Pharmacology Biochemistry & Behavior, Vol. 17, pp. 703–707, 1982. Printed in the U.S.A.

Similarities of the Cataleptic State Induced by β-Endorphin and Morphine[1]

D. B. RONDEAU[2]

Département de Psychologie, Université du Québec à Montréal, Montréal, P. Q., Canada H3C 3P8

AND

M. TURCOTTE, L. YOUNG[3] AND D. HEBERT[4]

Département de Psychologie, Université de Moncton, Moncton, N.B.

Received 18 February 1982

RONDEAU, D. B., M. TURCOTTE, L. YOUNG AND D. HEBERT. *Similarities of the cataleptic state induced by β-endorphin and morphine.* PHARMAC. BIOCHEM. BEHAV. 17(4) 703–707, 1982.—A variety of behavioral tests were used to characterize the cataleptic state induced by various treatments. Besides catalepsy, posture, locomotion, rigidity and the presence of reflexive responses were assessed. Measures of analgesia and body temperature were taken. The behavioral profiles of β-endorphin, morphine, etonitazene, haloperidol, arecoline and GABA were compared at the time maximal catalepsy scores were obtained. Results indicated that, for an equivalent degree of catalepsy, the profile of β-endorphin was similar to that of opiates, except for changes in body temperature; β-endorphin's profile differed markedly from that of haloperidol, arecoline and GABA. Catalepsy was less pronounced with the latter two drugs. There were similarities in the behavioral profile of haloperidol and arecoline.

Catalepsy Rigidity Behavioral effect β-Endorphin Morphine Etonitazene Arecoline GABA Haloperidol

EXPERIMENTAL catalepsy is a behavioral symptom assessed by imposing an abnormal posture to laboratory animals usually following a drug treatment. The intensity of the cataleptic state is measured by recording the length of time the animals maintain one or more imposed awkward postures. Haloperidol and morphine are drugs well-known to produce catalepsy and akinesia or lack of spontaneous locomotor activity. However, the other behavioral effects of these drugs, for instance analgesia, rigidity and physical resistance or clinging are quite different [13, 19, 23]. Recently, it has been reported that the nature of the cataleptic state induced by morphine is also different from the cataleptic state induced by haloperidol [9]. De Ryck *et al.* [9] performed a detailed analysis of neuroleptic and opiate catalepsy and demonstrated that haloperidol and morphine produced two distinct behavioral states with regard to postural support and locomotion mechanisms. The motor subsystem involved in locomotor activity was inhibited by both haloperidol and morphine, but could be activated readily by sensory stimulation in morphine-treated rats. The motor subsystem involved in stable static equilibrium was inhibited by morphine and remained intact in haloperidol-treated rats; the latter animals responded to sensory stimulation by displaying bracing reactions [9].

The purpose of the present experiment was to study in details the cataleptic state induced by intraventricular injection of β-endorphin and to compare various effects of the opioid peptide on motor behaviors to those produced by other cataleptogenic drugs, including morphine and haloperidol. Results indicated that the spectrum of effects of β-endorphin on motor behaviors is similar to that of morphine but not of haloperidol. Results indicated also that weaker catalepsy scores are obtained with two other cataleptogenic drugs, arecoline and GABA, and that the behavioral profile of these drugs differs markedly from that of morphine.

METHOD

Animals

Male Sprague-Dawley rats from Ferme et Laboratoire d'Elevage Ltd, St-Constant, P. Quebec, were used. Animals were housed in individual cages with food and water ad lib. They weighed between 250 and 350 g at the beginning of the experiment.

Treatments

Groups of six or more rats were administered the follow-

[1]This work was supported by the NSERC of Canada, grant A7474, and by Conseil de recherche de l'Université de Moncton.
[2]Send requests for reprints to D. B. Rondeau at above address.
[3]L. Y. was recipient of an NSERC undergraduate summer research award.
[4]Now at the Université de Montréal.

