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Spinal Paralysis and Catalepsy Induced by Intrathecal Injection of Opioid Agonists

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FRENK, H., J. MILLER, J. N. JOHANNESSEN AND D. J. MAYER. *Spinal paralysis and catalepsy induced by intrathecal injection of opioid agonists.* PHARMACOL BIOCHEM BEHAV **36**(2) 243–247, 1990.—The intrathecal administration of high (1.05 μmol) doses of D-Ala^2-Met^5-enkephalinamide (DAMA), D-Ala^2-Leu^5-enkephalinamide (DADLE), Try-D-Thr-Gly-Phe-Leu-Thr, MR2034-TA, dextrorphan tartrate, U50,488H, levorphanol tartrate, methadone hydrochloride, and 1-methyl-4-phenyl-4-propionoxy-piperidine induced spinal hypokinesia. The first 5 of these compounds caused spinal paralysis, whereas the other compounds and lower doses of the first 4 induced waxy catalepsy that was restricted to the hindquarters of rats. The paralysis induced by DAMA was not reversible by IT injections of 50 μg naltrexone, indicating, together with the paralytic effects of dextrorphan, that traditional opiate receptors are not involved in this behavioral effect. The spinal catalepsy induced by 0.26 μmol of DAMA was prevented by IT pretreatment with 10 μg of naltrexone. In view of this finding and the observation that spinal catalepsy can be induced by agonists of all opiate receptor classes, it seems likely that spinal catalepsy is produced by activation of specific opiate receptors, although the subtype remains to be established.

Opioids Multiple opiate receptor agonists Spinal paralysis Spinal catalepsy Hypokinesia Naltrexone

OPIATES have long been known to induce akinesia, catalepsy, or catatonia following systemic or intracranial administration to the rat (9). Recently it has been demonstrated that the intrathecal (IT) administration of high doses of dynorphin (10,21), D-Ala^2-D-Leu^5-enkephalin (24), D-Ala^2-Met^5-enkephalinamide (DADLE), methadone, but not morphine sulfate (6,28) induce akinesia in the hindbody of the rat. This manifestation of akinesia varies between total inability of the hind paws to support the body, or "paralysis" (6, 10, 21, 24, 28) to less severe "catalepsy," in which the hind paws support the body but can be made to assume bizarre postures that are maintained for prolonged periods (6,28).

The effects of opiate antagonists on these opiate-induced behavior patterns are controversial. Naloxone has been reported to reverse the paralysis induced by dynorphin (21) whereas others observed no effect of naloxone on paralysis induced by either dynorphin (10), or D-Ala^2-D-Leu^5-enkephalin (24). Naltrexone partially reversed catalepsy induced by other opiate agonists (28), suggesting that the motor impairment following high doses of IT opioids is a mixed opioid/nonopioid effect. However, the questions pertaining to the subtype(s) of opiate receptor involved and the stereospecificity of the effect are as yet unanswered, since no systematic comparison of high affinity agonists for different opiate receptors has been attempted. In fact, no systematic study exists demonstrating that opiate agonists other than those mentioned above are able to elicit spinal hypokinesia. The present study addresses these questions.

METHOD

Subjects

Adult male Sprague-Dawley rats (Harlan) weighing 350–500 g at the time of surgery were used in these experiments. Throughout the experiments animals were individually housed in stainless steel cages with a grid floor. Water and food pellets were available ad lib. The room was artificially lighted (light on from 0700 to 1900 hr).

Surgical Techniques and Drugs

All surgery was performed in animals anesthetized with 50 mg/kg sodium pentobarbital intraperitoneally (IP), supplemented with Metofane. To implant intrathecal catheters, the cisterna magna was exposed and incised. Saline-filled sterile polyethylene tubing (PE 10) was then inserted into the subarachnoid space and gently threaded 8.5 cm caudally such that the cut end of the catheter lay at the lumbosacral enlargement. The PE tubing was plugged at the rostral end with a sterile piece of 30-gauge wire and secured by flowing dental acrylic around the IT catheter and a

skull screw. All animals were treated postoperatively with gentamicin, as required. Five of the animals were also implanted with EEG recording cortical electrodes. The electrodes consisted of 4 stainless steel jeweler's screws soldered to stainless steel wire which was insulated with Teflon except at the tips. The screws were attached by means of the wire to connector pins (Amphenol). The screws were threaded into the skull bilaterally overlying the prefrontal and occipital cortex. The connector pins were then snapped into a nylon connector plug (Amphenol), and the whole assembly cemented to the skull with dental acrylic.

