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BRIEF COMMUNICATION

Paradoxical Opiate Specific Paralytic Effects of High Doses of Intracerebroventricular Etorphine and Fentanyl in Rats

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LESHEM, M., H. FRENK, R C COGHILL AND D. J. MAYER *Paradoxical opiate specific paralytic effects of high doses of intracerebroventricular etorphine and fentanyl in rats* PHARMACOL BIOCHEM BEHAV **38**(2) 475–478, 1991 —Injections of high doses of etorphine (0.0625, 0 25, or 1 0 μmol) or equimolar fentanyl into the cerebral ventricles of rats induced a sequence of motor effects including catatonia, a novel flaccid paralysis, and recurrent catatonia These effects were dose related, naloxone reversible, and reveal an opiate specific organization of a central motor hierarchy

Paralytic effects Opiate Etorphine Fentanyl

OPIATES produce muscular rigidity (catatonia) upon systemic administration at high doses in rats. This behavior has been demonstrated to be mediated by opiate-specific receptors located in supraspinal loci within the central nervous system (2). In addition, opiates induce motor deficiencies in the rat which may be mediated by spinal substrates (1). Intrathecal (IT) administration of high doses of delta (7) or kappa receptor (3) agonists produces a loss of muscle tonus (flaccid paralysis) that is restricted to the hindbody and not reversible by specific opiate antagonists such as naloxone and naltrexone. At lower IT doses, the delta receptor agonists, or high doses of mu-receptor agonists, produce a waxy catalepsy in the hindbody which is reversed by naltrexone (1).

We have also found that IT administration of etorphine hydrochloride produces behavioral effects which are different from those produced by other opiates. Within 15 s of administration etorphine produces a full body catatonic stretch which persists for 1.0–1.5 min (1). This catatonic state is followed by a flaccid paralysis resembling that induced by the high doses of kappa and delta agonists, except that it involves the entire body. Since etorphine has been shown to produce catatonia from supraspinal loci (6) and the catatonic response to etorphine involves the upper body (neck and forepaws), the catatonia could be due to rapid diffusion of the IT etorphine to supraspinal loci.

To our knowledge, flaccid paralysis induced by high doses of etorphine has not been previously reported. The present experiments were designed to test whether this phenomenon is indeed of supraspinal origin and is a result of specific action of etorphine on opiate receptors.

METHOD

Subjects and Surgery

Male Sprague-Dawley rats (average weight 480 g), housed singly with water and food ad lib, were implanted with IT catheters or with cannulae in the anterior third ventricle of the brain under pentobarbital (50 mg/kg) anesthesia. ICV guide cannulae were made from 11 mm long, 22 ga stainless steel tubing. Cannulae were positioned stereotaxically 2 mm above the third ven-

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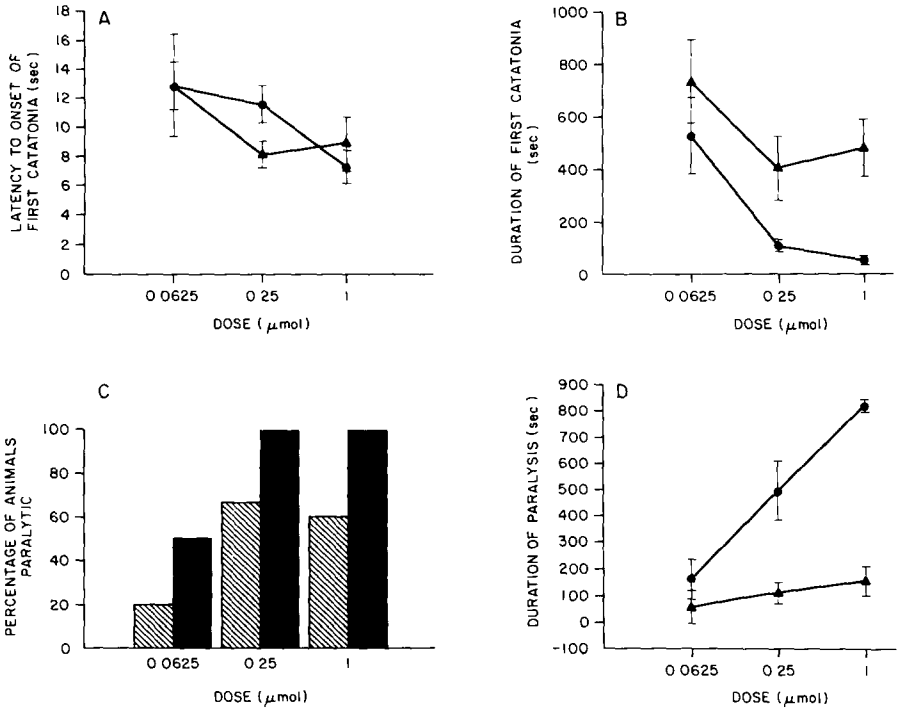

