*Pharmacology Biochemistry & Behavior*, Vol. 35, pp. 735–737. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

# Locomotion Induced by Ventral Tegmental Microinjections of a Nicotinic Agonist

ENRICO MUSEO AND ROY A. WISE[1]

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

Received 31 May 1989

MUSEO, E. AND R. A. WISE. *Locomotion induced by ventral tegmental microinjections of a nicotinic agonist.* PHARMACOL BIOCHEM BEHAV **35**(3) 735–737, 1990.—Bilateral microinjections of the nicotinic agonist cytisine (0.1, 1 or 10 nanomoles per side) into the ventral tegmental area increased locomotor activity. This increase in locomotion was antagonized by mecamylamine (2 mg/kg, IP), a nicotinic antagonist that readily crosses the blood-brain barrier, and by pimozide (0.3 mg/kg, IP), a central dopaminergic antagonist. Hexamethonium (2 mg/kg, IP), a nicotinic antagonist that, unlike mecamylamine, does not cross the blood-brain barrier, had no effect; this suggests that mecamylamine's attenuation of cytisine-induced locomotor activity resulted from a blockade of central and not peripheral nicotinic receptors. The data support the notion that nicotinic and dopaminergic substrates interact at the level of the VTA to produce increases in locomotor activity.

Cytisine Nicotine Ventral tegmental area Locomotion Dopamine

THE mesolimbic dopamine (DA) system has been implicated in locomotor behavior (4). Axons originating in the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAS) appear to be involved (6). The administration of compounds that increase activity in the mesolimbic DA system augment locomotor activity (12); systemically administered amphetamine, for example, increases NAS DA release (5) and facilitates locomotion (14).

The systemic administration of nicotine also enhances locomotor activity (1); the same effect has been reported following intra-VTA injections of cytisine, a potent nicotinic agonist (11). The effect on locomotion seems to result from the activation of the mesolimbic DA system; nicotinic binding sites have been localized in the VTA as well as the NAS (2,3), and the number of these binding sites is markedly reduced by 6-OHDA-induced degeneration of the medial forebrain bundle DA fibers (3). In addition, dopaminergic neurons in the VTA increase their rate of firing following either the iontophoretic (8) or systemic (10) administration of nicotine. Systemic nicotine also increases the release of DA in the NAS (5, 9, 13); this release is believed to be regulated by receptors located on cell bodies and terminals (2,15).

The present experiments were designed to further investigate the preliminary finding of Pert and Chiueh (11) that ventral tegmental injections of the nicotinic agonist cytisine stimulate locomotor activity. The basic finding was replicated and three pharmacological challenges were used to further characterize the mechanism of cytisine's effect.

## METHOD

Twenty-one male Long Evans hooded rats weighing 350–400 g were implanted bilaterally with 22-ga guide cannulae under pentobarbitol (60 mg/kg, IP) anaesthesia. The guide cannulae were aimed at the VTA with their tips located 1.5 mm above the final injection site. Guide cannulae were stereotaxically implanted at a 10 degree angle; with the incisor bar set 5 mm superior to the interaural line the coordinates were 2.5 mm posterior to bregma, 2.0 mm lateral to the midsagittal suture and 7.1 mm ventral to the skull surface. 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 a 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

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

connected via an electrical interface to a computer placed in an adjoining room. During habituation and test sessions the room was dark and white noise (60 dB) was continuously present.

Bilateral VTA injections were made while animals were free to move in a plastic container (20 × 40 × 16 cm). Drug or vehicle (sterile physiological saline) solutions were injected in a volume of 0.5 μl per side over 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. In order to maximize drug diffusion the injector cannulae were left in place for 60 sec following the injections. Cytisine, hexamethonium bromide and mecamylamine (Sigma) were each dissolved in sterile physiological saline, whereas pimozide (Janssen Pharmaceutica) was dissolved in a 0.1 molar solution of tartaric acid.

All animals were tested with each of two doses of cytisine [1 and 10 nanomoles (nmol)] and one vehicle injection in a randomized repeated measures design. Following the testing of these doses two additional doses (0.01 and 0.1 nmol) were tested in an attempt to determine a threshold dose below which locomotion was not facilitated. One day 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, and locomotor activity was recorded during a 60-minute session.

In a second experiment animals were randomly assigned to one of three groups. The first group (n = 6) received an injection of the nicotinic antagonist mecamylamine (2 mg/kg, IP) 20 minutes prior to an intra-VTA injection of cytisine (10 nmol); a second group (n = 7) was administered the nicotinic antagonist hexamethonium (2 mg/kg, IP) 20 minutes prior to an intra-VTA cytisine injection; and a third group (n = 7) received an injection of pimozide (0.3 mg/kg, IP) five hours prior to intra-VTA cytisine.

Following the experiments animals were anaesthetized (chloral hydrate: 400 mg/kg) and 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.

## RESULTS

Only animals with both injector tips located in the VTA were included in the data analyses. Of the 21 animals implanted with guide cannulae three were not included in the statistical analyses; one died following surgery and two had at least one injector tip located outside the VTA.

