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Neurobehavioral Evidence for Kappa Agonist Activity of the Morphinan Derivative 14-β-Methyl 8-Oxacyclorphan [BC (3016)]

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JOLICOEUR, F B , D MENARD, R RIVEST, S LEMAIRE AND B BELLEAU *Neurobehavioral evidence for kappa agonist activity of the morphinan derivative 14-β-methyl 8-oxacyclorphan [BC (3016)]* PHARMACOL BIOCHEM BEHAV **38**(2) 401–405, 1991 —The purpose of the present study was to determine if the in vivo neurobehavioral effects of the morphinan 14-β-methyl 8-oxacyclorphan, [BC (3016)], would reflect the kappa agonist activity found in our previous in vitro studies The effects of intracisternal administration of various doses (10–80 μg) of BC (3016) on body temperature, muscle rigidity, nociception of thermal, chemical and mechanical stimuli as well as its ability to induce catalepsy were examined The effects of intrathecal administration of the same doses of the compound on reactivity of animals to a thermal stimulus were also assessed Finally, the ability of BC (3016) to antagonize well known neurobehavioral effects of morphine was investigated Results indicate that the analgesic properties of BC (3016) resemble those of typical kappa agonists Intracisternal administration of the drug failed to affect nociception to an aversive thermal stimulus but markedly reduced the reactivity of animals subjected to noxious chemical or mechanical stimuli On the other hand, intrathecal administration of BC (3016) significantly attenuated nociception of animals to a thermal stimulus The in vivo neurobehavioral effects of BC (3016) appear to be kappa selective since the drug did not decrease body temperature, increase muscular tone or induce catalepsy, three effects generally attributed to μ agonists Furthermore, BC (3016) antagonized the immobility, trunk rigidity, catalepsy and analgesia induced by morphine In summary, the present results reveal that BC (3016) displays a profile of neurobehavioral effects similar to that of well known kappa agonists

BC (3016) Analgesia Kappa receptors Morphinan Mu receptors Nociception Aversive stimuli

SEVERAL years ago a novel opioid alkaloid of the morphinan class, 14-β-methyl 8-oxacyclorphan [BC (3016)], was synthesized and shown to be inactive as an analgesic in various animal species submitted to noxious thermal stimuli (9). However, the compound was found to be a very potent analgesic in the mouse acetic acid writhing test. Recently, our group has examined the ability of BC (3016) to depress the electrically evoked contractions of the guinea pig ileum and of the mouse vas deferens as well as to compete with the binding of selective ligands for the κ, μ and δ receptors in rat brain and guinea pig cerebellum membrane preparations (10). Together, the results of that study clearly indicated that BC (3016) has a high affinity for the kappa receptors and that its profile of pharmacological activity resembles that of other well known κ agonists as well as that of dynorphin-A (1–13), a putative endogenous κ receptor ligand (3).

In view of these results, the purpose of the present experiment was to determine if the in vivo neurobehavioral effects of BC (3016) would reflect the κ agonist activity of the compound found in our in vitro study. It is well known that typical kappa agonists, when injected centrally, are weak analgesic agents in nociception tests using aversive thermal stimuli but are very potent in reducing animals' reactivity to pain induced by chemical and mechanical stimuli such as those used in the acetic acid writhing and tail pinch tests respectively (15). On the other hand, kappa agonists do decrease nociception to thermal stimuli following spinal administration where a high density of κ receptors are located (1,5). In addition, while typical μ agonists such as morphine reduce body temperature (19), induce catalepsy (19), and muscle rigidity (12), κ agonists do not induce these effects. Furthermore, evidence has accumulated recently indicating that κ agonists can atagonize several pharmacological effects of typical μ agonists (2, 11, 16).