ing treatments: 20 mg/kg IP morphine sulfate (BDH Chemicals), 1 mg/kg SC haloperidol (McNeil Labs), 15 mg/kg IP arecoline (Sigma Chemicals), 2 g/kg gamma-aminobutyric acid, GABA, IP (Sigma Chemicals), 2 μg IVT etonitazene (generously supplied by the Bureau of Dangerous Drugs, Health and Welfare, Canada) and 6.25, 12.50 and 25.00 μg IVT β-endorphin (Sigma Chemicals). For peripheral administration, drugs were dissolved in 0.9% NaCl and the injection volume was 1 ml/kg, except for GABA, 10 ml/kg. For intraventricular administration, animals were anaesthetized with sodium pentobarbital (60 mg/kg IP), placed in a stereotaxic instrument and chronically implanted with a cannula into the left ventricle. Physiological saline was used as the vehicle and the injection volume was 10 μl given over a 3 min period via a 50 μl Hamilton microsyringe. Four rats which received either a peripheral or an intraventricular injection of the vehicle were used as a control group. Behavioral testing took place at least 74 hrs after surgery. Upon completion of the experiment animals were infused with methylene blue dye and the cannula placements were verified by gross dissection of the brain.

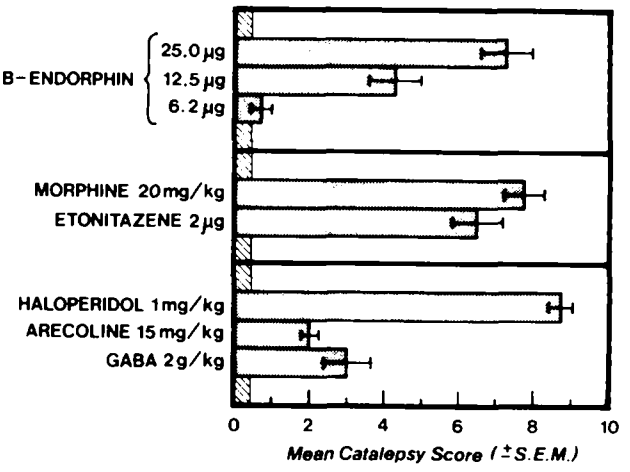

FIG. 1. Mean catalepsy scores (±S.E.M.) for the various treatments. S.E.M. scores of the control group are given horizontally.

Behavioral Procedures

Animals were tested on at least three occasions at either 10, 30, 60 or 90 min after the injection depending upon the treatment. Observations and measures were always taken according to the following sequence:

Posture and activity. Rats were placed in a 16 squares 88×88 cm open field and observed at distance for 5 min. Frequency of locomotor, rearing and grooming activities were recorded. The posture of the animals was examined taking into account the observations made by De Ryck [9] in morphine and haloperidol treated rats; position of the trunk, orientation of the head and the presence or not of wide-broad-based support as indicated by limb position was noted. These animals were removed from the open field and responses to a series of behavioral tests were assessed.

Catalepsy. Three tests were used; the 10 cm bar test and the 3 cm and 9 cm cork tests [20,22]. A catalepsy score, 0 to 3, was attributed according to the time rats maintained each of the imposed postures, up to a maximum of 60 sec. The maximum catalepsy score was 9 points.

Rigidity. Five tests were performed to evaluate rigidity; the rigidity score was the sum of the scores obtained on each test: (1) The "bridge test" [3] for which scores varied from 0 to 3 according to time limbs of the animal remained on the bars after being properly placed. (2) The "trunk test" for which scores of 0, 1 or 2 were based on the time rats remained in an upright posture when held above the knee joints of the hindlimbs [2,17]. (3) The "hindlimb rigidity test" scored 0 or 1, a modification of the system used by Barnett *et al.* [2]. (4) Stifness of the tail was assessed by lifting the tail from the base to its two thirds with a pencil; scores of 2 were given only when the tail could curl around the pencil. (5) Muscle tone was scored subjectively, 0, 1 or 2, by grasping the back of the animals. The maximum rigidity score was 10 points.

Clinging. The tonic grasping reflex was assessed by two tests. In the "vertical grid test" [19] the animal was placed in the center of a rectangular (36×40 cm) wire screen (1/4-inch mesh) and the time it remained stationary was recorded. In the second test [15] the rat was suspended by its front paws grasping a metal rod (0.5 cm diameter) which was held by the experimenter about 50 cm above the table. The length of time the animal remained suspended was noted. Results in sec up to a maximum of 60 sec, were converted into scores from 0 to 3. The maximum score for clinging was 6 points.