Drugs injected IT were selected according to their reported affinity to subtypes of opiate receptors. The agonists with a preferential affinity for mu-receptors were levorphanol tartrate (29), methadone hydrochloride (29), and 1-methyl-4-phenyl-4-propionoxypiperidine (11) (MPPP). Agonists with selective affinity for kappa-receptors were MR2034-TA (17), U50,488H (12) and ethylketocyclazocine (20) (EKC). Agonists with high affinity to both mu- and delta-receptors were D-Ala^2-Met^5-enkephalinamide (DAMA) and D-Ala^2-Leu^5-enkephalinamide (7) (DADLE). For a more specific delta-agonist, Try-D-Thr-Gly-Phe-Leu-Thr (30) (DTLET) was chosen. All agonists but ethylketocyclazocine were injected at doses of 0.066, 0.26, and 1.05 μmol. Ethylketocyclazocine, because of its low solubility, was only injected at the 0.066 μmol dose. Because all doses were injected IT at equimolar doses, the 0.066, 0.26, and 1.05 μmol doses shall be called Low, Intermediate, and High dose, respectively, throughout the remainder of the experiments. The animals receiving the High dose of DAMA were implanted with cortical recording electrodes. In addition to the agonists listed above, other animals were injected IT with dextrorphan tartrate or sulfated leucine-enkephalin (26) [0.066, 0.26, or 1.05 μmol hydrochloride (1.05 μmol)] or 20 μl of physiological saline. All of the drugs listed above were injected in a volume of 20 μl of physiological saline, and every dose group consisted of 6 animals. Where antagonism with naltrexone hydrochloride was attempted, this drug was administered IT in doses of 10 or 50 μg in 20 μl of saline, 10 min prior to DAMA.

Microinjection Technique

One week following surgery, animals were tested for motor impairment prior to injection. Those animals that showed any deficit in normal locomotion, or other signs of neuronal damage due to implantation, were discarded. Animals were then restrained in a Plexiglas tube which had an opening in the top through which the exterior end of the IT catheter protruded. This allowed for IT drug delivery without handling the animals. Animals were then injected with one of the treatment drugs or saline, and the catheter was flushed with 10 μl of saline (void volume of the catheter). Injections were delivered over 35–45 sec. Immediately following IT injection, animals were returned to their home cages, and a 30-min test period followed.

Behavioral Assessments

All animals were tested for the occurrence of analgesia, catalepsy and catatonia every min during the first 5 min, at 7.5 min, and 10 min following the IT injection. Analgesia was judged present if no vocalization, orientation, or struggling was observed in response to severe manual pinch. "Paralysis" was judged present in the hindbody of the animals if 1) the animals were unable to support this part of the body with their legs, 2) did not use their hind paws when walking, and 3) the hindbody was completely flaccid. Hindbody catalepsy was judged present if 1) the hindquarters of the animals could be manually rotated so that both hind paws lost contact with the grid floor and the animals

