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Differential Motor Effects of Intraventricular Infusion of Morphine and Etonitazene

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SHIZGAL, P., L. S. SKLAR, Z. W. BROWN AND Z. AMIT. *Differential motor effects of intraventricular infusion of morphine and etonitazene.* PHARMAC. BIOCHEM. BEHAV. 6(1) 17–20, 1977. – The motor effects produced by intraventricular infusions of morphine were compared to the effects of etonitazene. Despite the similarity in the peripheral actions of these drugs, motor effects of central infusions differed dramatically. Intraventricular morphine infusions resulted in explosive motor behavior whereas etonitazene produced extreme muscular rigidity. The periaqueductal grey (PAG) has been proposed as the substrate of morphine-induced explosive motor behavior. However, considerations of the dose of morphine and the mobility of this drug in tissue suggest that sites other than the PAG may also be involved in explosive motor behavior.

Morphine Etonitazene Motor effects Periaqueductal grey

DESPITE the fact that morphine appears to be reinforcing both when administered intravenously and intraventricularly, other effects of the drug appear to depend strongly on whether it is injected into the brain or the periphery. One such effect, violently exaggerated motor activity, has been obtained from centrally administered morphine [2, 3, 6, 7, 8]. In the present study we report a comparison of the motor effects produced by intraventricularly injected morphine and etonitazene, a powerful analgesic opioid. Despite the close similarity of the analgesic, reinforcing, and dependence inducing properties of these compounds [9], we demonstrate in this paper that the motor effects of central injections differ dramatically. In order to enhance a comparison of our results to those obtained by other authors from interstitial infusions [2, 3, 6] and in order to estimate the spread of the drug methylene blue was infused into the ventricles of several naive control animals prior to perfusion and brain removal. Histological analysis provided a crude index of the distribution in CSF of compounds administered under the conditions of our experiment.

METHOD

Male, Wistar rats (Canadian Breeding Farms) weighing 200–250 g at the time of surgery were used. A stainless steel guide cannulae (22 ga, Plastic Products) were stereotaxically aimed at the left lateral ventricle. A Harvard infusion pump was connected to the injection cannula by flexible PE tubing. A watertight fluid swivel [1] permitted the animals to turn freely without twisting the tubing.

Small doses of morphine were repeatedly injected into the ventricles at regular intervals. The series of infusions was continued until an explosive motor episode was observed or until the animal became stuperous to the point of being unable to maintain an upright posture. This procedure yielded an estimate of a dose threshold for the motor effect. Etonitazene was administered in a similar manner with the appropriate adjustments in dose as noted below.

Morphine sulphate was used throughout this study and all solution concentrations were expressed as the salt of the drug.

Infusions of a 2% w/v of morphine/Ringer's solution were delivered at 2 min intervals. The volume of the first infusion was 4 μl (80 μg of morphine) while in subsequent infusions, the volume was halved (40 μg of morphine in 2 μl). If neither explosive motor behavior nor stupor were observed by the time the cumulative dose approached 500 μg, then the injection volume was increased to at least 4 μl (80 μg) and to as much as 10 μl (200 μg). All infusions were delivered while the rats were free to move about a standard operant chamber (Ralph Gerbrands Inc.). Some additional behavioral observations were taken in a large open field.

Etonitazene was administered in a concentration of 0.02% (w/v). The potency of this compound which when injected peripherally, exceeds that at morphine by several orders of magnitude [9] was estimated from both its analgesic properties and dependence liability. Using this relationship as a rough guide, we chose doses of etonitazene to equal 1% of the morphine doses. The volume of the first infusion of etonitazene was 4 μl (0.8 μg). The volume of the second through the sixth infusions was 2 μl (0.4 μg) and the volume of the seventh through fourteenth infusions was 4 μl (0.8 μg). All infusions were delivered at a rate of 0.3 μl/sec and spaced at 2.0 min intervals.

Following termination of the morphine test session, the surviving animals were sacrificed with an overdose of ether. These animals were perfused intracardially with physiological saline followed by 10% Formalin. The brains were removed, frozen, and sectioned at a thickness of 40 μ. The rats that received etonitazene were retested with morphine before sacrifice.

Eight additional animals received infusions of 5% or 10% solutions of methylene blue. These animals were yoked to rats receiving morphine infusions i.e. a given animal in the methylene blue group received the same number and volume of infusions as a given animal in the morphine group. As soon as explosive motor behavior was manifested by the animals receiving morphine, the yoked animals (receiving methylene blue) were sacrificed by cervical dislocation. The brains were removed and fixed in 10% Formalin saline.