Reflexive responses. The presence or not of bracing reactions to passive displacement and to a 45° tilt was assessed [9]. Rats were inspected also for the following reflexes: righting, corneal, biting and reaching [19].

Analgesia. The tail flick test was used. It consisted in the measure of the latency, in sec, between placing the distal third of the rat's tail in 53°C water and noting the emergence of the tip of the tail from the water. Failure to respond in 20 sec resulted in termination of the test. Data were obtained on two occasions prior to any given treatment.

Body temperature. It was measured, at ambient temperature, using a thermistor probe (Yellowspring Instruments) inserted approximately 4 cm into the rectum. Pretreatment data were obtained.

Selection of the doses, routes of administration and times of behavioral testing was made according to reported effects of the treatments obtained with various behavioral measures relevant to those used in the present study [5, 7, 9, 21].

RESULTS

Mean results at the catalepsy and rigidity tests were compared within each treatment and the maximum effect times were determined. Except for morphine and haloperidol, the highest scores at these tests were obtained when rats were tested for the first time. Observations and results are reported only for the maximum effect times.

Mean catalepsy scores are presented in Fig. 1. A cataleptic state was induced by each of the substances examined. The effects of β-endorphin were clearly dose dependent; the lowest dose of the peptide was the only treatment which did not induce catalepsy. Intensity of catalepsy was comparable for morphine, etonitazene and 25 μg β-endorphin; it was slightly stronger for haloperidol but of much lower magnitude for arecoline and GABA. Scores of the latter two groups were significantly different from those of morphine, etonitazene, 25 μg β-endorphin and haloperidol (all $ps < 0.05$ or 0.01).

The maximal rigidity scores obtained after peptide or drug administration are presented in Fig. 2. β-Endorphin, in a

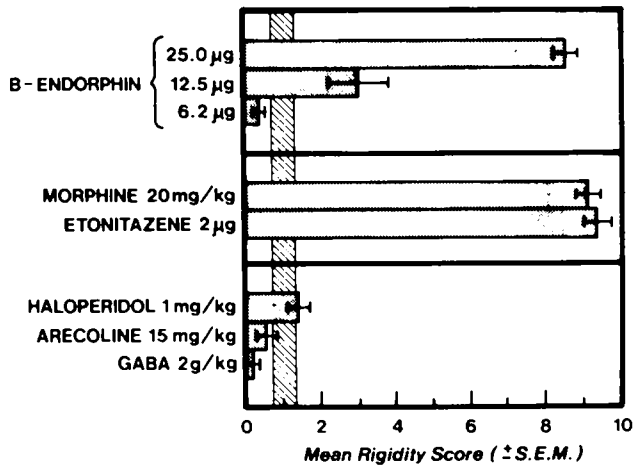

FIG. 2. Mean rigidity scores (± S.E.M.) for the various treatments. S.E.M. scores of the control group are given horizontally.

dose of 25 μg, morphine and etonitazene produced intense rigidity. Scores of the other groups were not significantly different from that of the control animals.

Qualitative behavioral observations concerning posture, reflexes and bracing reactions are presented in Table 1. There was variability in the behavior of animals treated with the two lower doses of β-endorphin; observations and also results of the measurement of analgesia, body temperature and clinging are not reported for the latter groups.

Control rats were very active during the 5 min period in the open field; mean number of squares crossed was 40.2, mean number of rearings was 15.0 and mean time spent in grooming activities was 28.0 sec. Akinesia or absence of spontaneous locomotor activity was a common effect of all treatments. Animals remained immobile in a corner of the open field. Except for most of the animals injected with arecoline, the akinesia was accompanied by a total disappearance of rearing and grooming behaviors during the 5 min. period. Tremors, lacrymation, piloerection, teeth grinding and salivation with associated grooming of the face were noted to occur within a few minutes in rats injected with the cholinergic agonist arecoline.

The posture assumed by animals treated with 25 μg β-endorphin and with the two narcotic drugs, morphine and etonitazene, had similar characteristics; limbs, which were adducted, did not support the body and the head was in contact with the floor. Rats injected with lower doses of β-endorphin did not show this lying posture although it was occasionally seen that head support was absent. On the contrary, limb and head support was typical of the posture assumed by haloperidol and arecoline treated rats. Front limbs were directed forward under the head while hindlimbs were laterally abducted; curvature of the trunk was marked in both cases. Animals injected with GABA were akinetic with respect to locomotor activity but were not as immobile as the other groups; a typical posture, different from that of control rats, could not be identified.