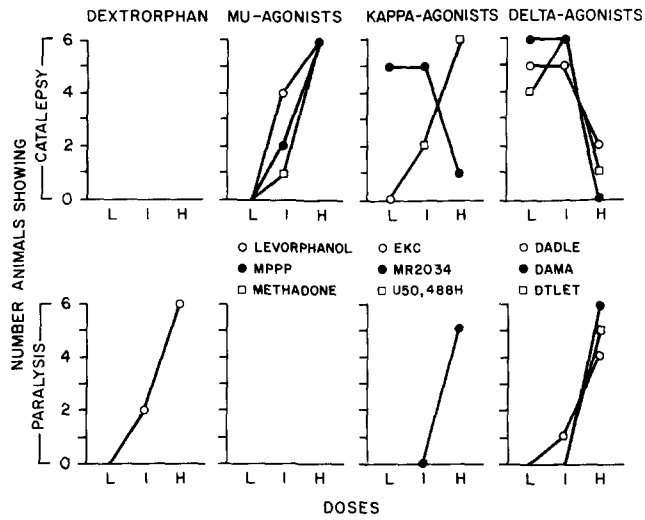

FIG. 1. Number of animals (n = 6) showing spinal catalepsy (top row) and paralysis (bottom row) following IT dextrorphan, or mu, kappa, and delta opiate agonists in doses of 0.066 (L), 0.26 (M) or 1.05 (H) μmol. For abbreviations, see text. Note that the reduction in animals showing catalepsy at the highest dose (C-D) is due to the increase in incidence of paralysis (G-H).

passively remained in that position for 1 min, or 2) the hind limbs of the animals could be manually flexed or extended completely without resistance from the animals. Catatonia was judged present in a part of the body if that region showed no spontaneous movement and remained rigid when attempts at flexion or extension were made. Latencies until the development of analgesia, catalepsy, catatonia, and paralysis were reported in the next section as mean and standard deviation.

RESULTS

The injection of nearly all opiate agonists examined in the present studies was followed by an impairment of motor behavior in a dose-related fashion.

Saline, Naltrexone, Sulfated Methionine-Enkephalin and Dextrorphan

Whereas the injection of 10 μl of saline, 1.05 μmol of naltrexone or sulfated methionine-enkephalin, or the lowest dose of dextrorphan did not alter hindbody motor behavior, the two highest doses of dextrorphan did (Fig. 1). With the High dose of dextrorphan, spinal paralysis was observed after 4.8 ± 2.8 min, and lasted for the remainder of the observation period. In none of these animals did either catalepsy or tail pinch analgesia occur.

Methadone, MPPP, and Levorphanol

None of the animals injected with the mu-receptor agonists displayed hindbody paralysis. However, the High dose of methadone, MPPP, and levorphanol induced hindbody catalepsy in all animals at 1.0 ± 0.0, 4.8 ± 2.6, and 3.1 ± 2.3 min, respectively. Whereas some animals injected with the Intermediate dose of these three substances displayed hindbody catalepsy, none of the Low dose groups showed any motor impairments (Fig. 1).

Analgesia was obtained in all animals injected with the High dose of methadone and MPPP, starting 1.2 ± 0.4 and 2.5 ± 3.7

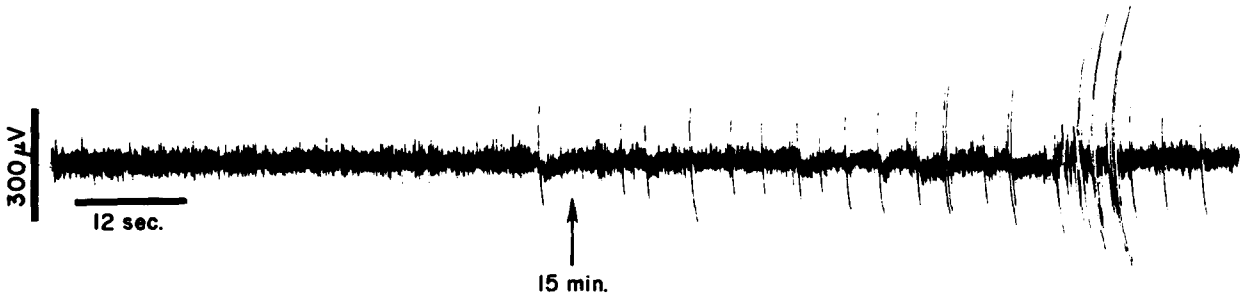

FIG. 2. Effect of the High (1.05 μmol) dose of DAMA on cortical EEG. Spikes appear at about 15 min following the IT injection demonstrating that the drug diffused to supraspinal areas.

min, respectively, following the injection, and in none of the other dose groups of these substances. Surprisingly, tail pinch analgesia was only noted in one of the animals injected with the High dose of levorphanol, and in none of the others.

