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Sensitization of Locomotion Following Repeated Ventral Tegmental Injections of Cytisine

ENRICO MUSEO[1] AND ROY A. WISE[2]

Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec, Canada H3G 1M8

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MUSEO, E. AND R. A. WISE. *Sensitization of locomotion following repeated ventral tegmental injections of cytisine.* PHARMACOL BIOCHEM BEHAV **48**(2) 521–524, 1994.—Systemic injections of nicotine increase locomotion, and repeating these injections brings about a sensitization of the locomotor response. Ventral tegmental injections of the nicotinic agonist cytisine also increase locomotion. In the present study cytisine was administered repeatedly into the ventral tegmentum to determine whether sensitization of its locomotor-activating effects would develop. Four groups of animals were tested; each group received a total of six injections at a rate of one injection every 48 h. Two of these groups received injections of cytisine (10 nmol/side): one group received injections into the ventral tegmentum, and, to insure the anatomical specificity of the locomotor effect, a second group received injections dorsal to the ventral tegmentum. The remaining two groups received vehicle injections: one group received injections into the ventral tegmentum, and the other received injections into more dorsal sites. The group of animals that received injections of cytisine into the ventral tegmentum locomoted more than any other group. In addition, only with this group was a progressive increase in the locomotor response evident across test days. These findings raise the possibility that a neural substrate in the ventral tegmentum mediates the locomotor-activating and sensitizing effects associated with the systemic administration of nicotine.

Nicotine Dopamine Mesolimbic Rat

THE systemic administration of nicotine enhances locomotor activity (2), and with repeated testing nicotine's locomotor-activating effects become more pronounced (9). Such progressive enhancement, commonly referred to as sensitization, has long been associated with the repeated systemic administration of other compounds such as amphetamine (19), cocaine (17), and morphine (1).

The intracerebral administration of certain drugs can also increase locomotion; in some instances, the repeated administration of these drugs is associated with a sensitization of the locomotor response. For example, the administration of morphine into the ventral tegmentum increases locomotion, and repeating these injections brings about a sensitization of this locomotion (20). The administration of the nicotinic agonist cytisine into the ventral tegmentum also increases locomotion (13,18). The present experiment was designed to determine whether the repeated administration of cytisine into the ventral tegmentum is sufficient to bring about a sensitization of the locomotor response. Vehicle and anatomical control groups were included to ensure the pharmacological and anatomical specificity of the observed effects.

METHOD

Male Long-Evans rats (Charles River Inc., Wilmington, MA) weighing between 400 and 500 g at the time of surgery were anaesthetized with sodium pentobarbital (60 mg/kg, IP) and placed in a stereotaxic instrument so that 22-gauge stainless steel guide cannulae could be implanted bilaterally. The tips of these guide cannulae were lowered to a location 1.5 mm short of the final injection sites. With the incisor bar set 5 mm above the interaural line and the angle of implantation

[1] Current address: Biological Psychiatry Branch, National Institute of Mental Health, Building 10, Room 3N212, 9000 Rockville Pike, Bethesda, MD 20892.

[2] Requests for reprints should be addressed to R. A. Wise, Department of Psychology, Concordia University, 1455 deMaisonneuve Blvd. West, Montreal, Canada H3G 1M8.

set at 10° off the vertical, the stereotaxic coordinates for the injection sites in the ventral tegmentum were as follows: 2.8 mm posterior to bregma, 2.0 mm lateral to bregma, and 8.6 mm below the skull surface. The stereotaxic coordinates for sites dorsal to the ventral tegmentum were as follows: 2.8 mm posterior to bregma, 2.0 mm lateral to bregma, and 7.1 mm below the skull surface. The guide cannulae were constructed from 22-gauge stainless steel tubing and were cut to a length of 15 mm. Acrylic cement was used to fix the guide cannulae in place once they were implanted; stainless steel screws threaded into the skull served as anchors for the acrylic cement. In addition, a plastic shield (15 × 15 mm) was partially embedded in the cement just posterior to the guide cannulae; this shield served to protect injector cannulae during the injection procedure. The injector cannulae were constructed from 30-gauge stainless steel tubing and cut to a length of 16.5 mm. Each injector cannula was slightly bent to ensure that there would be friction between the outer wall of the injector cannula and the inner wall of the guide cannula; this friction held the injector cannulae in place during the injection procedure. Following surgery, lengths of 30-gauge steel wire that were cut to extend 0.5 mm beyond the tip of the guide cannula were inserted into each guide cannula to keep the guide cannula patent. The animals were allowed at least one week to recover from the surgical procedure.

