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Brief Communications

Reversal of Akinesia and Release of Festination by Morphine or GABA Applied Focally to the Nucleus Reticularis Tegmenti Pontis

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Focal application of 5 μg of morphine sulfate to the nucleus reticularis tegmenti pontis (NRTP) in rats reversed the akinesia induced by 5 mg/kg systemic haloperidol or 40 mg/kg systemic morphine and released festinating forward locomotion. γ-Aminobutyric acid (200 μg) applied to this nucleus also reversed such akinesia. Intraventricular naloxone (10 μg) or picrotoxin (0.1 μg), respectively, blocked the effects of such focally applied drugs. Thus, morphine and γ-aminobutyric acid appear to act physiologically on the cells of the NRTP. The results suggest that systemic morphine, in addition to producing immobility, simultaneously facilitates a readiness for locomotion by inactivating a final common inhibitory system in the region of the NRTP.

In rats, sufficient amounts of systemically administered morphine can produce a dual effect: rigid akinesia that alternates with stimulation-induced bursts of running (De Ryck, Schallert, & Teitelbaum, 1980). De Ryck et al. noted that this alternation may be adaptive in predator/prey encounters because morphine-induced akinesia, which shares many similarities with death-feigning immobility (Carli, Farabollini, & Fontani, 1976; De Ryck et al., 1980; Klemm, 1971), is characterized by a posture that is compatible with an increased readiness to run when physically stimulated (e.g., if dropped by the predator). Morphine can also promote movement when applied intracranially to the periaqueductal gray matter (Jacquet & Lathja, 1974) or the ventral tegmental area (Joyce & Iversen, 1979).

Bilateral electrolytic lesions of the nucleus reticularis tegmenti pontis (NRTP) produce a form of galloping forward locomotion (Cheng, Schallert, De Ryck, & Teitelbaum, 1981). Therefore, the NRTP normally inhibits locomotion. We recently showed (Chesire, Cheng, & Teitelbaum, 1983) that after NRTP damage, systemically administered morphine or haloperidol no longer abolishes movement. Because morphine and haloperidol do not appear to act on any mechanism caudal to the NRTP that is sufficient to inhibit locomotion, we proposed (Cheng et al., 1981; Chesire, Cheng, & Teitelbaum, 1982, 1983) that, as far as these drugs are concerned, the NRTP is an integral part of a final common pathway for their inhibition of locomotion. If this is so, then neural systems that control the inhibition of locomotion should converge in the region of the NRTP and should act physiologically on its cells.

In this brief report, we verify our earlier demonstrations (Cheng et al., 1981, Chesire et al., 1983) that focal application of γ-aminobutyric acid (GABA) to the NRTP can reverse the akinesia produced by systemic haloperidol or morphine and can elicit galloping forward locomotion (a possible animal analogue of some forms of parkinsonian festination). We show here that morphine applied focally to the NRTP can also reverse both forms of akinesia. In addition, the releasing effects of these drugs applied locally in the NRTP were blocked by their antagonists applied at some distance, intraventricularly. Thus, intraventricular naloxone blocked the effects of morphine in the NRTP, and intraven-

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tricular picrotoxin blocked the effects of GABA in the NRTP. Therefore, the effects of morphine and GABA appear not to be due to indiscriminate tissue trauma at that locus but rather to a physiological inhibitory action on the cells of the NRTP (but see Discussion).

Method

Subjects

The animals were 22 male Long-Evans hooded rats (Blue Spruce) weighing 300–500 g. They were housed individually in suspended metal mesh cages with free access to laboratory chow and tap water. The colony had a 12:12 hr light/dark cycle with lights on at 0630, with the temperature maintained at 23 ± 2 °C.

Surgery and Postoperative Care

The animals were anesthetized with 50 mg/kg sodium pentobarbital and, by stereotaxic procedures, were chronically implanted with two bilateral 22-ga guide cannulas aimed at the NRTP alone (n = 11) or four cannulas, two aimed at the NRTP and two at the lateral ventricles (n = 11). With the incisor bar elevated 5 mm above the ear bars, coordinates for the tip of the guide cannulas aimed at the NRTP were 1 mm posterior to the interaural line, 1 mm lateral to the exposed midline sinus, and 3 mm ventral to the dura (the injector cannula was lowered an additional 6 mm). With the skull level between bregma and lambda, coordinates for the guide cannulas aimed at the lateral ventricles were 0.5 mm posterior to bregma, 1.5 mm lateral, and 2.5 mm ventral to the surface of the skull (the injector cannula was lowered an additional 2 mm). Following surgery, some animals were aphagic and adipsic for 1–2 days and required intragastric infusion of a liquid diet (Teitelbaum & Epstein, 1962; Teitelbaum & Stellar, 1954).