MR2034-TA, U50,488H, and Ethylketocyclazocine

Unlike the agonists with affinity for the mu-receptor, the High dose of MR2034-TA induced paralysis in the hindbody of all rats starting 1.8 ± 1.2 min postinjection. This condition remained unchanged in all but one animal throughout the observation period. Animals did not react to tail pinch with motor responses or vocalization.

Paralysis was not observed in the Intermediate dose group of MR2034-TA, nor in animals injected with the three doses of U50,488H (Fig. 1). All animals in the MR 2034-TA Intermediate dose group, and in the U50,488H High dose group showed hindbody catalepsy starting 4.3 ± 3.2 and 2.2 ± 0.4 min, respectively, postinjection. Catalepsy was also observed in some animals injected with the Intermediate dose of U50,488H. No motor changes were observed in any of the animals injected with low doses of either MR2034-TA, U50,488H, or ethylketocyclazocine. Analgesia to tail pinch was not observed in any animal injected with the kappa-receptor agonists in the Intermediate and Low dose groups.

DAMA, DADLE, and DTLET

DAMA, DADLE, and DTLET produced similar effects. Thus, at the High dose paralysis was induced in all animals by DAMA (latency 1.5 ± 0.8 min), in 4 of 6 animals by DADLE (latency 6.2 ± 3.0 min), and in 5 of 6 animals by DTLET (latency 6.2 ± 3.0 min) and lasted until the end of the test period. In those animals where paralysis did not follow the IT injection within one min, catalepsy preceded this effect.

The DAMA High dose group was monitored for changes in cortical EEG associated with spinal motor effects. EEG spikes were recorded in all animals, but were not concurrent with motor changes. Thus, cortical spikes (Fig. 2) started well after the onset of paralysis, and after the conclusion of the 10-min behavioral test period in all animals (spiking latency 14.0 ± 3.1 min). Concomitant with the EEG spikes, rigid forebody catatonia developed in all animals. In 2 of these animals this catatonia spread over the hind limbs as well, replacing the flaccid paralysis with a rigid extension of the hind paws. In the remaining animals, the posterior portion of the body remained flaccid in spite of the pronounced catatonia in forelimbs and head. This situation persisted for one hr after which observation was discontinued.

Hindbody catalepsy followed the administration of the Intermediate doses of DAMA, DADLE, and DTLET in all animals (latencies were 4.0 ± 6.3, 3.9 ± 1.9, and 4.1 ± 2.1 min, respectively). For one DTLET-treated animal this catalepsy preceded paralysis. Hindbody catalepsy occurred in all animals following the Low doses of DAMA and DADLE (latencies were 9.5 ± 1.0 and 6.2 ± 3.0 min, respectively). The Low dose of DTLET induced hindbody catalepsy in 4 and no motor changes in 2 animals.

In those animals where paralysis did not appear within the first min following the injection of DAMA, DADLE, and DTLET, analgesia was observed (latencies 1.0 ± 0.0, 3.0 ± 1.7, and 2.2 ± 0.4 min, respectively). All animals injected with the Intermediate dose of DAMA, DADLE, and DTLET (latencies 1.2 ± 0.4, 3.1 ± 3.4, and 2.3 ± 1.2 min, respectively) and Low dose (latencies 4.5 ± 2.7, 3.7 ± 3.1, and 6.0 ± 3.0 min, respectively) were analgesic until the end of the observation period.

Comparison of Cataleptic Effects at Different Doses

Qualitative and quantitative differences were apparent between the mu-, delta-, and kappa-agonists at different dose levels. Whereas a dose-response relationship was established for nearly all opiate agonists utilized (Fig. 1), only the delta-agonists and one kappa-agonist elicited paralysis at the High dose.

