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Both Nicotinic and Muscarinic Receptors in Ventral Tegmental Area Contribute to Brain-Stimulation Reward

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YEOMANS, J. AND M. BAPTISTA. *Both nicotinic and muscarinic receptors in ventral tegmental area contribute to brain-stimulation reward.* PHARMACOL BIOCHEM BEHAV **57**(4) 915–921, 1997.—Cholinergic neurons of the pedunculopontine tegmental nucleus (Ch5) and laterodorsal tegmental nucleus (Ch6) monosynaptically activate dopamine neurons of the substantia nigra and ventral tegmental area (VTA) via nicotinic and muscarinic receptors. The nicotinic receptors near the VTA have been proposed to be important for nicotine self-administration in rats and for tobacco smoking in humans. Nicotinic and muscarinic blockers were microinjected into the VTA of rats trained to lever-press for lateral hypothalamic stimulation via an ipsilateral electrode. The competitive nicotinic blocker dihydro-β-erythroidine (DHβE; 5–60 μg) shifted rate–frequency curves to the right by a mean of 6–27% in a dose-related manner; the noncompetitive nicotinic blocker mecamylamine (10–300 μg) produced similar shifts of 7–21%. Atropine (30 μg) shifted the curves to the right by a mean of 82% in three of the sites tested with DHβE. All blockers decreased maximum bar-pressing rates significantly in some sites when the shifts were large. Therefore, nicotinic receptors in the VTA make small contributions to the maintained rewarding effect of brain-stimulation reward in rats, but muscarinic receptors in the VTA appear to be more important. © 1997 Elsevier Science Inc.

Self-stimulation Dopamine Dihydro-β-erythroidine Mecamylamine Atropine Mesopontine Pedunculopontine Cholinergic

CHOLINERGIC input to ventral tegmental area (VTA) and substantia nigra (SN) dopamine cells occurs via monosynaptic projections from mesopontine cholinergic neurons of the pedunculopontine and laterodorsal tegmental nuclei (4,9,17–19,33). Both nicotinic and muscarinic receptors are found near dopamine cells of the VTA or SN, and these receptors or mRNA for these receptors is lost when VTA or SN dopamine cells are killed with 6-hydroxydopamine (11,42,44).

Nicotinic and muscarinic agonists both directly excite VTA or SN dopamine neurons in vitro, apparently via postsynaptic receptors on dopamine cells (5,27). Nicotinic and other cholinergic agonists (e.g., carbachol, neostigmine) applied to the VTA or SN in vivo increase dopamine efflux in the nucleus accumbens or striatum (2,3,20). Systemically applied nicotine increases dopamine release, especially in the nucleus accumbens (22), and this response is blocked by the nicotinic blocker mecamylamine infused into the VTA (34).

Muscarinic receptors in the VTA appear to be important to the reward functions of these dopamine cells. Carbachol (1 or 3 μg), a mixed muscarinic–nicotinic agonist, injected into the VTA induced a conditioned place preference (53). A muscarinic antagonist injected near the VTA reduced brain-stimulation reward lever-pressing rates (26), whereas injection of acetylcholine into the VTA increased rates (37). Muscarinic blockers introduced into the VTA increased the frequency required to produce a criterion rate for hypothalamic or dorsal tegmental brain-stimulation reward by 50–500% (24,25,53). These frequency increases induced by atropine (30 or 60 μg) in the VTA were reversed by pretreatment with carbachol (2 or 3 μg) in the VTA (24). The weak or nonexistent effects of atropine on peak bar-pressing rates were contrasted with the large shift in required frequency.

Recently, nicotinic receptors in the VTA have also been proposed to be important for reward (7). Lesions of mesolim-

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bic dopamine terminals reduced self-administration rates for intravenous (IV) nicotine (14). Introduction of the nicotinic blocker DHβE (18 and 30 μg) into the VTA decreased bar-pressing rates for IV nicotine, whereas the muscarinic antagonist atropine (18 and 30 μg) had no effect (13). The locomotor stimulating effects of nicotinic agonists in the VTA (8,32,36) did not appear to be responsible for these effects (13). These results suggest that nicotinic receptors in the VTA may be one major route by which self-administered nicotine in cigarettes leads to nicotine abuse in humans (7).

Unfortunately, the lack of a sharp dose–response curve for self-administration of nicotine in rats makes it difficult to determine the size of the reward-inhibiting effects of nicotinic antagonists (12,13,28). At both doses of DHβE, the rats self-administered nicotine at almost half-maximum rates on average, an effect not seen after 6-OHDA lesions of mesolimbic terminals, after which self-administration rates declined to operant levels over 5 days (13,14).

In the present experiments, brain-stimulation reward rate–frequency curves were measured following VTA administration of two nicotinic antagonists. The advantages of brain-stimulation reward are: a) the stability of the steep rate–frequency curve lasts for weeks of testing, allowing repeated tests in the same sites; b) the strength of the reward signal can be measured quantitatively by the lateral shift in the rate–frequency curve (6,16,47,50). Brain-stimulation reward rate–intensity or rate–frequency curves have been used to assess the weak or delayed reward-facilitating effects of single or repeated injections of systemic nicotine (1,10,15,21).