Procedure

In order to produce akinesia, the animals were first injected ip with 5 mg/kg haloperidol (5 mg/ml Haldol ampules; McNeil Laboratories) When they became akinetic (30–40 min postinjection), we released locomotion by injecting 200 μg of GABA (Sigma Chemicals) into the NRTP. In order to reverse the release of locomotion, 20–40 min after injection of GABA, rats with ventricular cannulas were injected with .1 μg of picrotoxin (Sigma) in each lateral ventricle. We also injected haloperidol-treated rats with 5 μg of morphine sulfate (Mallinckrodt) into the NRTP, followed by 10 μg of naloxone HCl (Endo Laboratories) intraventricularly. Identical procedures were followed for rats made akinetic by systemic administration of 40 mg/kg morphine. The intracranial drugs were injected in 1 μl of saline at a rate of 1 μl/min except picrotoxin which was injected at a rate of 1 μl/2 min. Cannulas were left in place 1–2 min following infusions. To control for unspecific actions of morphine and GABA, we injected 1 μl of isotonic saline into the NRTP of animals made akinetic by systemic haloperidol (n = 4) or morphine (n = 4). In animals with ventricular cannulas, we also infused 1 μl of saline intraventricularly to control for unspecific effects of naloxone and picrotoxin

Locomotor effects were quantified in drug-treated animals by counting the number of seconds required to run a distance of 300 cm on the floor of a large open room (M of five trials per animal per drug treatment). The open room was used because festinating rats run in a relatively straight line forward. Because normal (undrugged) rats frequently interrupt or change the direction of their locomotion in the open field, we estimated their speed of locomotion by counting the seconds required to traverse a distance of 77 cm through a hollow Plexiglas tube 6.2 cm in internal diameter (Chesire et al., 1983).

Histology

To mark the locus of drug delivery, we infused 1 μl of india ink through the guide cannulas of 6 rats at a rate of 1 μl/min. The animals were then overdosed with sodium pentobarbital and perfused through the heart with isotonic saline followed by 10% formol-saline. Frozen coronal sections 40 μm thick were cut, mounted, and stained with cresyl violet. In all 6 animals, the ink had diffused into the NRTP, with some additional diffusion into the surrounding region.

Results

In animals made akinetic by systemically administered haloperidol, 200 μg of GABA focally applied to the NRTP quickly reverses the akinesia and releases festinating straightforward locomotion (Cheng et al., 1981). We verified this finding. GABA reversed haloperidol akinesia within 15 min (typically 5–7 min), and the effect lasted as long as 2 hr. The average running speed of GABA-treated rats (n = 6) was 46 cm/s compared with 8 cm/s for 5 undrugged normal rats. Saline did not reverse haloperidol akinesia In rats with additional ventricular cannulas (n = 3), intraventricular picrotoxin blocked the forward locomotion released by GABA applied focally in the NRTP as soon as 2 min postinjection, and the animals again resumed the broad-based stance (De Ryck et al., 1980) characteris-

tic of haloperidol-induced catalepsy. Because its effect can be reversed by intraventricular picrotoxin, GABA applied intracranially appears to act physiologically, not primarily mechanically, on the cells of the NRTP.

Local application of GABA to the NRTP apparently did produce some mechanical trauma, because after repeated infusions (as few as two or three) the effects of systemic haloperidol were largely nullified (e.g., after systemic haloperidol, the animals no longer became akinetic. We did not observe any failure of haloperidol to produce akinesia after as many as five injections of morphine).

Morphine in the NRTP also reversed haloperidol-induced akinesia ($n = 12$) within 10 min, and galloping developed within 30 min (typically 15–20 min). The releasing effects of morphine in the NRTP usually lasted about 1.5 hr. The average running speed of rats treated focally with morphine was 51 cm/s compared with 11 cm/s for 5 undrugged control rats. Again, saline injected focally into the NRTP did not reverse haloperidol-induced akinesia ($n = 4$). Such saline-injected rats remained completely akinetic as long as the 9 haloperidol-treated control rats (2–3 hr).

In 6 rats made akinetic by haloperidol (Figure 1A) and released from their akinesia by morphine in the NRTP (Figure 1B), intraventricular infusion of 10 μg of naloxone reversed the release of locomotion (Figure 1C). Naloxone injected intraventricularly in 2 rats treated with systemic haloperidol alone did not alter the haloperidol catalepsy. Unlike GABA-treated rats reversed by picrotoxin, morphine-treated rats reversed by naloxone did not resume the posture characteristic of haloperidol-induced catalepsy. This may indicate that although some NRTP tissue is affected by both drugs, the areas may not overlap completely (Chesire et al., 1983). However, we cannot rule out the possibility that a higher dose of naloxone would have caused the animals to resume a posture typical of haloperidol-induced akinesia. In 3 of 5 rats tested, 200 μg of GABA injected into the NRTP also reversed the akinesia produced by systemically administered morphine within 15 min.