A dose-dependent effect on locomotion was revealed by an analysis of variance conducted on the mean locomotor counts for each of the treatments, $F(4,68) = 4.32$, $p<0.003$; each of the three highest doses was found to produce statistically reliable (Tukey test, $p<0.01$) increases in locomotion (Fig. 1). Animals administered the nicotinic antagonist mecamylamine prior to receiving intra-VTA cytisine locomoted less than when they were administered cytisine alone, $t(4) = 2.8$, $p<0.05$ (Fig. 2A). Hexamethonium, a nicotinic antagonist that does not have easy access to the CNS, did not attenuate cytisine-induced locomotion, $t(5) = 0.6$, $p>0.05$ (Fig. 2B). The DA antagonist pimozide blocked the increase in locomotion seen following intra-VTA cytisine, $t(6) = 6.8$, $p<0.01$ (Fig. 2C).

In order to ensure that the attenuation of cytisine-induced locomotion by mecamylamine and pimozide was not due to a general inhibitory effect these antagonists may have on locomotion, an additional experiment was conducted wherein 32 naive animals were randomly assigned to one of four treatments; in this way the effects of the three antagonists on spontaneous locomotor activity were compared to those of saline. While mecamylamine appeared to increase locomotion in comparison to saline, and

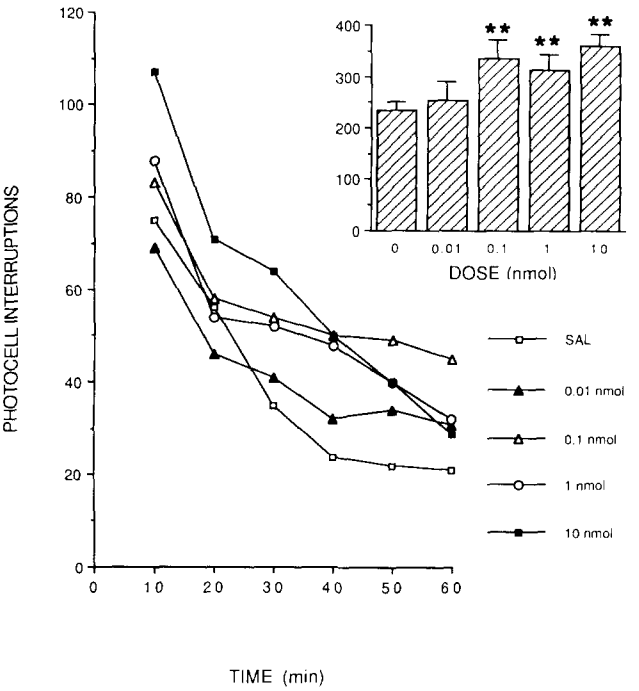


FIG. 1. Mean number of photocell interruptions for each of five intra-VTA treatments plotted in 10-min intervals. The inset figure shows mean total photocell interruptions for the 60-minute test sessions. $^{**}p<0.01$.

pimozide appeared to decrease locomotion, an analysis of variance conducted on the locomotor counts for each of the treatments revealed that these trends were not statistically significant, $F(3,28) = 2.46$, $p>0.05$.

## DISCUSSION

The systemic administration of nicotine is known to increase locomotor activity in rats (1). The present study confirms an earlier report that intra-VTA injections of the nicotinic agonist cytisine can have this behavioral effect (11). These data are in agreement with the notion that the mesolimbic DA system plays a role in mediating increases in locomotion produced by systemic nicotine. The behavioral activation by intra-VTA cytisine fits with autoradiographic and electrophysiological reports showing, respectively, the presence of nicotinic receptors in the VTA (2,3) and increased DA cell firing following the iontophoretic application of nicotine (8). In addition, evidence based on the use of the in vivo microdialysis technique shows that systemic nicotine increases the release of DA in the NAS (5,9) and that the release of DA in the NAS correlates with locomotor activity (7). Since the iontophoresis of nicotine onto DA cells in the VTA increases the firing of these cells, and since increased activity of the mesolimbic DA system is associated with hyperactivity, it would be predicted that the administration of a nicotinic agonist in the VTA would produce hyperactivity. We confirm that the administration of the nicotinic agonist cytisine into the VTA increases locomotor activity and that this effect on locomotion is seen across a large range of doses.