In the present experiment we examined the effects of intracisternal administration of various doses of BC (3016) on body temperature, muscular tone, nociception to a thermal stimulus as well as its ability to induce catalepsy The effects of intrathecal administration of the compound on the reactivity of animals to a thermal stimulus were also assessed. When significant effects were found, the ability of pretreatment with naloxone to antagonize these effects was evaluated. Finally, the effects of pretreatment

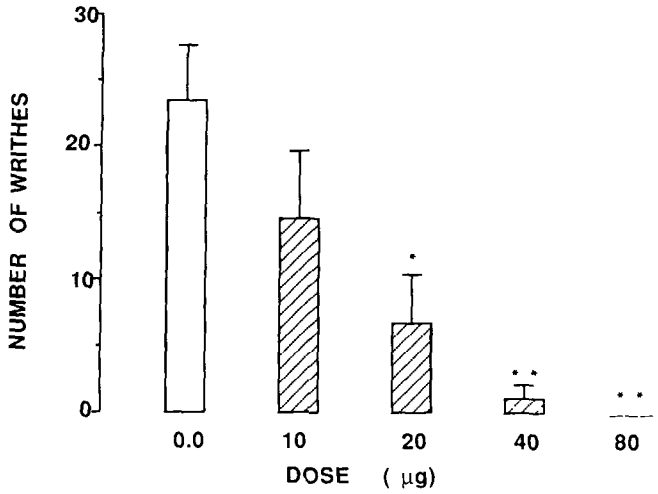

FIG 1 Effects of intracisternal administration of BC (3016) on number of writhes presented as a function of dose

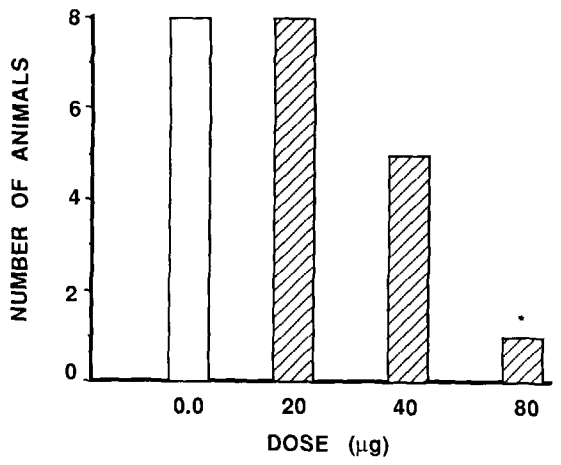

FIG 2 Effects of intracisternal administration of BC (3016) on number of animals in each group (n = 8) displaying normal nociception in the tail pinch test presented as a function of dose

with various doses of BC (3016) on immobility, trunk rigidity, catalepsy and analgesia induced by morphine were examined.

METHOD

Animals

Male hooded rats obtained from Canadian Breeding Farm (St. Constant, Québec) and weighing between 250–300 g were used They were housed in a temperature-controlled room having a 12-h light/dark cycle. Under ketamine/xylazine anesthesia, animals were implanted with indwelling polyethylene catheters (PE 10). For intracisternal administration, a hole was drilled in the middle of the posterior cranial suture and a 1 cm catheter was lowered into the cisterna magna. For intrathecal injections, a 7.5 cm catheter was inserted through a slit in the cisternal membrane, and pushed gently to reach the lumber subarachnoid space. For both types of cannulation, tubing was fixed to the skull with dental cement and protective 30-ga metal stylets were placed at the extremities of the catheters.