Brain stimulation in medial forebrain bundle sites activates reward mainly by directly activating myelinated axons (39,49). This reward can be blocked by local injections near dopamine cells or terminals (25,41,46,53), suggesting that the myelinated axons directly activated by brain-stimulation reward are excitatory afferents to dopamine cells (39). Because brain-stimulation reward can also be blocked by cholinergic blockers near cholinergic terminals on dopamine cells, or by cholinergic agonists near the mesopontine cholinergic cells (55), Ch5 and Ch6 cells may relay brain-stimulation reward signals from the directly activated myelinated axons of the medial forebrain bundle to the dopamine cells. Therefore, Ch5 and Ch6 cholinergic neurons may be a crucial link in the major reward systems of the medial forebrain bundle and dorsal tegmentum (49).

In this study, brain-stimulation reward rate–frequency curves are tested following VTA injections of nicotinic blockers DHβE and mecamylamine. Doses of DHβE were used that were effective in reducing self-administration rates for nicotine (13). As a control, a dose of atropine that was ineffective in altering nicotine self-administration (13), but effective in shifting brain-stimulation reward thresholds (24), was tested in the same VTA sites tested with DHβE.

METHODS

Surgery

Under pentobarbital anesthesia (60 mg/kg), 15 male Wistar rats (Charles River, Canada) were implanted with 250-μm-diameter monopolar stainless steel electrodes with hemispherical tips. These electrodes were aimed for the medial forebrain bundle at the level of the lateral hypothalamus (2.6 mm posterior to bregma, 1.8 mm lateral to the midline, 9.2 mm below the dura, with the lambda–bregma line placed horizontally). A guide cannula was implanted into the VTA (4.8 mm posterior to bregma, 0.8 mm lateral to the midline, 8.5 mm below the dura) ipsilateral to the electrode.

Procedure

All rats were allowed 1 week to recover from surgery before training began. They were placed in a Plexiglas operant chamber (30 × 30 × 28 cm) with a 5-cm-wide lever protruding 4 cm into the chamber. Each rat was trained to bar-press for 0.5-s trains of monophasic, 0.14-ms-duration, constant-current cathodal pulses. All rats pressed at rates over 40/min and received at least five sessions of training or baseline testing before formal testing began. During these preliminary sessions, currents were determined that evoked rate–frequency curves that rose rapidly at frequencies near 40 Hz. These currents, which ranged from 250 μA to 850 μA in different animals, were then held constant for each rat for all subsequent formal testing, during which only the frequency of stimulation was varied.

The stimulation parameters used in this experiment (large-surface-area electrodes, short duration pulses, and moderate currents) were chosen to maximize the direct activation of myelinated axons of the medial forebrain bundle (i.e., axons with absolute refractory periods of 0.4–1.2 ms and conduction velocities of 1–8 m/s) (31,39,47,48,56) known to be critical for brain-stimulation reward, and to minimize the direct activation of unmyelinated axons of the medial forebrain bundle (e.g., dopaminergic axons with refractory periods of 1.2–2.5 ms, conduction velocities below 1 m/s, and long-duration action potentials, or similar cholinergic axons) (18,23,49,54).

The frequencies used in this experiment ranged from 20 to 200 Hz and were varied via a computerized system described previously (6). Trials began with the entry of a retractable lever into the operant chamber and the simultaneous delivery of a 0.5-s train of pulses. A given frequency was available for the 70-s trial. Bar-presses were not counted during the first 10 s (due to variability in the distance and movement of the animal to the bar), so that bar-presses were recorded only in the last 60 s of each trial. At the end of the trial, the lever retracted from the chamber. After a 10-s pause, a new trial began with a new frequency available. The frequencies varied randomly from trial to trial until all frequencies in a range (say, 25–100 Hz) were tested at 0.1 log unit steps (e.g., 25, 32, 40, 50, 63, 79, 100 Hz). Stimulation parameters were monitored on an oscilloscope, and lever-presses for each trial recorded by an Apple 2E computer.

Baseline rate–frequency curves were measured for 30 min following vehicle injections (at least 3 complete curves), then for 90 min following drug injections (at least 10 complete curves). The effect of the injections was measured by comparing rate–frequency curves before and after injections. For atropine, only the 60 min following the start of testing after each injection are reported, because the atropine effect peaks between 10 and 40 min and declines quickly between 45 and 90 min (24,53). Each session began 5–10 min after the beginning of the injections, to allow for closing of the cannula, transfer of the rat to the cages, and start-up of the program; the beginning of the postinjection session is reported as time 0.

Drugs

All drugs were dissolved in sterile physiological saline (0.9% NaCl). Injectors were sterilized in ethanol, then rinsed in sterile saline, before and after each injection. All injections were made slowly over a 90-s period in a volume of 0.5 μl using a Hamilton microsyringe. No animal was given a drug test more often than once every 48 h. Doses of the competitive nicotinic antagonist DHβE (5, 15, 30, 60 μg, corresponding to 27–320 mM/0.5 μl in the injector) were tested in four to seven

rats in an ascending order. For the first three rats, only the doses (15, 30 μg) nearest those used by Corrigall et al. (13) were tested, to follow the procedure of that paper, then for subsequent rats the range of doses was expanded (5–60 μg).