Animals were tested using boxes (20 × 41 × 25 cm) made of wood, except for their Plexiglas fronts and wire-grid floors. Two photocells, each positioned 4 cm above the floor, separated the test compartment along its longest side into three equal areas; the photocells were connected via an electrical interface to a computer located in an adjoining room.

Two days prior to the beginning of the experiment each animal was familiarized with the testing environment by being placed in an activity box for 60 min. Animals were subsequently divided into four groups; the members of each group were injected six times at a rate of one injection every two days. Two groups of animals were injected with a concentration of cytisine (Sigma Chemical Co., St. Louis) that increases locomotion when administered into the ventral tegmentum (10 nmol/0.5 μl/side) (13). Two other groups were injected with saline, the drug vehicle (0.5 μl per side). Of the two groups that were repeatedly injected with cytisine, one group received injections into the ventral tegmentum and the other received injections into sites dorsal to the ventral tegmentum. This latter group of animals (referred to as a dorsal, or anatomical, control group) was included so as to assess whether the diffusion of drug to sites dorsal to the ventral tegmentum might by itself be sufficient to account for the locomotor-activating effects associated with ventral tegmental injections of cytisine. Of the two groups that were repeatedly injected with saline, one group received injections into the ventral tegmentum, and the other received injections into more dorsal sites. These two groups were used as vehicle control groups and served to determine whether the repeated administration of the vehicle alone could account for any of the behavioral effects associated with the repeated administration of cytisine.

Bilateral injections were made with 1-μl glass syringes that were connected to injector cannulae with polyethylene tubing (PE-20). Each pair of injections was made over a 100-s period using an electric injection pump. Immediately after the injection procedure was over, each animal was placed in an activity cage and locomotor activity was measured for 60 min.

Following the completion of the experiment, animals were anaesthetized with chloral hydrate (400 mg/kg) and perfused transcardially with 50 ml of saline followed by 50 ml of 10% formalin. Each brain was then frozen with dry ice and sliced with a microtome to obtain 40-μm-thick coronal sections that were collected on glass slides. The location of the tip of each injection cannula was determined by visual inspection of a magnified projection of the brain section. The injection sites were in a region between 2.4 and 3.2 mm posterior to bregma, and within 2 mm of the midline. Ventral tegmental sites were all ventral to the medial lemniscus, and dorsal injection sites were all at the level of the medial lemniscus or less than 1.5 mm dorsal to it (15).

The data were analyzed using the two-way analysis of vari-

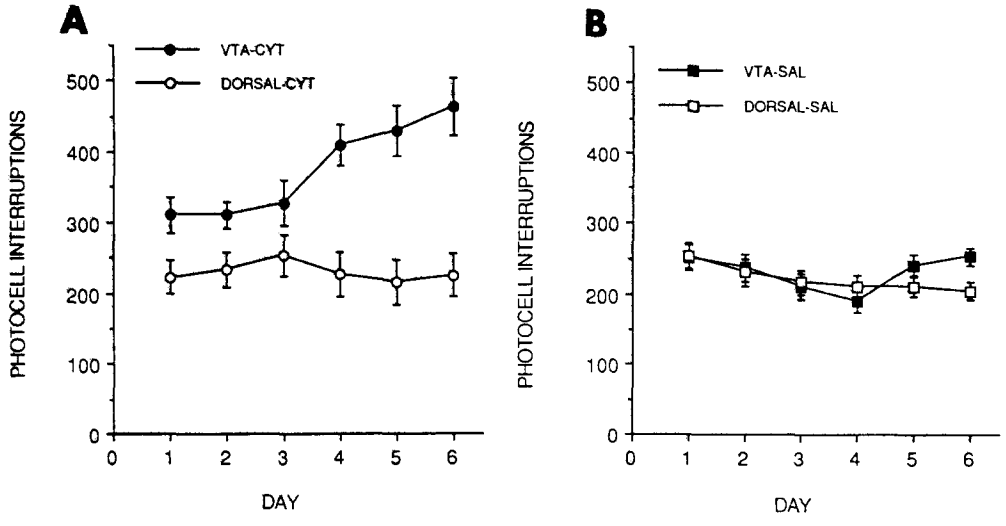

FIG. 1. (A) Locomotor effects associated with repeated injections of *cytisine* into either the ventral tegmentum (●) or more dorsal sites (○). (B) Locomotor effects associated with repeated injections of saline into either the ventral tegmentum (■) or more dorsal sites (□).

ance (ANOVA). Only animals with both injector tips located in the ventral tegmentum or sites dorsal to it were included in the data analyses.