Examination of reflexes and bracing reactions revealed a similar profile for β-endorphin (only at 25 μg), morphine and etonitazene; animals lost most of the reflexes tested and did not show bracing reactions when passively displaced or tilted. Opposite observations were made with haloperidol, arecoline and GABA; bracing reactions were always present and, with the exception of the loss of the biting reflex in haloperidol injected rats, animals retained all the reflexes (Table 1).

Results of the measurement of analgesia, body temperature and clinging at the time when cataleptic state was maximal for the various treatments are presented in Table 2. Arecoline and GABA did not modify any of these three measures. β-Endorphin, in a dose of 25 μg, morphine and etonitazene produced a strong analgesic effect. The signifi-

TABLE 1

BEHAVIORAL PROFILE OF β-ENDORPHIN AS COMPARED TO OTHER CATALEPTOGENIC DRUGS

	Treatments						
	Vehicle	β-Endorphin 25 μg	Etonitazene 2 μ	Morphine 20 mg/kg	Haloperidol 1 mg/kg	Arecoline 15 mg/kg	GABA 2 g/kg
Time After Injection (min)	30	30	10	60	60	10	30
Akinesia	–	++	++	++	++	++	++
Posture							
Lying	–	++	+	++	–	–	–
Head Downward	–	++	++	++	–	–	-
Limb Adduction	–	++	++	++		–	+
Reflexes (loss of. . .)							
Corneal	–	++	++	++	–	–	-
Righting	–	+	+	+	–	–	–
Reaching	–	+	++	+	–	–	-
Biting	-	++	++	++	++	–	–
Bracing Reactions							
Passive Displacement	++	–	–	–	++	++	++
Tilt	++	–	–	–	++	++	++

++Observed in 80–100% of the animals.
+Observed in 50–80%of the animals.
- Observed in less than 10% of the animals or never observed.

TABLE 2

OTHER MEASURES OF THE BEHAVIORAL PROFILE OF β-ENDORPHIN AS COMPARED TO OTHER CATALEPTOGENIC DRUGS

Treatments	Analgesia (Changes in reaction time, sec)*	Body Temperature (Changes in degrees, C)*	Clinging (Max score=6)*
Vehicle	0.4 ± 0.4	+0.2 ± 0.20	1.4 ± 0.6
β-Endorphin 25 μg	13.6 ± 2.4	−1.2 ± 0.20	0
Morphine 20 mg/kg	16.0 ± 0.8	+0.9 ± 0.45	0
Etonitazene 2 μg	15.0 ± 1.0	+0.4 ± 0.20	0
Haloperidol 1 mg/kg	2.0 ± 0.5	+0.1 ± 0.32	4.4 ± 1.5
Arecoline 15 mg/kg	0.2 ± 0.2	−0.1 ± 0.48	1.0 ± 0.4
GABA 2 g/kg	0.8 ± 0.6	−0.1 ± 0.12	0.3 ± 0.3

*Mean results ±S.E.M.

cant increases in reaction time at the tail flick test for the latter three treatments were of comparable magnitude (p's<0.01); many rats did not respond within 20 sec. However, only 25 μg β-endorphin and morphine produced changes in body temperature; the observed changes were in the opposite direction. Morphine exerted a significant hyperthermia effect; this effect was present 30 min after administration and persisted for at least 3 hrs. On the other hand, β-endorphin's significant hypothermic effect was transient; mean changes in body temperature recorded at 30 min intervals after injection of the peptide were −1.2, −1.1, −0.4, −0.1 and 1.0 degrees C. It should be noted that signs of catalepsy, rigidity and akinesia in these two groups had completely disappeared 2 hrs following the injection. Lower doses of β-endorphin, 12.5 and 6.25 μg, did not significantly modify body temperature and produced weaker analgesic effect (data not presented). Haloperidol was the only treatment to increase significantly the scores obtained at the two clinging tests ($p<0.01$). On the opposite, scores were decreased significantly in rats injected with 25 μg β-endorphin and with the opiates (ps<0.01). Animals slided off immediately from the grid or from the bar indicating that the grasping response had totally disappeared.