FIG 1 (A) Latencies to initial catatonia produced by etorphine (circles) and fentanyl (triangles) (B) Duration of this catatonia for both compounds Note that since catatonia in many animals is terminated by paralysis, and that more animals in the higher dose groups than in the lowest showed paralysis, a seemingly inverse dose response is obtained (C) Percentage of animals that became paralytic following etorphine (solid) and fentanyl (hatched) (D) Duration of paralysis for both compounds

tricle (AP +0.8, L 0, DV −4.2) (5) through a hole drilled directly above the target coordinates Each guide cannula was plugged with 27 ga stainless steel wire to prevent occlusion or infection The injection cannulae were made from 27 ga stainless steel tubing and were designed to protrude 2 mm out of the guide cannula when fully inserted. Proper positioning of the injection cannula was verified by dye injection.

To implant IT catheters, the cisterna magna was exposed and incised. Gentamycin-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 piece of 30 ga wire and secured by flowing dental acrylic around the IT catheter and a skull screw All animals were treated postoperatively with Combiotic to prevent infection

Drugs

Etorphine hydrochloride (0 0625, 0.25 or 1 μmol) or equimolar doses of fentanyl were dissolved in physiological saline. IT injections were delivered in 20 μl boluses over 30 s, and equivolume ICV injections were delivered over the same period. Naloxone (50 mg/ml/kg), fentanyl, or saline were injected intraperitoneally.

Experimental Design and Analysis

Seven days after surgery, each subject was removed from his home cage and randomly assigned to a treatment group Dose response studies were conducted using 3 doses of both etorphine and fentanyl that were injected via the ICV route To administer the drug, animals were manually restrained for the removal of the wire plug and insertion of the injector. Animals were then released on a flat raised platform (160 × 100 cm) and injected while freely moving. Onset of catatonia was operationally defined as a sudden loss of locomotor behavior, accompanied by the appearance of a Straub tail. Operational definition of the beginning of flaccid paralysis was a sudden relaxation of the straub tail Termination of the paralytic stage was delineated by a reemergence of catatonia, as evidenced by the recurrence of straub tail and muscular rigidity. Behavioral observation was terminated if an animal remained in the same behavioral phase longer than 900 s, except for the highest dose groups of fentanyl and etorphine which were monitored for 90 min following injection

To determine opioid specificity we injected IP naloxone or equivolume saline five minutes after the onset of flaccid paralysis induced by etorphine (1 μmol) Behavioral observation was terminated 90 min following injection of naloxone or saline, or when animals recovered normal locomotion, as was the case for naloxone-treated animals

Fentanyl (1 μmol) was injected IP to determine whether the effect was systemically or centrally mediated. In this experiment only initial catatonia was recorded.

Etorphine (1 μmol) was injected, either IT or ICV, to determine whether the paralytic effect was spinally or supraspinally mediated In this experiment, only latencies to initial catatonia and paralysis were recorded.