RESULTS AND DISCUSSION

Animals that received injections of cytisine into the ventral tegmentum locomoted more than animals injected into more dorsal sites, $F(1, 13) = 15.00, p < 0.002$. In addition, overall activity increased across days, $F(5, 65) = 4.60, p < 0.001$, but only in the ventral tegmental group was there a progressive increase, $F(5, 65) = 7.55, p < 0.001$ (Fig. 1A). Repeated saline injections into the ventral tegmentum produced no more locomotion than did similar injections into sites dorsal to the ventral tegmentum, $F(1, 10) = 0.24, p > 0.05$. The animals in both vehicle groups locomoted less and less with repeated testing, $F(5, 50) = 2.75, p < 0.025$ (Fig. 1B).

It is apparent, then, that the locomotor-activating effects associated with ventral tegmental injections of cytisine, but not saline, became progressively stronger with repeated testing. To the extent that injections dorsal to the ventral tegmentum were not associated with the development of sensitization, it appears that the sensitized response resulted from the actions of cytisine in the ventral tegmentum, and not from the actions of cytisine in a region dorsal to the ventral tegmentum. These actions in the ventral tegmentum may account for some of systemic nicotine's effects on locomotion.

It has been suggested that the progressively stronger locomotor-activating effects associated with repeated systemic injections of nicotine are the byproduct of the tolerance that develops to the inhibitory effects of nicotine (2); the present findings, although not inconsistent with the view that tolerance to the inhibitory effects can partly account for the sensitized locomotor response associated with repeated injections of nicotine, suggest that a second mechanism is possible. Since the administration of cytisine into the ventral tegmentum did not appear to have any depressant effects on locomotion, it is unlikely that tolerance to any inhibitory effect accounted for the sensitized response associated with the repeated administration of cytisine; rather, it appears that some action at the level of the ventral tegmentum was, by itself, sufficient to bring about a progressive increase in locomotion, independent of any tolerance to an inhibitory action.

The effects reported in the present experiment are similar to the effects observed when morphine is administered into the ventral tegmentum; as with repeated morphine injections into the ventral tegmentum (16,20), repeated injections of cytisine produce progressively stronger effects on locomotion. In addition, as with repeated morphine injections at sites dorsal to the ventral tegmentum (20), cytisine injections at sites dorsal to the ventral tegmentum failed to induce locomotion in the present experiment.

The sensitization of the locomotor-activating effects associated with ventral tegmental injections of morphine and other opiates is believed to be mediated by the mesolimbic dopamine (DA) system (8,20). Opiates bind to sites in the ventral tegmentum (5), and when administered iontophoretically, morphine increases the firing of the mesolimbic DA cells (7); this effect is thought to account for the facilitation of DA release in the nucleus accumbens that accompanies both systemic (4) and ventral tegmental (10) injections of morphine—treatments that enhance locomotion.

A parsimonious explanation of the present findings is that cytisine acts on a neural substrate in the ventral tegmentum to increase locomotion and that its repeated actions on this substrate also produce a gradual strengthening of the locomotor response. The parallels between the effects of nicotine and the effects of morphine on the mesolimbic DA system suggest that the sensitizing effects on locomotion of systemic nicotine or ventral tegmental cytisine may also be mediated by the mesolimbic DA system. This notion is consistent with various findings. For example, nicotine (3) and cytisine (14) bind to sites in the ventral tegmentum; in addition, the systemic administration of nicotine increases the firing of mesolimbic DA cells (6,11) as well as the release of DA in the nucleus accumbens (4,12). The release of DA in the nucleus accumbens is also enhanced following the administration of nicotine into the ventral tegmentum (12). Lastly, the locomotor-activating effects associated with ventral tegmental injections of cytisine are blocked by systemic injections of the nicotinic antagonist mecamylamine as well as by systemic injections of the DA antagonist pimozide (13). These findings, in addition to the results of the present experiment, raise the possibility that the mesolimbic DA system mediates the locomotor-activating effects as well as the sensitizing effects associated with the systemic administration of nicotine.

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