Procedure

Following at least four days of recovery from surgery, five groups of animals (n = 8) received an intracisternal injection of either 0.9% NaCl, 10, 20, 40 or 80 μg BC (3016). Immediately prior to, and at 6, 12, 18, 24 and 30 min following injections, animals were submitted to a battery of neurobehavioral tests consisting of measurements of body temperature, muscular tone, catalepsy and reactivity to various noxious stimuli. Most of these tests have been described in detail elsewhere (6,7). Briefly, body temperatures were recorded by means of a thermistor probe (Yellow Springs Instruments) inserted approximately 4 cm in the rectum. For muscular tone, animals were suspended by their front paws grasping a metal rod (0 5 cm diameter) which was held by the experimenter about 50 cm above a table. The time the animal remained on the bar (maximum 60 s) was recorded. A prolonged grasping response has been correlated with direct measures of muscle rigidity (14). Also, animals were held by the hind legs and held upright for a period of 5 s. The presence of rigidity was recorded if an animal kept its trunk in the upright position during this period. Trunk rigidity has been shown to be a good index of muscular rigidity induced by μ agonists (12). For catalepsy, an animal's front paws were placed on a wooden bar (1 cm in width) suspended 10 cm over the table. The time spent in that position, up to a maximum of 60 s, was recorded.

Nociception was assessed using the tail immersion and the tail pinch tests. The tail immersion test we used is a variant of a procedure previously described (4): the animal held by the experimenter was lowered so that its tail would be immersed in a bath of water maintained at a constant temperature of 49°C. The time for the nociceptive response (tail flick or whole body jerk) to occur was noted. In the tail pinch procedure, mild pressure was applied to an animal's tail by means of a pencil. A normal response was recorded if, within 5 s, the animal either jumped, vocalized, struggled or turned to bite the pencil

Five additional groups received the same intracisternal injection treatments and five min later were administered 5 ml/kg of a 1% acetic acid solution intraperitoneally Number of writhes observed during the following 30-min period were recorded To assess the effects of intrathecal administration of BC (3016) on nociception of thermal stimuli, five other groups of animals received either 0.9% NaCl or one of the same drug doses via this route of administration and subjected to the tail immersion test. For all the above neurobehavioral tests, experimenters were unaware of the injection treatments

When significant changes were found, the effects of pretreatment with 2.5 mg/kg naloxone were evaluated. The antagonist was given subcutaneously, 30 min prior to intracisternal or intrathecal injections

To assess possible μ antagonist properties of BC (3016), separate groups were administered intracisternally 0.9% NaCl or various doses of the compound 15 min prior to the intracisternal injection of 60 μg morphine. An additional group of control animals were given two intracisternal injections of 0.9% NaCl. Catalepsy, trunk rigidity, nociception in the tail immersion test were then measured, as described above, at 15-min intervals for 1 h following morphine administration. Immobility was also evaluated by recording the time animals would leave a 30 by 30 cm square.

BC (3016) was synthesized according to a previously reported procedure (9). Naloxone hydrochloride was generously provided by Endo Laboratories of Canada. All drugs were dissolved in 0.9% NaCl solutions. Volumes of both intracisternal and in-

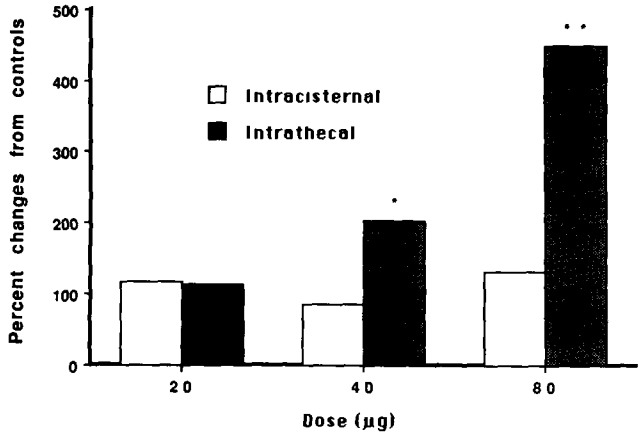

FIG 3 Percent changes from respective controls for intracisternal (open columns) and intrathecal (shaded columns) administration of BC (3016) presented as a function of dose

trathecal injections were 10 µl administered over a 30-s period by means of a 50 µl Hamilton syringe. Naloxone was injected in a volume of 1 ml/kg.