Following DHβE tests, atropine (30 μg, or 44 mM) was tested in three of the rats previously tested with DHβE. In two of these rats, saline in the VTA was tested followed by saline 30 min later, to determine whether the order of injections affected the results. Finally, cytisine (4 μg, 21 mM) or nicotine (0.4 mg/kg SC) was tested in two rats each.

In an independent group of three rats, four doses of the noncompetitive nicotinic antagonist mecamylamine (10, 30, 100, and 300 μg, or 98–2940 mM in the injector) were tested in an ascending order for each rat.

Histology

After all behavioral testing, the rats were euthanized with pentobarbital and perfused with saline and 10% formalin. The brains were fixed in 10% formalin saturated with sucrose and allowed to soak for 24 h. The brains were then sectioned at 40 μm and stained with cresyl violet to determine electrode and cannula tip sites.

RESULTS

Baseline and Saline Tests

All rats were trained for at least 20 h over several days. During this period, maximum bar-pressing rates increased gradually and error bars decreased. Then, baseline rate–frequency curves were tested repeatedly until similar responding at each frequency was observed on at least two consecutive days. Complete rate–frequency curves are shown for two representative animals in Fig. 1. In all cases, bar-pressing rates rose quickly, in a sigmoidal fashion, as frequency increased. These rate–frequency curves were quite similar following two saline injections into VTA sites separated by 30 min, suggesting that the effect of order of injection is minimal in these conditions (Fig. 1). Previous studies have found similar results for repeated injections into the VTA of saline or of atropine on separate days (24,25). Means across animals are shown in Fig. 4 in the vehicle conditions.

Locations of electrodes in the lateral hypothalamus and cannulae near the VTA are shown in Fig. 2. The brains for M11 and M46 were lost.

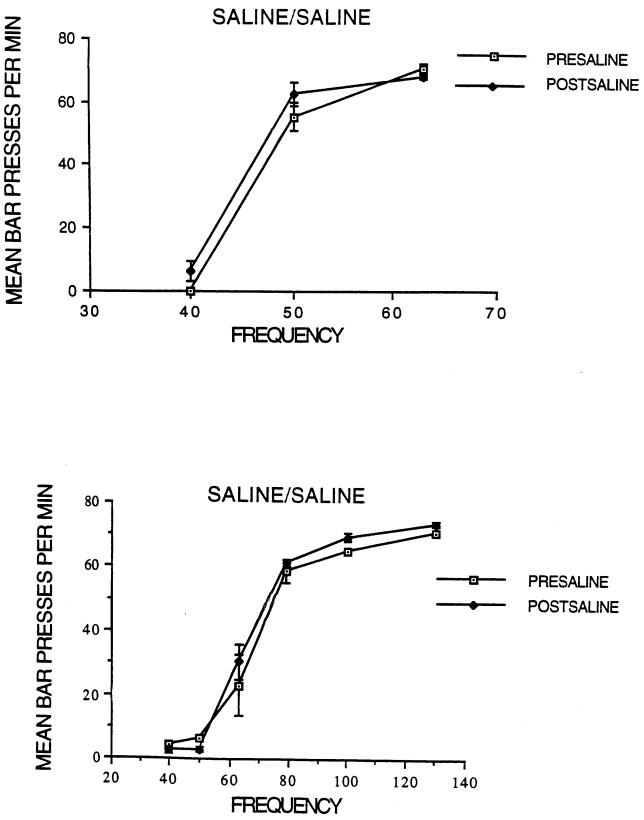

FIG. 1. Rate–frequency curves for two rats (upper and lower panels) following two saline injections into the VTA separated by 30 min. Presaline, mean results following first saline injection; postsaline, mean results following second saline injection.

Dihydro-β-Erythroidine

Injections of the competitive nicotinic blocker DHβE shifted rate–frequency curves to the right reliably [general linear model, $F(4, 20) = 13.84$, $p < 0.0001$]. Figure 3A shows one example (rat M13) after a dose of 30 μg DHβE. The required frequency (measured at half the maximum bar-pressing rate) shifted by 28.1% vs. the saline control. For this rat at this dose, the maximal bar-pressing rate declined significantly, from 68 to 52 bar-presses/min.

The mean shift in required frequency (measured at half the maximum bar-pressing rate on each curve) at all doses is shown in Fig. 4. The consistency of the results between sites is shown by the small error bars. The lowest dose of DHβE shifted the rate–frequency curves slightly to the right (mean of 6.2% for the five rats tested) relative to the saline control condition. No reliable effect on peak bar-pressing rate was observed (mean 56.3/min for DHβE vs. 54.8/min for saline).