When the effects of the Low dose were compared, it became

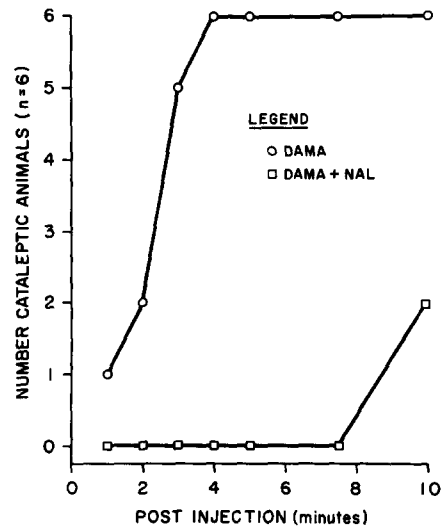

FIG. 3. Pretreatment with 10 μg IT of naltrexone (NAL) blocks the spinal catalepsy induced by the Medium (0.26 μmol) dose of DAMA injected via the same route ($p<0.01$).

evident that the enhanced potency of the delta-agonists in causing motor impairment existed also at this dose level. Thus, more DAMA- and DADLE-, but not DTLET-treated animals (Fisher Exact Probability Test, two-tailed, $p<0.002$, $p<0.017$, and $p<0.078$, respectively) (25) showed catalepsy without paralysis than animals treated with mu-agonists, U50,488H, and control substances. MR2034-TA, but not U50,488H, was significantly more potent at this dose than the mu-agonists and control substances ($p<0.017$).

Antagonism With IT Naltrexone

Animals injected with either the High or the Intermediate dose of DAMA were pretreated either with saline, or with naltrexone IT in an attempt to block paralysis or spinal catalepsy. Naltrexone (50 μg) pretreatment 10 min prior to the IT administration of the High dose of DAMA was ineffective in preventing the development of paralysis. By contrast, naltrexone (10 μg, IT) potently reduced the incidence of catalepsy induced by the Intermediate dose of DAMA (Fig. 3).

DISCUSSION

Nine of the ten mu, delta, and kappa opiate receptor agonists injected IT in the present experiments produced motor impairments that were restricted to the hindquarters of rats. The only opioid (ethylketocyclazocine) that did not produce these motor effects could only be administered at the lowest molar dose because of problems of solubility.

A strong case can be made that the cataleptic effects observed following lower doses of the kappa- or delta-receptor agonists, or the highest dose of all the other agonists can be ascribed at least in part, to specific opiate receptors, thus confirming similar previous observations (28). Spinal catalepsy was elicited by opiates with both the peptide and the nonpeptide structure, but not following the administration of high doses of the sulfated enkephalin or naltrexone, suggesting that the effect is not peculiar to any one chemical class but common to all opiates. Furthermore, opiate-induced spinal catalepsy was stereospecific, being elicited by levorphanol but not dextrorphan. Finally, the cataleptic effects of DAMA were virtually eliminated by low doses of intrathecal naltrexone.

Our data are less conclusive in regard to the opiate receptor type that mediates spinal catalepsy. At the lowest dose used (66 nmol), none of the mu-agonists produced this behavior, suggesting that the mu-receptor is not implicated in this behavior. On the basis of the observations that ethylketocyclazocine and U50,488H did not produce this behavior at this dose, whereas all three delta-agonists did, it is tempting to postulate a role for the delta-receptor in spinal catalepsy. However, MR2034TA, a potent kappa-agonist (15), produced pronounced spinal catalepsy at the Low dose, suggesting that either receptors of the kappa type are involved, or that this compound also has affinity for the delta-receptor.

The most severe hypokinesia consisted of a flaccid paralysis that has been previously observed following IT administration of dynorphin (10,21) and DAMA (6). In the present experiments paralysis was reliably induced by 1.05 μmol of DAMA, DADLE and DTLET [all delta-receptor agonists (5, 7, 30)] and the kappa-receptor agonist MR2034-TA (18).

