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Microinjections of a Nicotinic Agonist Into Dopamine Terminal Fields: Effects on Locomotion

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MUSEO, E. AND R. A. WISE. *Microinjections of a nicotinic agonist into dopamine terminal fields: Effects on locomotion.* PHARMACOL BIOCHEM BEHAV **37**(1) 113–116, 1990.—Nicotine induces locomotion, a behavior associated with the mesocorticolimbic dopamine system. The present study determined the effects on locomotion of direct microinjections of the nicotinic agonist cytisine into four DA terminal fields where nicotinic receptors have been localized: nucleus accumbens (NAS, n = 20), caudate putamen (CPU, n = 9), olfactory tubercle (OT, n = 8), and medial prefrontal cortex (MPC, n = 12). Male Long-Evans rats were injected with cytisine (0.1, 1, 10 and 100 nanomoles per 0.5 μl per side) or vehicle through indwelling cannulae, and locomotor activity was recorded during a 60-minute test session; each animal was tested with each dose in counterbalanced order. NAS injections of the three highest doses of cytisine increased locomotion relative to vehicle injections; injections in the CPU, dorsal to the NAS, were ineffective, as were MPC and OT injections. The data support the notion that systemic nicotine may interact with dopaminergic projections to the NAS to produce increases in locomotor activity.

Cytisine Nicotine Nucleus accumbens Locomotion Dopamine

THE mesocorticolimbic dopamine (DA) system is thought to be important for the normal expression of locomotion (2,10). Mesocorticolimbic DA fibers originate primarily in the ventral tegmental area (VTA) and terminate in areas such as the nucleus accumbens (NAS), olfactory tubercle (OT) and medial prefrontal cortex (MPC). Changes in locomotor activity have been reported following the administration of six-hydroxydopamine (6-OHDA) into the VTA, a treatment that brings about the degeneration of the mesocorticolimbic DA system; the subsequent reinnervation of the NAS or MPC using DA tissue transplants normalizes activity (9). At least in the case of the NAS, the local release of DA and the formation of DA metabolites correlate highly with locomotion (11). Moreover, compounds that alter activity in the mesocorticolimbic DA system influence locomotor activity. For example, systemic injections of amphetamine increase DA release (8) and also produce hyperactivity (26), whereas pretreatment with DA antagonists attenuates amphetamine's effect on locomotion (15,23), presumably by competing for receptors critical for locomotor activation. Microinjections of DA antagonists directly into the NAS also reduce locomotor activity (1), while injections of DA (7,22) and DA agonists (3,21) into this structure increase activity. Thus, the NAS is involved not only in spontaneous locomotion but also in the locomotion induced by psychomotor stimulants.

Nicotine has been reported to influence locomotor activity (4), and a number of experimental findings support the notion that nicotine's effect, like those of other drugs that enhance locomotion, is mediated, at least in part, by the mesocorticolimbic DA system. Nicotinic binding sites have been localized autoradiographically in the VTA, NAS, caudate putamen (CPU), OT and MPC (6), and, at least in the case of the VTA and NAS, nicotine binding is markedly reduced by 6-OHDA-induced degeneration of the mesocorticolimbic and nigrostriatal DA fibers (5). DA neurons in the VTA increase their rate of firing following either the systemic (16) administration of nicotine. This increase in the rate of firing presumably leads to the increases in NAS DA levels that have been found with the technique of in vivo microdialysis (14).

Two mechanisms of action involving the mesolimbic DA system have been proposed to account for nicotine's effect on locomotor activity. The first takes into consideration nicotine's ability to bind to receptors on DA cell bodies in the VTA and to increase cell firing; presumably, this brings about an increase in the release of DA at efferent sites and thereby facilitates locomotion. Support for this notion comes primarily from studies in electrophysiology (13,16) and autoradiography (5). Reports of increases in locomotor activity following intra-VTA injections of cytisine, a potent nicotinic agonist, also support the notion that nicotine can act at the level of the cell body to influence DA activity (18,20). A second way nicotine can potentially facilitate locomotor activity is by enhancing DA release by acting directly on receptors on DA terminals. The idea that nicotine may act at the

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level of DA terminals to influence DA release is based largely on the use of synaptosomal and tissue-slice preparations (12, 24, 25, 27); in addition, Misfud *et al.* (17) reported that the administration of nicotine by way of a microdialysis probe positioned in the NAS increased the release of DA in the NAS. It therefore appears that nicotine can act at more than one point in the mesolimbic DA system.