The doses of DHβE (15 and 30 μg) that previously inhibited self-administration rates for nicotine (13), also shifted rate–frequency curves for brain-stimulation reward to the right reliably. Required frequencies were increased by a mean of 21.9% at 15 μg, and by 25.5% at 30 μg. Both doses also reduced peak bar-pressing rates (mean 62.8/min for 15 μg DHβE and 71.1/min for saline; mean 44.3/min for 30 μg DHβE and 52.7/min for saline) significantly in three of six sites in both groups.

The highest dose of DHβE (60 μg) shifted rate–frequency curves by a similar mean amount, but the results were more inconsistent. That is, for three of four rats the peak rates were strongly reduced (means 44.2/min for 60 μg DHβE and 63.8/min for saline), and two of the rats showed signs of toxicity or aversion, including Straub tail elevation. For these latter two rats, thresholds were elevated on subsequent days of testing, so no further testing was carried out. No further rats were tested at this 60-μg dose. For the first two rats, no such signs of toxicity and aversion were observed, and baseline thresholds were unaltered on subsequent days.

Individual comparisons indicated that the 5-μg dose of DHβE elevated required frequency by less than the 15-μg dose (HSD = 15.66, $p < 0.01$, using the Tukey–Kramer test), 30-μg dose (HSD = 19.3, $p < 0.01$), or 60-μg dose (HSD = 20.8, $p < 0.01$). Therefore, the effects of DHβE were dose related.

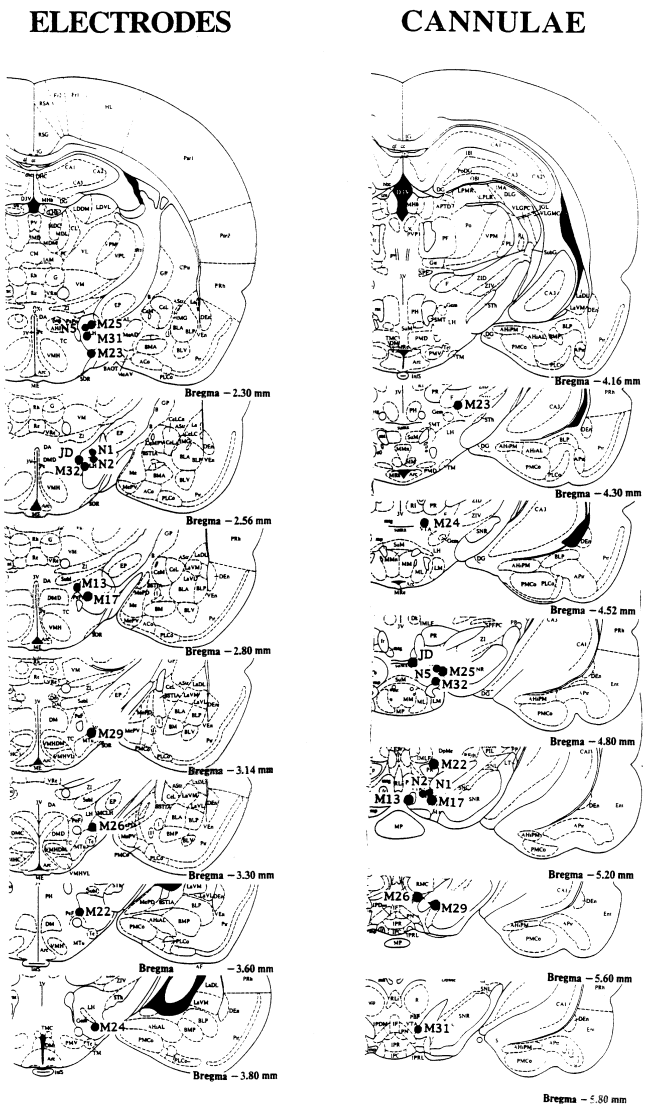

FIG. 2. Histologically determined sites of electrode and cannula tips shown on modified Paxinos and Watson (35) atlas sections. Left panels show electrode tip sites in the lateral hypothalamus; right panels show injection sites in and around the VTA.