Because focally administered morphine appears to act physiologically on the cells of the NRTP (its action is reversed by naloxone), it appears that part of morphine's systemic action may be to potentiate locomotion by inhibiting the NRTP while simultaneously inhibiting locomotion by acting elsewhere. If this is so, then focal application of morphine to the NRTP should alter the balance produced by systemically administered morphine, and should eliminate the akinesia, which would leave only the potentiation of movement. In one rat tested so far, morphine applied to the NRTP partially reversed the akinesia produced by systemically administered morphine, and released continuous alternate or synchronous hindlimb movement. The releasing effects of morphine in this rat were also blocked by intraventricularly administered naloxone. This result requires further study.

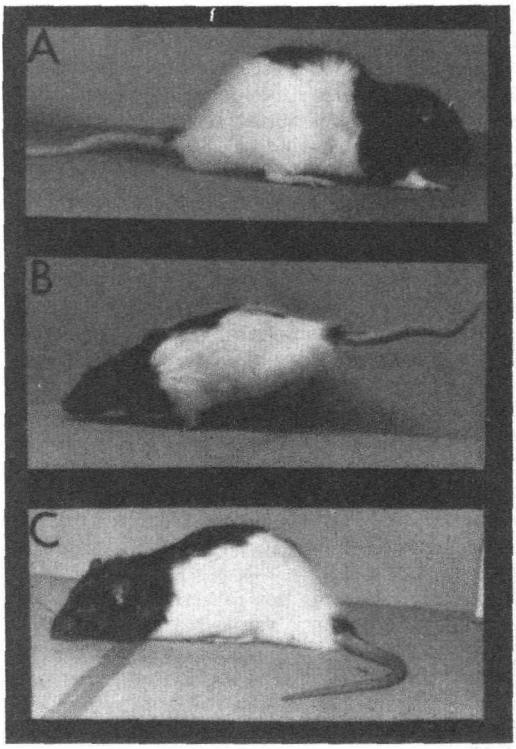

Figure 1. A: A rat with bilateral cannulas in the NRTP and the lateral ventricles 30 min following 5 mg/kg ip haloperidol. (The animal is completely akinetic and, left untreated, would remain so for up to 3 hr.) B: The same rat 10 min after 5 μg of morphine was infused into the NRTP. (After a brief period of walking and running, the animal gallops and is unable to inhibit its locomotion, so it collides with obstacles or runs off the edge of the table.) C: The same rat 15 min after 10 μg naloxone was infused into the lateral ventricles. (Naloxone caused the animals to become akinetic and lose postural support for frequent periods [up to 20 s] between brief episodes of walking or running.)

Discussion

We have demonstrated that two forms of drug-induced akinesia (produced by systemically administered morphine or haloperidol) can be reversed by focal application of drugs (morphine or GABA) to the NRTP. We suggest that GABA and morphine can act physiologically at syn-

apses there, presumably on cells of the NRTP, because the release of locomotion produced by focal application of these drugs can be reversed by intraventricular infusion of the appropriate antagonist whereas saline has no effect. This agrees with earlier findings by us that electrolytic damage of the NRTP abolished the locomotor inhibition and catalepsy produced by haloperidol or morphine (Chesire et al., 1983). Alternatively, the antagonist drugs picrotoxin and naloxone administered intraventricularly may exert their effects indirectly via other neural systems. The possibility that their action is not specific should be tested by administering them directly to the NRTP. However, in two rats tested so far, intraventricularly administered picrotoxin did not reverse the effects of morphine applied to the NRTP, and intraventricularly administered naloxone did not reverse the effects of focally applied GABA. Furthermore, when GABA is focally applied in the region of, but not into the NRTP, haloperidol-induced catalepsy is not reversed. Instead, the animals lose postural support and righting reflexes (personal observations, 1982). Although preliminary, these results argue that some specific physiological action of morphine and GABA takes place on the cells of the NRTP.

The results also suggest that as far as the systemic action of morphine or haloperidol is concerned, the cells of the NRTP form part of a final common pathway for the inhibition of locomotion. Our results indicate that these cells are inhibited by at least two types of receptors, opiate and GABAnergic, suggesting that neurons using these transmitters may converge on the NRTP. Finally, we suggest that systemically administered morphine, while inactivating locomotion by acting elsewhere in the nervous system, simultaneously potentiates movement by inactivating the inhibitory system of which the NRTP is a crucial part. The combined antagonistic effects may be useful in death feigning, which systemically administered morphine appears to mimic.

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