Data Analysis

Data obtained on body temperature, grasping time and catalepsy as well as in the tail immersion test were analyzed by means of individual two-way ANOVA's for repeated measures on one factor (17). Factors included in each analysis were groups and test periods. Significant group by test period interactions were further analyzed by simple main effect analyses at each level of each factor. Differences between control and treated groups at each test period as well as within group differences between baseline and each postinjection test period were assessed by Dunnett tests. Total number of writhes obtained following intracisternal injections were analyzed by a single factor ANOVA Data collected in the trunk rigidity and tail pinch tests constituted nonparametric data and were analyzed by Fisher exact probability tests (13).

RESULTS

Analysis of the data of animals administered various doses of

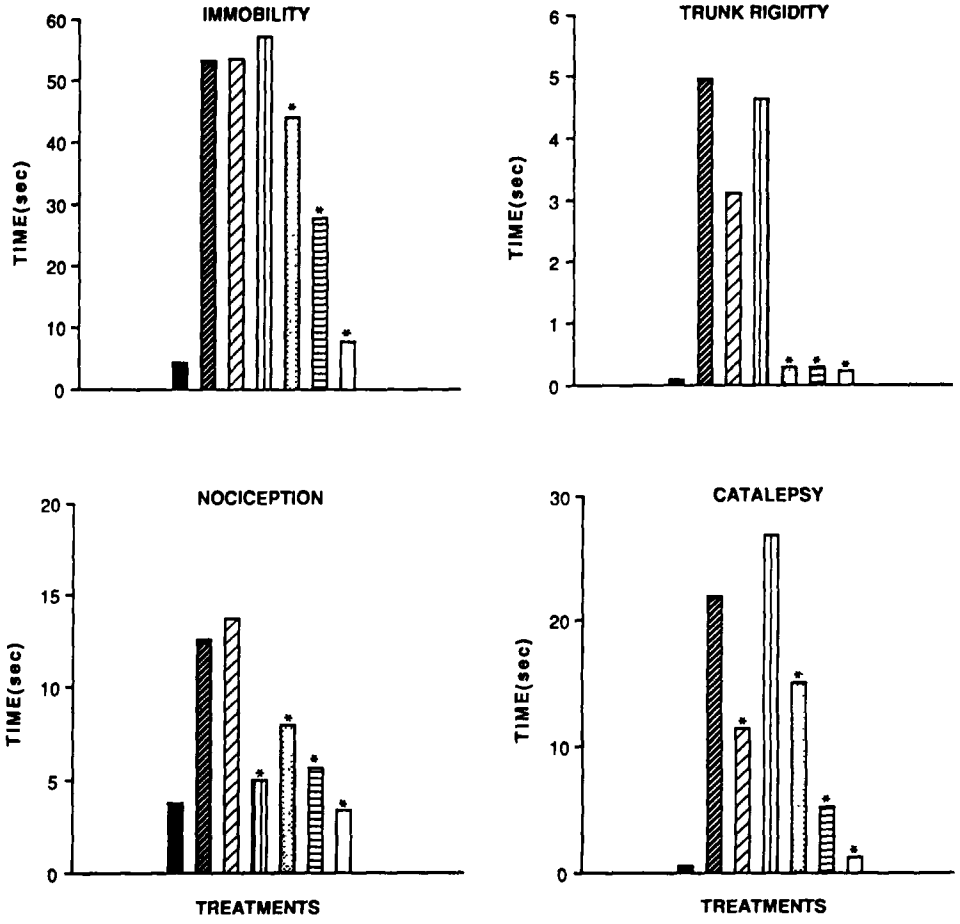

FIG 4 Effects of BC (3016) on morphine-induced immobility, trunk rigidity, nociception and catalepsy. Data obtained with control animals receiving two injections of 0.9% NaCl are illustrated by the solid column. Effects of the following treatments prior to the administration of morphine (60 µg) are illustrated by columns 0 9% NaCl (solid hatched) or 10 (open hatched), 20 (vertical stripes), 40 (dotted), 80 (horizontal striped) and 120 µg (open) of BC (3016)