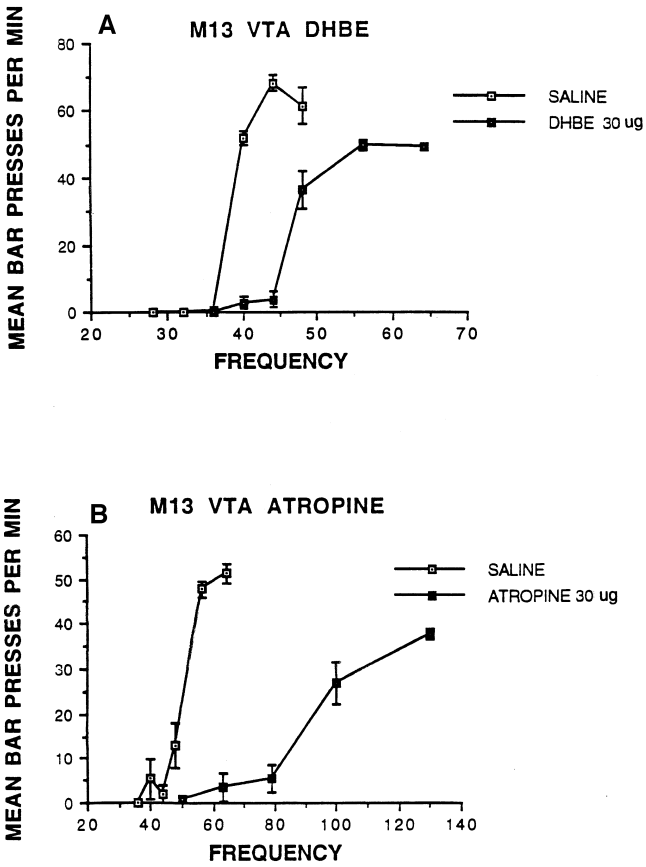

FIG. 3. (A) Rate–frequency curves for rat M13 following injections into the VTA of saline, then 30 μg DHβE, on a single day. (B) Effects of saline, then 30 μg atropine (both injected 2 days after the study in panel A).

Atropine

In three of the rats tested with two or four injections of DHβE, atropine (30 μg) was injected into the VTA site on another test day. For each of these sites, the rate–frequency curve shifted strongly to the right (i.e., required frequency increased by a mean of 82.3%) and shifted by more than for any DHβE dose. Figure 3B shows one example, in a site where DHβE was much less effective at the same dose.

Individual comparisons showed that atropine was more effective than all doses of DHβE (HSD = 76.13, 60.47, 56.83, and 55.33 for atropine vs. 5, 15, 30, and 60 μg DHβE, respectively, all $p < 0.01$). Peak bar-pressing rates were lowered substantially in all three sites tested (mean 34.5/min for 30 μg atropine and 51.4/min for saline). Because of the large shifts in required frequency, however, we may not have tested frequencies that were high enough to obtain the highest possible bar-pressing rates in all cases (e.g., Fig. 3B).

Nicotinic Agonists

Injections of nicotine (0.4 mg/kg SC, $n = 2$) had no apparent effect on rate–frequency curves. Required frequencies shifted by less than 2% in both cases. Cytisine in the VTA (4 μg, $n = 2$) raised required frequencies by 6.8%. Peak bar-pressing rates were not significantly altered.

Mecamylamine

The noncompetitive nicotinic antagonist mecamylamine increased required frequencies by amounts similar to those for DHβE, but less consistently. That is, at 10 μg the mean increase was 13% (SEM 9%), at 30 μg the shift was 21% (SEM 21%), at 100 μg the shift was 17% (SEM 15%), and at 300 μg the shift was 7% (SEM 23%) (Fig. 4). No reliable effect on peak bar-pressing rate was observed, as shown for one rat in Fig. 5.

GENERAL DISCUSSION

Nicotinic and Muscarinic Antagonists

The competitive nicotinic blocker DHβE raised required frequencies by up to 27% in a dose-related manner, suggest-

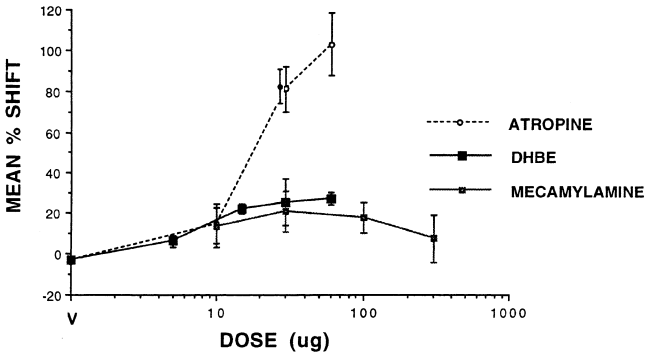

FIG. 4. Dose–response curves for DHβE, mecamylamine, and atropine injected near the VTA. Mean required frequencies for drug conditions, relative to same-day saline controls, are shown at each dose tested (±SEM). Previous results for atropine (24,25,55), using slightly different methods, are shown connected by dashed lines. The present results with atropine at a dose of 30 μg are shown as a solid circle. V, vehicle.

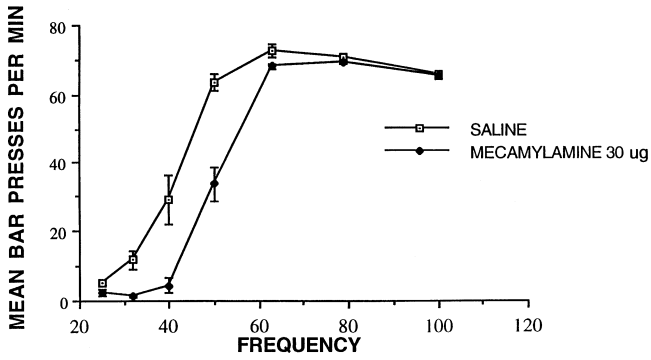

FIG. 5. Rate–frequency curve shift for mecamylamine (30 μg in the VTA) for one rat.

ing that nicotinic receptors in the VTA contribute to brain-stimulation reward. These effects appear to reach an asymptote in the range of doses used here, and the highest dose (60 μg) appeared to be toxic in two sites. The noncompetitive nicotinic blocker mecamylamine had effects of similar size, although less consistent. These results suggest that nicotinic receptors can contribute only up to 23% of the rewarding effect of brain-stimulation reward in the conditions of this experiment.