Latencies and durations of the various behavioral phases were analyzed separately using a 2 (drugs) by 3 (doses) analysis of variance (ANOVA). Single factor ANOVA was employed to compare IT and ICV etorphine and to assess the effect of naloxone Since not all animals in every experimental group became paralytic, the Fisher Exact Probability test was utilized to deter-

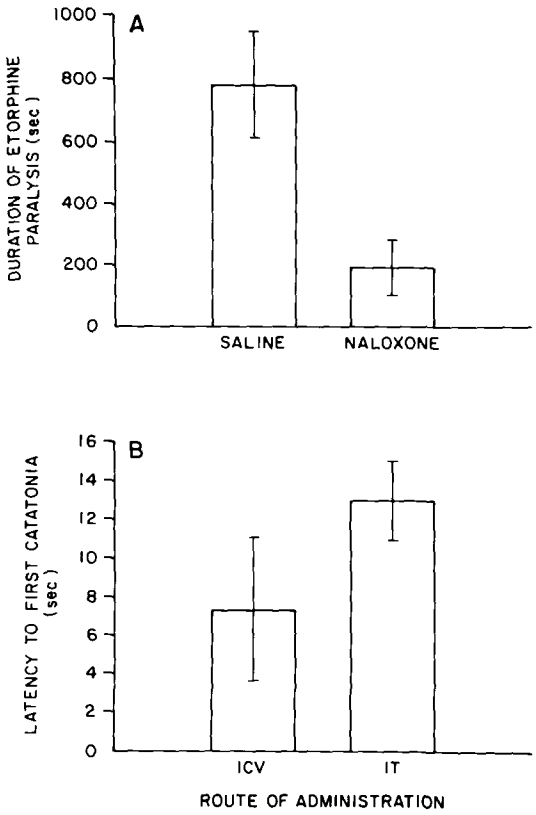

FIG 2 (A) Effect of naloxone on the duration of paralysis Saline and naloxone were administered five min following the onset of paralysis in these animals Duration of paralysis was measured following the injection of naloxone and its vehicle (B) Latencies to the onset of the first catatonia following ICV or IT administration of etorphine (1 μmol)

mine differences between doses in the number of animals reaching paralysis. Estimates of the variance in text and figure legends consist of the Standard Error of the Mean.

RESULTS

Comparison of Etorphine and Fentanyl

ICV injection of both etorphine and fentanyl produced a dramatic catatonic response. Within 20 s of administration, all animals suddenly stopped moving and displayed a straub tail, sometimes freezing in midstride. As Fig. 1A shows, both etorphine and fentanyl produced this response with no distinguishable differences in the time of onset, $F(1,46)=0.168$, n s. However, this latency was inversely proportional to dose, $F(2,46)=4.5$, $p<0.02$, such that high doses of either compound elicited catatonia more rapidly than the 2 lower doses. The duration of fentanyl-induced catatonia was longer than that produced by etorphine, $F(1,46)=14.5$, $p<0.0007$ (Fig. 1B). Additionally, the duration of catatonia induced by either fentanyl or etorphine was dose dependent such that high doses resulted in shorter catatonia, $F(2,46)=9.095$, $p<0.0007$.

During the first minutes of this catatonic stage, respiration was notably depressed, resulting in cyanosis in some animals in all dose groups, and in death of one animal at the highest dose of etorphine. Cyanosis disappeared and slow respiration returned in all remaining animals within five minutes.

The catatonic phase was abruptly terminated by the appearance of a flaccid paralysis in most but not all animals. This behavior was characterized by sudden dissipation of the muscular rigidity and loss of muscle tone. Despite this, normal ventilatory patterns returned. We analyzed the frequency of animals failing to show flaccid paralysis before the 900 s cutoff and found a significant effect of the treatments ($p<0.01$) (Fig. 1C). All animals receiving the two highest doses of etorphine became paralytic, while 50% of those in the lowest dose failed to display paralysis Fentanyl-treated groups showed less paralysis than animals receiving etorphine with 60% of the highest, 66% of the intermediate, and 20% of the lowest dose group displaying paralysis. The higher potency of etorphine was also reflected in its effects on the duration of paralysis Etorphine produced a paralysis of greater duration than that produced by fentanyl, $F(1,39)=23.9$, $p<0.001$, although paralysis produced by either compound was found to be directly dose dependent such that highest doses elicited a paralysis of longer duration, $F(2,39)=6.5$, $p<0$ 005, Fig. 1D. At the end of paralysis, all animals entered another pronounced catatonic phase which lasted for more than 90 min in all animals of the highest dose groups of both compounds

