and D-4 subtypes of dopamine receptors in rat striatal and bovine caudate nucleus membranes. Japan J Pharmacol 40:417, 1986.

4. Barasi S, Ben-Sreti MM, Duggal KN, Gonzalez JP, Sewell RDE : Antinociceptive activity on intravenous and intrathecal dopamine agonists in naive and supersensitive rats. Br J Pharmac 84: 166 P, 1985.

5. Barasi B, Ben-Sreti MM, Clatworthy AL, Duggal KN, Gonzalez JP, Robertson J, Rooney KF, Sewell RDE : Dopamine receptor-mediated spinal antinociception in the normal and haloperidol pretreatment rat: effects of sulpiride and SCH 23390. Br J Pharmac 90:15, 1987.

6. Barasi S, Duggal KN : The effect of local and systemic application of dopaminergic agents on the tail flick latency in the rat. Eur J Pharmac 117:287, 1985.

7. Ben-Sreti MM, Gonzalez IP, Sewell RDE : Differential effects of SKF 38393 and LY 141865 on nociception and morphyne analgesia. Life Sci 33:665, 1983.

8. Ben-Sreti MM, Gonzalez IP, Sewell RDE : The influence of selective dopaminergic and cholinergic agonists on precipitated opiate abstinence. Alcohol Alcoholism 18:353, 1983.

9. Bhargava NH : Cyclo (leucylglycine) inhibits the development of morphine induced analgesic tolerance and dopamine receptor supersensitivity. Life Sci 27:117, 1980.

10. Chiodo LA, Antelman SM, Caggiula AR, Lineberry CG : Sensory stimuli alter the discharge rate of dopamine (DA) neu-

rons. Evidence for two functional types of DA cells in the substantia nigra. Brain Res 189:544, 1980.

11. Dackis CA, Gold MS : Bromocriptine as treatment of cocaine abuse. Lancet 11:1151, 1985.

12. Ensinger H, Majewski H, Hedler L, Starke K : Neuronal and postjunctional components in the blood pressure effect of dopamine and bromocriptine in rabbits. J Pharmacol Exp Ther 234:681, 1985.

13. Fleetwood-Walker SM, Hope PJ, Molony V : Dopamine inhibits the responses of multireceptive dorsal horn neurones to noxious but not innocuous, cutaneaus stimulation in the rat and Cat. J Physiol 354:95, 1984.

14. Guaza C, Torrellas A, Borrell S, Borrell J : The effects of acute and chronic administration of morphine on the turnover of brain and adrenal catecholamines in rats. Pschopharmacology 68:43, 1980.

15. Harris LS, Puison AK : Some narcotic antagonists in the benzomorphan series. J Pharmacol Exp Ther 143:141, 1964.

16. Huang JT, Yano I, Tokemori AE : Effect of various central biogenic aminemodifiers and ambient temperature on the naloxane induced Jumping in morphine depend mice. In Factors Affecting the Action of Narcotics, ed by MW Adler, L Manara and R Saminin, pp 495-509, Raven Press, New York, 1978.

17. Ishikawa K, Shibanoki S, Mc Gaugh JL : Direct correlation between levels of morphine and its biochemical effect on monoamine systems in mouse brain. Biochem Pharmacol 32:1473, 1983.

18. Iwamoto ET, HOIK, Way EL : Elevation of brain dopamine during naloxone-precipitated withdrawal in morphine dependent mice and rats. J Pharmacol Exp Ther 187:558, 1973.

19. Jensen TS, Yaksh TL : Effects of an intrathecal dopamine agonist apomorphine on the thermal and chemical evoked noxious responses in rats. Brain Res 296:283, 1984.

20. Kebabian JW, Calne DB : Multiple receptors for dopamine. Nature 277:93-96, 1979.

21. Litchfield ST, Wilcoxon F : A simplified method of evaluating dose effective experiments. J Pharmacol Exp Therap 25:99-113, 1949.

22. Lokhandwala MF, Tadepalli AS, Jandhyala BS : Cardiovascular ctions of bromocriptine; Evidence for a neurogenic mechanism. J Pharmacol Exp Ther 211: 620, 1979.

23. Martin JR, Takemori AE : Modification of the development of acute opiate tolerance by inreased dopamine receptor sensitivity. J Pharmacol Exp Ther 241:48, 1987.

24. Moore RV, Bloom FE : Central catecholamine neuron systems anatomy and physiology of the dopamine systemns. Ann Rev Neurosci 15:129, 1978.

25. Paalzow GHM, Paalzow LK : Opposing effects of apomorphine on pain in rats. Evaluation of the dose-response curve. Eur J Pharmac 88:27, 1983.

26. Pay S, Barasi S : A study of the connections of nociceptive substantia nigra neurones. Pain 12:75, 1982.

27. Ritzmann RF, Lee JM, Fields JZ : Peptide supersensitivity. Life Sci 31: 2287, 1982.

28. Ritzmann RF, Walter R, Bhargara HN, Flexner LB : Blockage of narcotic-incluced dopamine receptor supersensitivty by cyclo (leu-gly). Proc Natl Acad Sci USA 76:5997, 1979.

29. Rossier J, Bloom F : Central neuropharmacology of endorphins. Adv Biochem Psychopharmacol 20:165, 1979.

30. Saarnivaara L: Analgesic activity of some sympathetic drugs and their effect on morphine analgesia in rabbits. Ann Med Exp Biol Tenn 47:180,1969.

31. Sewell RDE, Spencer PSJ : Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. Neuropharmacology 15:683, 1976.

32. Spampinato U, Inveernizzi R, Nowakowska E: Reduction of morphine's effect on striatal dopamine metabolism in rats treated with a low dose of apomorphine of agents increasing serotonin transmission. Biochem Pharmacol 33:163, 1984.

33. Spano PF, Stefanini E, Trabucchi M, Fresia P : Stereospecific interaction of sulpiride with striatal and nostriatal dopamine receptors. In sulpiride and Other Benzamides, ed by PF Spoxano Tratucchi, GU Corsini and GL Gessa. pp 11-31, Italian Brain Research Foundation Press, Milan, 1979.

34. Stoof JC, Kebabian JW : Opposing roles for D-1 and D-2 dopamine receptors in efflux of cyclic AMP from rat neostriatum. Nature 294:366, 1981.

35. Takagi H, Kuriki H : Suppressive effect of tetrabenazine on the development of tolerance to morphine and its reversal by DOPA. Int J Neuropharmacol 8:195, 1969.

36. Takagi H, Takashima T, Kimura K : Antagonism of the analgesic effect morphine in mice by tetrabenazine and reserpine. Acrh Int Pharmacodyn Ther 149:484,1964.

37. Urwyler S, Tabak off B : Stimulation of dopamine synthesis and release by morphine and D-A1A 2D-LEU 6 enkephalin in the mouse striatum in vivo. Life Sci 28:2277, 1981.

38. Way EL, Loh HH, Shen FH : Simultaneous quantitative assessment of morphine tolerance and physical dependence. J Pharmacol Exp Ther 167:1, 1969.

39. Wood PL, Scotland M, Richard JW, Rachham A : Actions of mu, Kappa, Sigma, delta and agonist/antagonist opiates on striatal dopaminergic junction. J Pharmacol Exp Ther 215:697, 1980.

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