Although one factor involved in nicotine's facilitation of locomotion is its excitatory effect at the level of the VTA, the notion that nicotine can also increase the release of DA by acting via nicotinic receptors located on DA terminals makes it of interest to determine whether or not direct microinjections of a nicotinic agonist into various regions containing DA terminals can lead to hyperactivity. In this way, the contribution each of these terminal areas makes with regards to mediating nicotine's excitatory effect on locomotion can be evaluated. To test the hypothesis that systemic injections of nicotine can increase locomotion by acting directly on one of several structures efferent to the VTA, microinjections of several doses of cytisine were made into the NAS, OT, MPC or CPU.

METHOD

Sixty naive male Long-Evans hooded rats weighing 350–450 g were used for the series of experiments. They were housed individually in steel cages and had free access to food and water. Lights were on between 0800 and 2000 hr. All animals were implanted bilaterally with 22-ga guide cannulae while under pentobarbitol (60 mg/kg, IP) anaesthesia. The tips of the guides were located 1.5 mm above the final injection sites. Stereotaxic surgery was performed with the incisor bar set 5 mm superior to the interaural line and, with the exception of animals in the MPC group, cannulae were implanted at a 10 degree angle (from the vertical plane and with respect to bregma) in order to avoid puncturing the ventricles. The coordinates for the injection sites were the following: NAS: 3.2 mm anterior to bregma (AP), 2.2 mm lateral to the midsagittal suture (ML) and 7.8 mm ventral to the skull surface (DV); CPU: (AP = 3.6, ML = 2.2, DV = 5.8); OT: (AP = 3.6, ML = 3.5, DV = 9.2); MPC: (AP = 4.5, ML = 0.7, DV = 3.5). The guide cannulae were constructed from 22-ga stainless steel tubing and were cut to a length of 15 mm. The injector cannulae were constructed from 30-ga stainless steel tubing and were cut to a length of 16.5 mm. Loose-fitting cannulae were slightly bent to create friction between the outer wall of the injectors (and the obturators) and the inner wall of the guide; this held the cannulae in place during and between injections. 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 was partially embedded in the cement and served to protect injector cannulae during brain microinjections. Thirty-ga stainless steel obturators the length of the guide cannulae were inserted following surgery and were removed when injections were made. The animals were allowed at least one week to recover from the surgical procedure.

Twelve activity boxes were used to measure locomotor activity. Each box (20 × 41 × 25 cm) was constructed of wood except for the Plexiglas front and a wire grid floor. Two photocells were positioned 4 cm above the floor and separated the compartment along its longest side into three equal areas. The photocells were connected via an electrical interface to a computer placed in an adjoining room; red lights were used to activate the photocells. During habituation and test sessions the room was dark and sounds were masked with white noise. Testing and habituation sessions took place between 1000 and 1600 hr.

Bilateral injections were made while the animals were free to move in a plastic container (20 × 40 × 16 cm). Drug or vehicle solutions (sterile physiological saline) were injected in a volume of 0.5 μl per side in a period of 100 sec. Thirty-ga injector cannulae were connected to 1-μl syringes via PE-20 tubing and were used to make the intracranial injections. The injector cannulae were left in place for 60 sec following the injection in order to allow for drug diffusion; upon the removal of the injector cannulae, obturators were replaced. Cytisine (Sigma) was dissolved in sterile physiological saline.

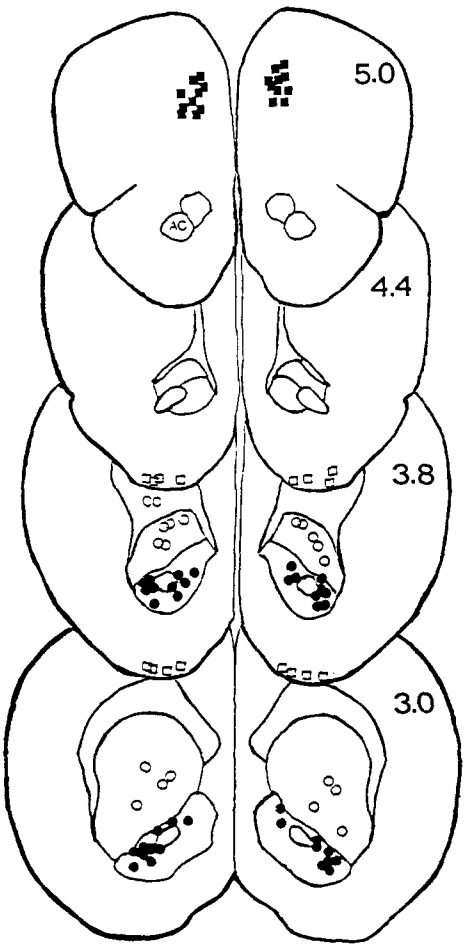

FIG. 1. Schematic drawings showing the location of injector cannulae tips in all animals included in the statistical analyses: nucleus accumbens (●), caudate putamen (○), medial prefrontal cortex (■), olfactory tubercle (□). For purposes of illustration points representing the location of cannulae tips were collapsed along the anterior-posterior plane. CC, corpus callosum; AC, anterior commissure. Numbers on brain sections refer to the approximate location of the coronal sections (mm anterior to bregma) (19).