DISCUSSION

Results of the present experiment confirmed previous observations that β-endorphin, morphine and etonitazene produce similar effects on motor behaviors [4, 5, 10, 16, 19]. The similarity of the undisturbed posture adopted by β-endorphin treated rats and the absence of grasping and bracing reactions in these animals suggest that the peptide may exert, like morphine [9], an inhibitory action on the motor subsystem involved in stable static equilibrium in addition to a blockade of locomotor mechanisms.

The cataleptic state induced by those three substances was of similar intensity. It was accompanied by identical changes at various tests: akinesia, rigidity, loss of several reflexes, clinging and analgesia. However, changes in body temperature differed following treatment. Morphine produced hyperthermia, 25 μg β-endorphin produced hypothermia while etonitazene had no effect. Although the thermal effects of morphine are confusing because of a plethora of conflicting reports [24] there is indication that low doses of morphine cause hyperthermia in Sprague-Dawley rats [8]. The hypothermia obtained with β-endorphin has been observed previously [6]. The fact that intense catalepsy can be accompanied by opposite changes in body temperature as well as by no changes at all, as in cases of etonitazene and haloperidol [6], do not provide support to the point of view that the posture of catalepsy may be useful in thermoregulation [18]. The possible link between catalepsy and regulation of body temperature has to be explored further.

Results of the present experiment confirmed observations on the differentiation of neuroleptic and opiates cataleptic state [9, 10, 12, 13, 19]. The akinesia and catalepsy induced by haloperidol was not accompanied by rigidity, analgesia or, except for biting, loss of reflexes; moreover, the effects on clinging were in the opposite direction.

Although cholinergic agonists, such as arecoline, do induce catalepsy [7,14] the spectrum of effects on motor behaviors of this group of drugs has not been compared to that of other cataleptogenic drugs. The present results indicated that arecoline induced a much weaker cataleptic state than haloperidol and opiates. It should be noted that cholinergic signs, such as piloerection, lacrymation and tremors were present when animals were tested for catalepsy. Tremors had disappeared twenty minutes after injection of the drug and there was then no sign of catalepsy. Besides catalepsy, and clinging which were less intense, the behavioral profile of arecoline resembled that of haloperidol: similar posture, no loss of reflexes, presence of bracing reactions and absence of rigidity, analgesia and changes in body temperature. Some of these observations suggest that arecoline may not affect the motor subsystem involved in stable static equilibrium even though tremors are present. Hypothermia was not observed after arecoline treatment in the present experiment; such effect has been reported for a higher dose of the drug [25].

GABA, a substance known to produce sedation and a depression in locomotor activity [11,21] has been found also to induce catalepsy at a high dose [1]. In the present experiment, catalepsy scores obtained after GABA treatment were only moderate; as for arecoline, GABA induced a much weaker cataleptic state than haloperidol and opiates. A typi-

cal posture resembling that produced by other drugs could not be identified; animals showed no locomotor activity but changed posture frequently and were flaccid when handled. With the exception of clinging, the effects of GABA on the other tests were similar to those of arecoline.

In general, the results of this experiment demonstrated that the cataleptic states induced by various treatments are not equivalent. However, a single dose of each drug was employed in the present experiment, except for β-endorphin. The possibility that the observed differences at the various behavioral tests may reflect differences in potency cannot be ruled out. It remains that assessment of catalepsy should be accompanied by an examination of a variety of motor responses, including rigidity and physical resistance or clinging.