Atropine (30 μg) elevated required frequency by 82%. We have analyzed the results of three previous studies (24,25,53) that measured the effects of atropine (10, 30, and 60 μg) near the VTA on required frequency. To allow comparisons with the present results, all results (i.e., percent shift in required frequency relative to baseline) in the period 10–70 min postinjection were included for all animals with injection sites within 2 mm of the VTA and electrode sites in the lateral hypothalamus. Figure 4 (dashed lines) shows the dose–response curve for atropine in these previous studies. Vehicle injections (18 rats, 3 using Ringer's and 15 using artificial cerebrospinal fluid) in the VTA shifted thresholds hardly at all (mean shift of −3% relative to baseline). Ten micrograms of atropine shifted frequencies by 20%. Thirty micrograms of atropine shifted frequencies by 45–125% in seven rats, with a mean shift of 81%, which is consistent with the present results (mean shift 82%). Sixty micrograms of atropine shifted frequencies by a mean of 102% in 14 rats. At this highest dose, for the three rats with the strongest effects, however, the rats would not bar-press for 20–40 min postinjection, so 102% underestimates the size of the atropine effects at 60 μg. Therefore, the dose–response curve for atropine suggests no asymptotic limit.

One criticism of the atropine results is that atropine can be a weak local anesthetic. The effect of 60 μg atropine on brain-stimulation reward thresholds is blocked, however, by pretreatment with the muscarinic/nicotinic agonist carbachol (24). Because atropine has poor nicotinic binding affinity (45), muscarinic-like receptors appear to be the main receptors for the reward-blocking effects of atropine. Furthermore, atropine in the VTA at doses of 30 and 60 μg had no effect on nicotine self-administration, in sites where DHβE was effective (13). Therefore, local anesthesia cannot account for the main effects of atropine. The critical site for the atropine effect on brain-stimulation reward is localized to the lateral half of the VTA near dopamine cells (25), consistent with the locations of the cannulae in the present experiment.

These results suggest that both nicotinic and muscarinic receptors in the VTA are involved in brain-stimulation reward. Under the conditions of the present experiment, muscarinic receptors appear to be more important than nicotinic receptors to the maintenance of brain-stimulation reward, due to the stronger effect of atropine than either nicotinic blocker in the same sites and in the same dose ranges. This appearance may be misleading, however, because: a) the diffusability and receptor binding of the different blockers in vivo may not be directly comparable (45), and b) non-receptor-specific effects of the drugs, such as the weak local anesthetic effect of atropine, cannot be completely discounted (24).

Muscarinic receptors may be more important than nicotine receptors to brain-stimulation reward because of the lasting, repeatable effect of muscarine on dopamine cells, compared with the desensitizing effect of nicotine over repeated injections (5). Because the VTA injection sites were near the interpeduncular nucleus, nicotinic presynaptic receptors in the interpeduncular nucleus may have been blocked by DHβE (30).

Bar-Pressing Rates

The cholinergic blockers that were most effective in increasing required frequencies reduced peak bar-pressing rates in several sites as well. Kofman and colleagues (24,25,53) previously did not find changes in peak bar-pressing rates using doses of atropine of 30 and 60 μg, but did not run complete rate–frequency curves in any conditions. The present results support the association between activation of mesopontine cholinergic systems and locomotion (29), between nicotinic agonists in the VTA and locomotion (8,32,36), and between VTA dopamine activation and locomotion (46).

Circuits for Brain-Stimulation and Nicotine Reward

The present results further support the idea that brain-stimulation reward requires the maintained activation of mesopontine cholinergic neurons that monosynaptically activate dopamine neurons. The release of endogenous acetylcholine near dopamine cells should activate both nicotinic and muscarinic receptors. Muscarinic receptors remain sensitive after repeated testing (which contrasts with the decreased sensitivity of nicotinic receptors in activating dopamine cells) (27). The

maintained sensitivity of muscarinic receptors to acetylcholine release may explain the importance of muscarinic receptors to brain-stimulation reward over hours of testing in this paradigm.

By contrast, VTA nicotinic receptors, and not VTA muscarinic receptors, are important for the rewarding, dopamine-releasing, and locomotor effects of systemic nicotine (13,34, 36). Therefore, these effects of nicotine are not likely to occur via activation of mesopontine cholinergic cells (as appears to occur in brain-stimulation reward), but rather directly via nicotine receptors near dopamine cells. Corrigall et al.'s (13) preliminary results that partial ibotenate lesions of the pedunculopontine nucleus failed to inhibit nicotine self-administration similarly indicate that the cholinergic neurons are not necessary if postsynaptic nicotine receptors are intact.

By contrast, Yeomans et al. (55) found that cholinergic cells in the mesopontine region are important for brain-stimulation reward. Injections of carbachol (1–4 μg) near the pedunculopontine tegmental nucleus raised required frequency by 100% to over 400% in a dose-related manner, whereas scopolamine reduced required frequency by 20–80%. Heavy anatomical projections from the lateral and rostral hypothalamus to the pedunculopontine and laterodorsal tegmental nuclei via the medial forebrain bundle may be important (38,40). We tentatively propose that myelinated axons of the medial forebrain bundle may activate Ch5 and/or Ch6 cholinergic neurons, which, in turn, activate VTA dopaminergic neurons to induce brain-stimulation reward.