In addition, we report that the nicotinic antagonist mecamylamine antagonized intra-VTA cytisine's facilitation of locomotion, whereas the nicotinic antagonist hexamethonium did not. Although these results are preliminary, they suggest that mecamylamine's attenuation of cytisine-induced locomotion was centrally

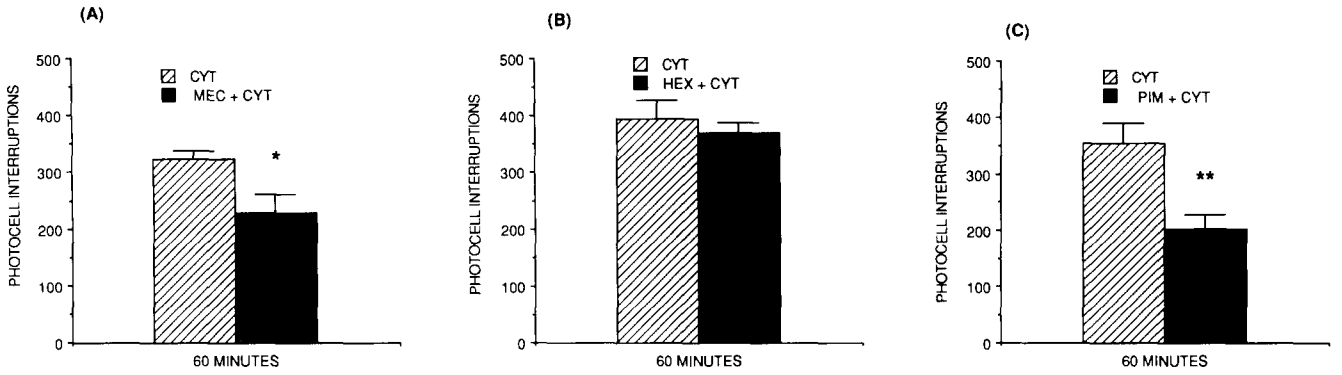


FIG. 2. Effects of (A) mecamylamine (MEC: 2 mg/kg), (B) hexamethonium (HEX: 2 mg/kg) and (C) pimozide (PIM: 0.3 mg/kg) on the mean number of photocell interruptions produced by bilateral intra-VTA injections of cytisine (CYT: 10 nmol). *$p<0.05$; **$p<0.01$.

mediated: both mecamylamine and hexamethonium block peripheral nicotinic receptors, but only mecamylamine has easy access to the CNS. On the basis of the tests with these drugs alone, the decrease in cytisine-induced locomotor activity seen in animals pretreated with mecamylamine or pimozide is interpreted as reflecting the attenuation by these drugs of cytisine's excitatory effect, and is not likely to be due to a general inhibitory effect on locomotion, especially in the case of mecamylamine. Taken together, the data reported here support the notion that nicotinic and dopaminergic substrates interact at the level of the VTA to influence locomotor activity.

## REFERENCES

1. Clarke, P. B. S.; Kumar, R. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78: 329–337; 1983.
2. Clarke, P. B. S.; Pert, A. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res. 348:355–358; 1985.
3. Clarke, P. B. S.; Pert, C. B.; Pert, A. Autoradiographic distributions of nicotine receptors in rat brain. Brain Res. 323:390–395; 1984.
4. Costall, B.; Naylor, R. J. The behavioural effects of dopamine applied intracerebrally to areas of the mesolimbic system. Eur. J. Pharmacol. 32:87–92; 1975.
5. Di Chiara, G.; Imperato, A.; Mulas, A. Preferential stimulation of dopamine release in the mesolimbic system: a common feature of drugs of abuse. In: Sandler, M., *et al.*, eds. Neurotransmitter interactions in the basal ganglia. New York: Raven Press; 1987: 171–182.
6. Fink, J. S.; Smith, G. P. Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res. 199:359–384; 1980.
7. Freed, C. R.; Yamamoto, B. K. Regional brain dopamine metabolism: a marker for the speed, direction, and posture of moving animals. Science 229:62–65; 1985.
8. Grenhoff, J.; Aston-Jones, G.; Svennson, T. H. Nicotinic effects on the firing pattern of midbrain dopamine neurons. Acta Physiol. Scand. 128:351–358; 1986.
9. Imperato, A.; Mulas, A.; Di Chiara, G. Nicotine preferentially stimulates dopamine release in the limbic system of the freely moving rat. Eur. J. Pharmacol. 132:337–338; 1986.
10. Mereu, G.; Yoon, K. P.; Boi, V.; Gessa, G. L.; Naes, L.; Westfall, T. C. Preferential stimulation of ventral tegmental dopaminergic neurons by nicotine. Eur. J. Pharmacol. 141:395–399; 1987.
11. Pert, A.; Chiueh, C. C. Effects of intracerebral nicotinic agonists on locomotor activity: involvement of mesolimbic dopamine. Soc. Neurosci. Abstr. 12:917; 1986.
12. Pijnenberg, A. J. J.; Honig, W. M. M.; Heyden, J. A. M.; Van Rossum, J. M. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. Eur. J. Pharmacol. 35: 45–58; 1976.
13. Rowell, P. T.; Carr, L. A.; Garner, A. C. Stimulation of [$^3$H] dopamine release by nicotine in rat nucleus accumbens. J. Neurochem. 49:1449–1454; 1987.
14. Segal, D. S. Behavioral characterization of d- and l-amphetamine: Neurochemical implications. Science 190:475–477; 1975.
15. Westfall, T. C. Effect of nicotine and other drugs on the release of $^3$H-norepinephrine and $^3$H-dopamine from rat brain slices. Neuropharmacology 13:693–700; 1976.