Analysis of the brain sections indicated that the cannulae in all animals penetrated into the lateral ventricle. Examination of the brains from the animals that received methylene blue infusions showed a left–right assymmetry in the forebrain distribution of the dye which was much more concentrated on the side of the infusion. The walls of the fourth ventricle, aqueduct of Sylvius, third ventricle, and left lateral ventricle were all heavily stained, the presence of the dye was observed as far as the central canal of the spinal cord. However, little penetration occurred into tissue. Figure 1 shows three representative sections from one brain.

RESULTS

Explosive motor behavior was seen in 7 of 9 animals receiving morphine infusions. The animals generally became quite still following the first few infusions. The full-blown syndrome was witnessed after additional infusions. In the small operant chamber, this syndrome appeared as violent twisting and jumping movements that occurred with such speed and power that the motion of individual limbs could not be clearly discerned. The rats crashed into the walls and ceiling of the chamber.

In the open field, the character of the explosive motor behavior appeared to be somewhat different. Vigorous running movements were observed instead of the leaping seen in the small box. (We use the term running movements because coordination was sometimes poor and at times rapid stepping movements occurred while the belly was still in contact with the substrate.) When the running movements did result in forward progression, they followed a circular course. The rats did not circle consistently toward one side or the other. Running was sometimes accompanied by twisting of the body causing the animal to roll over. In one case a full-blown tonic-clonic seizure was observed.

The episodes of explosive motor activity sometimes appeared to occur spontaneously but could also be elicited by shaking keys or flashing a bright light. These periods of violent activity were interspersed with periods of quiescence during which time laboured breathing and vocalizations could be heard. A period of immobility and depression usually followed the explosive motor behavior. The remaining animals demonstrated explosive motor activity following total doses of 160–385 μg of morphine. Two animals failed to show this effect even after infusion of 1000 μg of morphine. These animals became steadily more stuperous.

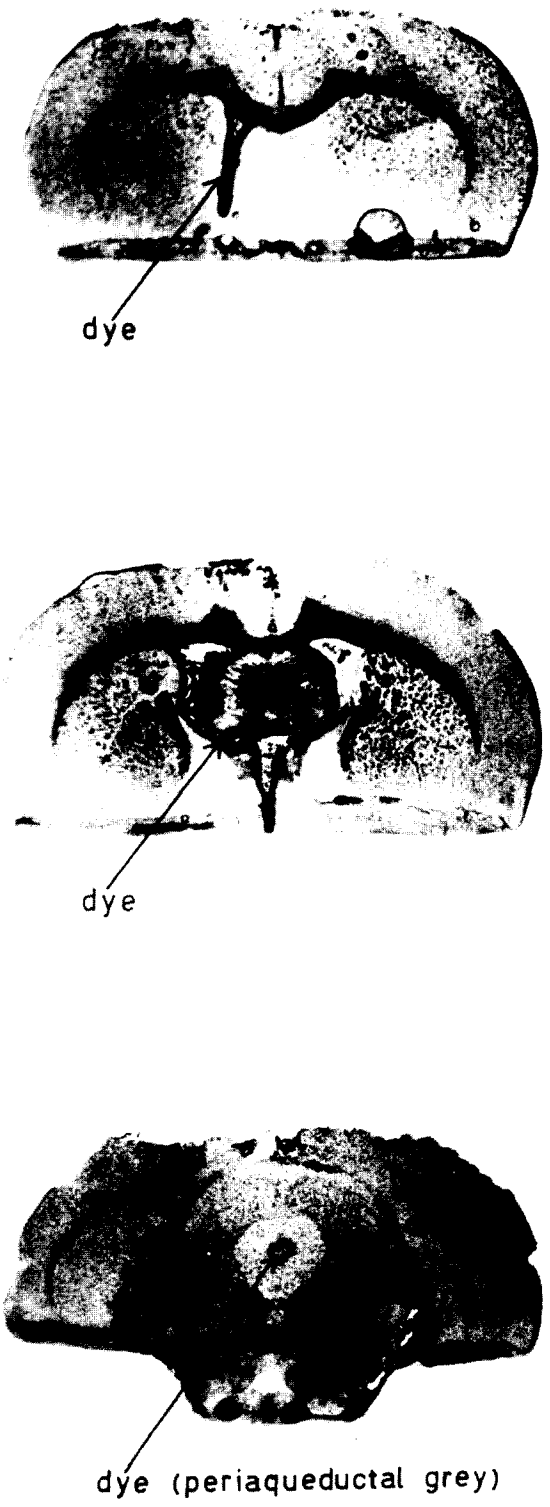

FIG. 1. Three sections from the brain of a representative animal that received intraventricular infusions of methylene blue. Top: anterior to the cannula plane. Middle: just posterior to the cannula plane. Bottom: more posterior to the cannula plane.