Each animal received four doses of cytisine [0.1, 1, 10 and 100 nanomoles (nmol) per side], in addition to vehicle. The pH of the injections ranged from 6 (vehicle) to 10 (100 nmol). The sequence of injections was counterbalanced with respect to order. One to three days prior to the beginning of the experiment, animals were habituated to the apparatus for one hour. On test days animals were administered one of the five treatments following a 30-minute habituation period; for each animal the 60-minute test session began immediately following the injection procedure.

Following the experiment, and while under deep chloral hydrate anaesthesia, animals were perfused transcardially with 50

ml of saline followed by 50 ml of 10% formalin. A thionin solution was then injected intracranially in a volume of 0.5 μl in order to help locate the injection site. Cannula placements were determined from 40 μ frozen coronal sections.

RESULTS

The locations of the injector cannulae tips are illustrated in Fig. 1. Only animals with both injector tips located in a given structure are shown and included in the statistical analyses; twelve animals were excluded on the basis of this criterion. The effects of each of the treatments at each of the four sites are shown in Fig. 2. The data pertaining to each site were analyzed separately, and only in the case of the NAS was a dose effect revealed by a one-way analysis of variance with repeated measures conducted on the mean locomotor counts, $F(4,76)=5.97$, $p<0.01$. Each of the three highest doses was found to produce statistically reliable increases in locomotion (Tukey's Honestly Significant Difference test, $p<0.05$). The administration of cytisine into CPU sites (dorsal to the NAS) did not produce effects on locomotion significantly different from those of the vehicle solution, $F(4,32)=0.13$, $p>0.05$. Also ineffective were injections into the OT, $F(4,28)=0.86$, $p>0.05$, and MPC, $F(4,44)=0.76$, $p>0.05$.

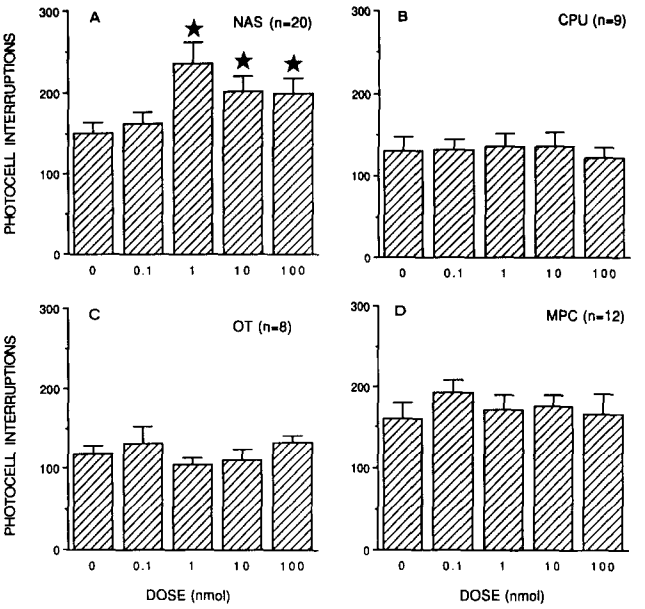

FIG. 2. Mean number of photocell interruptions for each of five treatments (doses expressed as nmol per side) during 60-minute test sessions: (A) nucleus accumbens, (B) caudate putamen, (C) olfactory tubercle, (D) medial prefrontal cortex. *Significantly different from saline ($p<0.05$, Tukey test).

DISCUSSION

The systemic administration of nicotine is known to increase locomotor activity in rats (4). The results of the present study support the view that the mesolimbic DA system plays a role in mediating the increases in locomotion produced by systemic nicotine. Although previous studies have shown that intra-VTA injections of the nicotinic agonist cytisine elicit hyperactivity (18,20), it appears that actions in the NAS may also be directly involved in mediating nicotine's locomotor-activating effects. This fits with a recent finding obtained with the use of the in vivo microdialysis technique that shows that the direct administration of nicotine into the NAS increases the local release of DA (17). Since increased activity of the mesolimbic DA system is associated with hyperactivity, it would be predicted that the administration of a nicotinic agonist into the NAS would produce hyperactivity. Our data support this prediction.

Taken together, the data reported here support the notion that nicotine can interact with dopaminergic substrates at the level of the NAS to influence locomotor activity. Thus, nicotinic stimulation appears to have psychomotor stimulant effects involving the NAS DA terminals, in addition to those involving the VTA DA cell bodies.

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