The role of an opiate receptor in opiate-induced paralysis is by no means self-evident. Clearly the lack of protection by naltrexone pretreatment for high doses of DAMA in the present experiment and of the kappa-receptor agonist dynorphin or DADLE in others (10,24) together with our observation that dextrorphan also induces paralysis indicates that activation of traditional opiate receptors is not required. On the other hand, there seem to be certain structural requirements which suggest some degree of specificity. Sulfated enkephalin, which has no affinity for the opiate receptor (27), did not elicit any discernible behavioral change even at the highest dose. This indicates that paralysis in our study is not a nonspecific peptide effect nor attributable exclusively to the high concentrations of the compounds used.

Some evidence suggests that paralysis may be produced by activation of the sigma-receptor by some of the compounds used in the present study (16). Different opiates, particularly the benzomorphans (31), as well as the dissociative anesthetic PCP and its analogues (27) bind to this receptor. Yet, neither naloxone, naltrexone, morphine, nor etorphine bind to the sigma-receptor (8, 18, 31). Therefore, behavior mediated by this receptor type would be resistant to these opiate antagonists, and not likely to be produced by mu-receptor agonists. Such is the case for opiate-induced spinal paralysis. In addition, dextro-rotary opiate isomers, including dextrorphan, compete for sigma-receptors (18). Consistent with this, in our study, dextrorphan was relatively potent in producing paralysis.

Additional evidence for the involvement of the sigma-receptor derives from the finding that both benzomorphans with sigma-agonist action (2,13) and the dissociative anesthetics (1) inhibit N-methyl-D-aspartate (NMDA)-induced neuronal excitation in the spinal cord. Since there is no competitive binding between the dissociative anesthetics and NMDA agonists (13, 22, 31), they seem to bind to different receptors. It has therefore been suggested that the NMDA and sigma-receptors form a receptor-receptor complex (1, 15, 29). This has been confirmed by the finding that PCP binding is dependent on the NMDA agonist l-glutamate, and that this effect was reversible by the NMDA antagonist 2-amino-7-phosphonoheptanoate (14). Hence, it is possible that in the present study opiate agonists and particularly the dextroisomer dextrorphan block NMDAergic transmission by activating a sigma-receptor. Consistent with this hypothesis, the NMDA antagonist 2-amino-5-phosphonovalerate produces spinal paralysis upon IT administration (3,4) which is indistinguishable from that produced in the present study.

On the other hand, delta-receptor agonists, which are not known to bind to sigma-receptors, also induced strong paralysis. This effect was not mediated by traditional opiate-receptors, since it was not blocked by high doses of naltrexone. Although this observation does not invalidate a role for the sigma-receptor in paralysis when produced by other opiates, it suggests that behavioral indistinguishable paralysis may be produced by the delta-agonists through a different mechanism.

In addition to cataleptic and paralytic effects the present study also tested tail pinch analgesia. Tail pinch analgesia was only observed following the IT injection of the highest doses of methadone and MPPP. These results are consistent with previous studies (29), in which similar doses of methadone or 25 μg of IT morphine failed to induce tail pinch analgesia within 20 min. Interestingly, all delta-receptor agonists induced tail pinch analgesia at much lower doses. The higher analgesic potency of delta-receptor agonists as compared to mu-receptor agonists upon IT administration has been previously observed by others (24) on cutaneous-thermal elicited pain.

In conclusion, the present study demonstrates that hindbody paralysis can be elicited by the intrathecal injection of a variety of opioid peptides with affinities predominantly to delta- and possibly also kappa-receptors. This effect is not sensitive to opiate antagonists and is produced by dextrorphan. These observations indicate that this effect is likely to be mediated by other than the traditional opiate receptors. Spinal catalepsy, on the other hand, can be elicited by intrathecal injections of all classes of opiate agonists, both peptides and nonpeptides. Although high doses of

agonists are required to produce this effect, it is blocked by low doses of naltrexone. It seems likely that simultaneous activation of specific opiate receptors produce spinal catalepsy.

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