REFERENCES

1. Balsara, J. J., J. H. Jadhav and A. G. Chandorkar. Effect of intraperitoneally administered GABA on haloperidol-induced catalepsy in the rat. *Psychopharmacology* **68:** 105–107, 1980.
2. Barnett, A., J. Goldstein, E. Fiedler and R. Taber. Etonitazene-induced rigidity and its antagonism by centrally acting muscle relaxants. *Eur. J. Pharmac.* **30:** 23–28, 1975.
3. Bloom, F., D. Segal, N. Ling and R. Guillemin. Endorphins: Profound behavioral effects in rats suggest new etiological factors in mental illness. *Science* **194:** 630–632, 1976.
4. Browne, R. G. and D. S. Segal. Behavioral activating effects of opiates and opioid peptides. *Biol. Psychiat.* **15:** 77–86, 1980.
5. Browne, R. G., D. C. Derrington and D. S. Segal. Comparison of opiate and opioid-peptide-induced immobility. *Life Sci.* **24:** 933–942, 1979.
6. Clark, W. Changes in body temperature after administration of amino acids, peptides, dopamine, neuroleptics and related agents. *Neurosci. Biobehav. Rev.* **3:** 179–231, 1979.
7. Costall, B. and J. E. Olley. Cholinergic- and neuroleptic-induced catalepsy: modification by lesions in the caudate-putamen. *Neuropharmacology* **10:** 297–306, 1971.
8. Cox, T. F. Lee and M. J. Vale. Effects of morphine and related drugs on core temperature of two strains of rats. *Eur. J. Pharmac.* **54:** 27–36, 1979.
9. De Ryck, M., T. Schallert and P. Teitelbaum. Morphine versus haloperidol catalepsy in the rat: a behavioral analysis of postural support mechanisms. *Brain Res.* **201:** 143–172, 1980.
10. De Wied, D., G. L. Kovacs, B. Bohus, J. M. Van Ree and H. M. Greven. Neuroleptic activity of the neuropeptide β-LPH_{82-77}. *Eur. J. Pharmac.* **49:** 427–436, 1979.
11. Freed, W. J. and E. K. Michaelis. Effects of intracisternal GABA or glutamic acid upon behavioral activity in the rat. *Pharmac. Biochem. Behav.* **5:** 11–14, 1976.
12. Head, M., H. Lal, S. Puri, C. Manitione and D. Valentino. Enhancement of morphine analgesia after acute and chronic haloperidol. *Life Sci.* **24:** 2037–2044, 1979.
13. Lal, H., G. Gianutsos and S. K. Puri. A comparison of narcotic analgesics with neuroleptics on behavioral measures of dopaminergic activity. *Life Sci.* **17:** 29–34, 1975.
14. Mason, S. T. Central noradrenergic-cholinergic interaction and locomotor behavior. *Eur. J. Pharmac.* **56:** 131–137, 1979.
15. Molinengo, L. and M. Orsetti. Drug action on the "grasping" reflex and on swimming endurance: an attempt to characterize experimentally antidepressant drugs. *Neuropharmacology* **15:** 257–260, 1976.
16. Pert, A., L. A. De Wald, H. Liao and C. Sivit. Effects of opiates and opioid peptides on motor behaviors: sites and mechanisms of action. In: *Endorphins in Mental Health*, edited by E. Usdin, W. E. Bunney and N. S. Kline. New York: Oxford University Press, 1979, pp. 45–61.
17. Rackham, A. Opiate-induced muscle rigidity in the rat: effects of centrally acting agents. *Neuropharmacology* **19:** 855–859, 1980.
18. Schallert, T., I. Q. Whishaw, M. De Ryck and P. Teitelbaum. The postures of catecholamine-depletion catalepsy: Their possible adaptive value in thermoregulation. *Physiol. Behav.* **21:** 817–820, 1978.
19. Segal, D. S., R. G. Browne, F. Bloom, N. Ling and R. Guillemin. β-Endorphin: Endogenous opiate or neuroleptic? *Science* **198:** 411–414, 1977.
20. Simon, P., R. Langwinski and J. R. Boissier. Comparison de différents tests d'évaluation de la catalepsie chez le rat. *Thérapie* **24:** 985–995, 1969.
21. Smialowski, A., M. Smialowska, K. Reichenberg, B. Byrska and J. Vetulani. Motor depression and head twitches induced by IP injection of GABA. *Psychopharmacology* **69:** 295–298, 1980.
22. Waldmeier, P. C. and A. A. Delini-Stula. Serotonin-dopamine interactions in the nigrostriatal system. *Eur. J. Pharmac.* **55:** 363–373, 1979.
23. Wauquier, A., C. J. E. Niemegeers and H. Lal. Differential antagonism by naloxone of inhibitory effects of haloperidol and morphine on brain self-stimulation. *Psychopharmacologia* **37:** 303–310, 1974.
24. Yehuda, S. and A. J. Kastin. Peptides and thermoregulation. *Neurosci. Biobehav. Rev.* **4:** 459–471, 1980.
25. Zetler, G. Pharmacological differentiation of "nicotinic" and "muscarinic" catalepsy. *Neuropharmacology* **10:** 289–296, 1971.