Receptor Subtypes

Because muscarinic receptors in the VTA are able to activate dopamine cells strongly for long periods and are critical in brain-stimulation reward, the identification of that muscarinic receptor type is especially important for understanding dopamine functions in drug and natural rewards and in chronic diseases (schizophrenia, Parkinson's disease) (51).

The genetic muscarinic receptor subtype most clearly identified in the VTA and substantia nigra, zona compacta is the rare m5 type. Messenger RNA for the m5 receptor is found in association with dopamine cells and D2 receptors (42,44). Evidence using antisense oligonucleotides indicates that this m5 receptor is important for brain-stimulation reward (52). Therefore, drugs that target this m5 receptor selectively might be useful for controlling drug abuse and schizophrenia.

Several nicotinic receptor subunits are found in the SN and the VTA, especially the α4 and β2 subunits [e.g., (43)]. The functions of these nicotinic subtypes in VTA have not yet been explored.

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REFERENCES

1. Bauco, P.; Wise, R. A.: Potentiation of lateral hypothalamic and midline mesencephalic brain stimulation reinforcement by nicotine: Examination of repeated treatment. J. Pharmacol. Exp. Ther. 272:294–301; 1994.
2. Blaha, C. D.; Allen, L. F.; Das, S.; Inglis, W. L.; Latimer, M. P.; Vincent, S. R.; Winn, P.: Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact, pedunculopantine tegmental nucleus-lesioned and laterodorsal tegmental nucleus-lesioned rats. J. Neurosci. 16:714–722; 1996.
3. Blaha, C. D.; Winn, P.: Modulation of dopamine efflux in the striatum following cholinergic stimulation of the substantia nigra in intact and pedunculopontine tegmental nucleus-lesioned rats. J. Neurosci. 13:1035–1044; 1993.
4. Bolam, J. P.; Francis, C. M.; Henderson, Z.: Cholinergic input to dopaminergic neurons in the substantia nigra: A double immunocytochemical study. Neuroscience 41:483–494; 1991.
5. Calabresi, P.; Lacey, M. G.; North, R. A.: Nicotinic excitation of rat ventral tegmental neurons in vitro studied by intracellular recording. Br. J. Pharmacol. 98:135–140; 1989.
6. Campbell, K. A.; Evans, G.; Gallistel, C. R.: A microcomputer-based method for physiologically interpretable measurement of the rewarding efficacy of brain stimulation. Physiol. Behav. 35: 395–403; 1985.
7. Clarke, P. B. S.: Mesolimbic dopamine activation—The key to nicotine reinforcement? In: Bock, G. and Marsh, J., eds. The biology of nicotine dependence. Chichester, UK: Wiley; 1990:153–168.
8. Clarke, P. B. S.; Fu, D. S.; Jakubovic, A.; Fibiger, H. C.: Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant actions of nicotine in rats. J. Pharmacol. Exp. Ther. 246:701–708; 1988.
9. Clarke, P. B. S.; Hommer, D. W.; Pert, A.; Skirboll, L. R.: Innervation of substantia nigra neurons by cholinergic afferents from pedunculopontine nucleus in the rat: Neuroanatomical and electrophysiological evidence. Neuroscience 23:1011–1019; 1988.
10. Clarke, P. B. S.; Kumar, R.: Effects of nicotine and amphetamine on intracranial self-stimulation in a shuttle box in rats. Psychopharmacology 84:109–114; 1983.
11. Clarke, P. B. S.; Pert, A.: Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic neurons. Brain Res. 348: 355–358; 1985.
12. Corrigall, W. A.; Coen, K. M.: Nicotine maintains robust self-administration in rats on a limited access schedule. Psychopharmacology 104:473–478; 1989.
13. Corrigall, W. A.; Coen, K. M.; Adamson, K. L.: Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res. 653:278–284; 1994.
14. Corrigall, W. A.; Franklin, K. B. J.; Coen, K. M.; Clarke, P. B. S.: The mesolimbic dopaminergic system is implicated in the reinforcing effects of nicotine. Psychopharmacology 107:285–289; 1992.
15. Druhan, J.; Fibiger, H. C.; Phillips, A. G.: Differential effects of cholinergic drugs on discriminative cues and self-stimulation produced by electrical stimulation of the ventral tegmental area. Psychopharmacology 97:331–338; 1989.
16. Edmonds, D. E.; Gallistel, C. R.: Reward versus performance in self-stimulation: Electrode specific effects of α-methyl-*p*-tyrosine on reward in the rat. J. Comp. Physiol. Psychol. 91:962–974; 1977.
17. Fujimoto, K.; Ikeguchi, K.; Yoshida, M.: Decrease and recovery of choline acetyltransferase activity in medial thalamus and ventral tegmental area after destruction of pedunculopontine areas in the rat. Neurosci. Res. 9:48–53; 1990.
18. Futami, T.; Kitai, S. T.; Takakusaki, E.: Glutamatergic and cholinergic inputs from the pedunculopontine tegmental nucleus to dopamine neurons in the substantia nigra pars compacta. Neurosci. Res. 21:331–342; 1995.
19. Gould, E.; Woolf, N. J.; Butcher, L. L.: Cholinergic projections to the substantia nigra from the pedunculopontine and laterodorsal tegmental nuclei. Neuroscience 28:611–623; 1989.
20. Hernandez-Lopez, S.; Gongora-Alfaro, J. L.; Martinez-Fong, D.; Aceves, J. A.: Cholinergic input to the substantia nigra pars com-