BC (3016) intracisternally revealed no significant changes in body temperature, catalepsy or grasping response time. Also, the nociceptive response time in the tail immersion test was not significantly affected by BC (3016). On the other hand, starting with 20 μg of the compound, total number of writhes in the acetic acid test was significantly decreased and totally abolished with the largest dose administered. These effects are shown in Fig. 1 where total number of writhes after intracisternal injections are presented as a function of dose. Also, a significant reduction in number of animals displaying normal nociceptive responses in the tail pinch test was found with 80 μg of BC (3016). This is illustrated in Fig. 2 where numbers of animals in each group (n = 8) displaying normal responses are presented as a function of dose

Following intrathecal injections, a significant analgesic effect in the tail immersion test was found first with the 40 μg dose of BC (3016). The effect was already present at 12 min, maximum at 18 min, and dissipated slightly at 24 and 30 min following injections. A more pronounced analgesic effect was found with the largest (80 μg) dose although the temporal course of effects was similar to that seen following 40 μg A direct comparison of the effects of intracisternal and intrathecal injections in the tail immersion test can be seen in Fig. 3 where percent changes from respective controls are presented as a function of dose. None of the above significant effects were altered significantly by naloxone.

Finally, pretreatment with BC (3016) significantly antagonized all neurobehavioral effects of morphine in a dose-related fashion. Immobility, trunk rigidity, analgesia and catalepsy were inhibited by BC (3016), starting at doses of 80, 40, 20 and 40 μg respectively. These results are illustrated in Fig 4 where the dose-related effects of BC (3016) on each morphine-induced behavior are presented.

DISCUSSION

The results of the present in vivo study confirm and extend our previous observations that BC (3016) possesses kappa properties in in vitro preparations (10). The in vivo kappa agonist activity of BC (3016) is eloquently revealed by the analgesic characteristics of the compound, in terms of both the types of noxious stimuli and the routes of administration. Central administration of the compound failed to affect nociception to a thermal stimulus but significantly reduced reactivity of animals subjected to chemical or mechanical stimuli (Figs. 1 and 2). On the other hand, spinal administration of BC (3016) markedly decreased nociception to a thermal stimulus. As mentioned above this profile of analgesic properties is typical of kappa agonists (1, 5, 15).

The in vivo neuropharmacological effects of BC (3016) appear to be kappa selective since the drug did not decrease body temperature, increase muscular tone or induce catalepsy, three effects generally attributed to μ agonists (12,19). It is noteworthy that in our previous in vitro study, BC (3016) displayed good affinity for μ receptor. Since we did not find μ agonistic activity in our in vivo study, the binding of BC (3016) might indicate that the compound is a μ antagonist. Indeed, in the first study on BC (3016) it was observed that the drug antagonized the effects of the μ agonist oxymorphone in the mouse Straub tail test (9)

Naloxone did not reverse any of the analgesic effects of BC (3016) obtained in this study. That naloxone did not affect the analgesic effect of BC (3016) in the writhing test is not surprising since the administration of naloxone has been shown to actually increase number of writhes in this test (8). However, naloxone has been shown to be effective in antagonizing kappa agonist-induced analgesia in other tests (18). It is possible that the experimental procedures, in terms of dose and route of administration, utilized in the present study account for the lack of effect of naloxone. In that respect it has been shown that systemic injection of similar doses of naloxone did not affect the analgesic effect of intrathecally administered dynorphin-A (1–13), a putative endogenous kappa agonist (5). However, intrathecal administration of the antagonist markedly attenuated dynorphin-induced analgesia. Therefore, it remains to be determined if pretreatment with intracisternal or intrathecal administration of naloxone would alter the analgesic effects of BC (3016) that we obtained after injections via these two routes.