Naloxone Reversibility of Paralysis

Naloxone reversibility of the paralysis was attempted only with the highest dose of etorphine Administration of naloxone five minutes after the onset of the paralytic stage reliably reduced the duration of flaccid paralysis, $F(1,8)=11$ 9, $p<0.01$, Fig. 2A, when compared to saline-injected controls. Paralysis ended with the naloxone-treated animals showing a second vigorous catatonic response similar to the one immediately following the administration of etorphine. This catatonic response was short-lived in the case of naloxone-treated animals and ended in the spontaneous righting of these animals after 247 0 ± 218 s and a return of normal locomotion. Since none of the saline controls showed spontaneous righting within 90 min, this difference was significant ($p<0.00001$)

Peripheral Versus Central and Intrathecal Versus Intracerebroventricular Injections

To determine whether peripheral or central sites mediated behaviors induced by fentanyl, we compared the onset to catatonia elicited by IP and ICV injections of the compound. ICV administration of fentanyl produced catatonia 5 times more rapidly than via the IP route, $F(1,8)=59$ 0, $p<0$ 0001 IP fentanyl never produced paralysis. To determine whether spinal or supraspinal sites mediated behaviors induced by etorphine, we compared the onset of catatonia and paralysis elicited by IT and ICV administration. ICV administration of etorphine produced catatonia more rapidly than IT administration, $F(1,16)=11.8$, $p<0$ 005, Fig. 2B. There were no differences in the latency to paralysis between the IT and ICV injected groups.

DISCUSSION

Following ICV injection, the opiate agonists etorphine and fentanyl produced a behavioral sequence not, to our knowledge, previously reported. This sequence consists of a brief catatonia followed by a paralytic phase. The paralytic phase is followed by the reemergence of catatonia, which persists for at least 90 min.

These phenomena appear to be mediated by supraspinal structures. First, IP-administered fentanyl produced catatonia 5 times slower than via the ICV route and never produced paralysis, demonstrating that these behaviors are centrally mediated. Second, ICV administration of etorphine produced catatonia more rapidly than IT administration. Third, IT administration of etorphine pro-

duced whole body catatonia, followed by whole body paralysis, instead of behavioral changes that were restricted to the hindbody. Such catatonia has only been elicited by intracerebral injections (2). In addition, the spread of catatonia to the forelimbs and head following IT opiates was correlated with the occurrence of spikes in the cortical EEG (1), suggesting that the presence of opiates in the brain accompanies whole body catatonia. Although the latency to catatonia after IT etorphine was very short, the extremely lipid soluble nature of etorphine could account for quick diffusion to the brain.

As for mechanisms that produced opiate paralysis, it is unlikely that it was produced by the severe anoxia that occurred during the preceding catatonia. The duration of paralysis was inversely proportional to the duration of catatonia. Together with the observation that not all animals that became catatonic became paralytic, this strongly suggests that impairment of ventilation during catatonia was not responsible for paralysis.

There is strong evidence indicating that the paralysis produced by etorphine, in spite of the high doses necessary to obtain this effect, is mediated by specific opiate receptors. First, paralysis was also produced by another opiate agonist in the present study, namely fentanyl. Second, it was obtained in a dose-related fashion. Finally, it was fully reversible by naloxone. Together with our observation that opiate paralysis originates in brain sites, this suggests that specific supraspinal areas are involved in this phenomenon.

The most interesting observation in the present study was that injection of naloxone during paralysis did not prevent recurrence of catatonia. When paralytic animals were injected with naloxone, they became catatonic prior to righting spontaneously. The catatonic state was also always seen before paralysis occurred in all animals treated by either etorphine or fentanyl. It seems, therefore, that catatonia is a necessary transitory stage to and from paralysis; thus suggesting that a behavioral hierarchy exists such that the catatonia is superceded by the paralytic stage.

In conclusion, we report a novel, opiate-specific flaccid paralysis distinct from the known opiate-induced catatonia. This paralytic effect is produced supraspinally and mediated by specific opiate receptors. The specific anatomical sites at which these opiates act within the brain to produce paralysis remain unknown. Because fentanyl and related compounds are utilized at high doses for clinical anesthesia in man (4), our findings may allow refinement in the use of these compounds. For example, it is not clear whether catatonia or paralysis is produced by doses normally used in man. An increase or decrease in the dose may result in a more desirable motor state during surgical anesthesia in man

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