Three animals received infusion of etonitazene 10 days prior to the morphine infusions. The effects of this opioid were in sharp contrast to the effects of morphine. All three rats became increasingly rigid as additional doses of etonitazene were delivered. The degree of stiffness after a total dose of 9.2 μg was remarkable. The tail was held almost straight out from the body. The back was so rigid that the animals could be balanced on a small bottle with almost no drooping of fore or hind-quarters. No spontaneous or reflex movements were observed. Despite the severity of these symptoms, recovery was extremely rapid. Within approximately 30 min of maximal stiffness, the animals appeared grossly normal. These results have since been replicated in 5 additional animals. Ten days after the etonitazene infusions, all three animals manifested explosive motor behavior in response to intraventricular infusions of morphine.

DISCUSSION

The explosive motor behavior observed in this study is a most striking, phenomenon especially in the light of the generally depressant properties of peripherally administered morphine. In an unpublished study that involved measurement of the LD 50 of intraperitoneally injected morphine, two of the present authors had the opportunity to observe the motor behavior of rats receiving high, peripherally administered doses of the drug. Although they observed occasional abrupt movements in response to auditory stimuli, no prolonged motor excitation was seen in any of the animals in the present experiment. In contrast, explosive motor behavior in the present experiment was exhibited by 7 of the 9 animals that received intraventricular infusions of morphine. Jacquet and Lajtha [4] also failed to observe explosive motor behavior in rats receiving high doses of morphine IP.

Reports of other experiments involving intraventricular administration of morphine are consistent with our findings. Tanaka and Kadowaki [8] observed restlessness, motor excitement and convulsions in the rabbit while Stern and Gauthier [7] reported motor agitation and convulsions following injection of morphine under the dura or into the fourth or lateral ventricles of rabbits. Explosive motor behavior has also been produced by interstitial microinjections of morphine into the periaqueductal grey (PAG) [2, 3, 6]. The proximity of this structure to the ventricular system suggests that the effects of intraventricularly administered morphine may be due to the action of the drug on the PAG. As Fig. 1 shows, methylene blue, can reach this site after infusion into the lateral ventricles according to a dose schedule that produced explosive motor behavior in response to morphine.

However, there is at least one reason for suspecting that structures other than or in addition to the PAG may be involved. Pert and Yaksh [5], reported on the basis of tracer studies, that "less than 10% of an injected dose of morphine sulfate (40 μg). . . reaches the ventricular system during the first 2 hr following an injection approximately 1–1.5 mm from the ventricular wall". Jacquet and Lajtha [4] reported similar results from a study of the diffusion of morphine in the PAG. We know of no reason to assume that the mobility of morphine from the ventricles to the PAG should differ from its mobility in the opposite direction. Given that our morphine infusions were diluted by the volume of the CSF and that the PAG is some distance from the site of injection, this low rate of mobility suggests that only very small amounts of morphine penetrated the PAG following our infusions. On the basis of this inference, we suspect that structures other than the PAG may have been responsible for the effects we observed. Perhaps explosive motor behavior may be produced by morphine action at more than one brain site.

The explosive motor behavior produced by morphine appears to be all the more paradoxical in the light of the results obtained with intraventricular infusions of etonitazene, a drug that so closely resembles morphine in many of its actions. Rats previously made dependent on morphine will drink more etonitazene than nondependent animals [9]. Intake of etonitazene during withdrawal from morphine suppressed withdrawal symptoms while a morphine-like abstinence syndrome could be demonstrated after rats were pretreated with etonitazene injections. These injections produced an intoxication similar to that observed following administration of morphine [9]. Given this evidence of pharmacological similarity, it is surprising that there is such a striking difference in the motor effects produced by intraventricular administration of morphine and etonitazene. This difference cannot be due to cannula placement. All three animals that showed profound stiffness in response to etonitazene subsequently manifested explosive motor behavior following intraventricular administration of morphine. It is possible that the differential effects of these two drugs may be related to differences in lipid solubility and consequent different distribution in neural tissue. This stiffness may resemble Jacquet and Lajtha's [3] report of catatonia produced by peripherally administered levorphanol and etorphine (both compounds are also analgesic opioids) and intracerebral etorphine. Paradoxically, these investigators also found that naloxone blocked both the explosive motor behavior following morphine and the stiffness following levorphanol and etorphine! We cannot, at present, account for all of these findings but are actively investigating the relation of the motor effects produced by etonitazene and morphine.

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