Finally, the intracisternal administration of 80 μg BC (3016) significantly decreased the immobility, catalepsy, muscular rigidity, and the analgesia induced by 60 μg of morphine. Thus BC (3016) displays, similarly to known kappa agonists, the ability to antagonize μ agonist actions (2, 11, 16).

In summary, the present results reveal that BC (3016) displays a profile of neuropharmacological effects similar to that of well known kappa agonists.

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REFERENCES

1 Attali, B , Gouarderes, H , Mazarguil, H , Audigier, Y , Cross, J Differential interaction of opiates to multiple "kappa" binding sites in guinea pig lumbosacral spinal cord Life Sci. 31 1371–1375, 1982
2 Friedman, H J , Jen, M , Chang, J K., Lee, N M , Loh, H H Dynorphin a possible modulatory peptide on morphine or B-endorphin analgesia in mouse Eur J Pharmacol 69 357–360, 1981
3 Izumi, K , Munekata, E , Yamamoto, H , Nakanishi, T , Barbeau, A Effects of taurine and α-aminobutyric on akinesia and analgesia induced by d-ala-2-met-enkephalinamide in rats Neuropharmacology, in press, 1989
4 Janssen, P A J , Niemegeers, C. J E , Diny, J G H The inhibitory effect of fentanyl and other morphine-like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittelforschung 13 502–507, 1963
5 Jhamandas, K , Sutak, M , Lemaire, S Comparative spinal analgesic action of dynorphin-(1–8), dynorphin-(1–13) and a kappa receptor agonist, U50,488 Can J Physiol Pharmacol 64 263–268, 1986
6 Jolicoeur, F B , Barbeau, A , Rioux, F , Quirion, R , St-Pierre, S Differential neurobehavioral effects of neurotensin and structural analogues Peptides 2 171–175, 1981
7 Jolicoeur, F B , Rondeau, D B , Hamel, E , Butterworth, R F , Barbeau, A Measurement of ataxia and related neurological signs in the laboratory rat Can J Neurol Sci 6 209–215, 1979
8. Kokka, N , Fairhurst, A. S Naloxone enhancement of acetic acid-induced writhing in rats. Life Sci 21 975–980, 1977
9. Lambert, Y , David, J P , Monkovic, I Analgesics and narcotic antagonists in the benzomorphan and 8-oxamorphinan series J Med Chem 21 423, 1978
10 Lemaire, S , Dumont, M , Jolicoeur, F B , Belleau, B 14-β-methyl-8-oxacyclorphan (BC-3016), a morphinan derivative with high affinity for kappa opioid receptor comparison with dynorphin-A (1–13) Can J Physiol Pharmacol 64 707–711, 1986
11 Porreca, F , Tortella, F C Differential antagonism of mu agonists by U50,488 H in the rat Life Sci 41 2511–2516, 1987.
12 Rackham, A Opiate-induced muscle rigidity in the rat· Effects of centrally acting agents. Neuropharmacology 19 855–859, 1980
13. Siegel, S Nonparametric statistics for the behavioral sciences New York McGraw-Hill Series in Psychology, 1956
14. Steg, G Efferent muscle innervation and rigidity Acta Physiol Scand 61(Suppl. 225) 5–53, 1964
15 Tyers, M B A classification of opiate receptors that mediate antinociception in animals Br J Pharmacol 69 503–512, 1980

16 VonVoigtlander, P F , Lahti, R A , Ludens, J H U-50,488 A selective and structurally novel non-mu (kappa) opioid agonist J Pharmacol. Exp Ther 224 7–12, 1983
17 Winer, B J Statistical principles in experimental design New York McGraw-Hill Book Company, 1971
18 Wood, P L Multiple opiate receptors Support for unique mu, delta and kappa sites. Neuropharmacology 21 487–497, 1982
19 Wood, P L. N-tetrahydrofurfuryl benzomorphans Comparison of a kappa and an agonist/antagonist analgesic Neuropeptides 5 323–326, 1985