pacta increases striatal dopamine metabolism measured by in vivo voltammetry. Brain Res. 598:114–120; 1992.
21. Huston-Lyons, D.; Kornetzky, C.: Effects of nicotine on the threshold for rewarding brain stimulation in rats. Pharmacol. Biochem. Behav. 41:755–759; 1992.
22. Imperato, A.; Mulas, A.; Di Chiara, G.: Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur. J. Pharmacol. 132:337–338; 1986.
23. Kayama, Y.; Ogawa, T.: Electrophysiology of ascending, possibly cholinergic neurons in the rat laterodorsal tegmental nucleus: Comparison with monoamine neurons. Neurosci. Lett. 77:277–282; 1987.
24. Kofman, O.; McGlynn, S.; Olmstead, M. C.; Yeomans, J. S.: Differential effects of atropine, procaine and dopamine in the rat ventral tegmentum on lateral hypothalamic rewarding brain stimulation. Behav. Brain Res. 38:55–68; 1990.
25. Kofman, O.; Yeomans, J. S.: Cholinergic antagonists in ventral tegmentum elevate thresholds for lateral hypothalamic and brainstem self-stimulation. Pharmacol. Biochem. Behav. 31:547–559; 1989.
26. Kraus, V. A.; Lapina, I.: Effect of direct electrical and chemical stimulation on the excitability of the hypothalamic nuclei and the self-stimulation reaction in dogs. Zh. Vyssh. Nervn. Deyat. Im. I. P. Pavlova 22:1226–1232; 1972.
27. Lacey, M. G.; Calabresi, P.; North, R. A.: Muscarine depolarizes substantia nigra zona compacta and ventral tegmental neurons in vitro through M1-like receptors. J. Pharmacol. Exp. Ther. 253: 395–400; 1991.
28. Latiff, A. A.; Smith, L. A.; Lang, W. J.: Effects of changing dosage and urinary pH in rats self-administering nicotine on a food delivery schedule. Pharmacol. Biochem. Behav. 13:209–213; 1980.
29. Mathur, A.; Yeomans, J. S.: Locomotion and stereotypy induced by microinjections of scopolamine into the pedunculopontine nucleus: Involvement of dopamine cells. Soc. Neurosci. Abstr. 19:758; 1993.
30. McGehee, D. S.; Heath, M. J. S.; Gelber, S.; Devay, P.; Role, L. W.: Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269:1692–1696; 1995.
31. Murray, B.; Shizgal, P.: Behavioral measures of conduction velocity and refractory period for reward-relevant axons in the anterior LH and VTA. Physiol. Behav. 59:643–652; 1996.
32. Museo, E.; Wise, R. A.: Locomotion induced by ventral tegmental microinjections of a nicotinic agonist. Pharmacol. Biochem. Behav. 35:735–737; 1990.
33. Niijima, K.; Yoshida, M.: Activation of mesencephalic dopamine neurons by chemical stimulation of the nucleus tegmenti pedunculopontinus pars compacta. Brain Res. 451:163–171; 1988.
34. Nisell, M.; Nomikos, G. G.; Svensson, T. H.: Systemic nicotine induced dopamine release in the rat nucleus accumbens is regulated by nicotinic receptors in the ventral tegmental area. Synapse 16:36–44; 1994.
35. Paxinos, G.; Watson, C.: The rat brain in stereotaxic coordinates, 2nd ed. Sydney: Academic Press; 1986.
36. Reavill, C.; Stolerman, I. P.: Locomotor activity in rats after administration of nicotinic agonists intracerebrally. Br. J. Pharmacol. 99:273–278; 1990.
37. Redgrave, P.; Horrell, R. I.: Potentiation of central reward by localized perfusion of acetylcholine and 5-hydroxytryptamine. Nature 262:305–307; 1976.
38. Semba, K.; Fibiger, H. C.: Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: A retro- and antero-grade transport and immunohistochemical study. J. Comp. Neurol. 323:387–410; 1992.
39. Shizgal, P.; Bielajew, C.; Corbett, D.; Skelton, R.; Yeomans, J. S.: Collision-like effect from concurrent electrical stimulation of two rewarding sites of the brain. J. Comp. Physiol. Psychol. 94:227–237; 1980.
40. Steininger, T. L.; Rye, D. B.; Wainer, B. H.: Afferent projections to the cholinergic pedunculopontine nucleus and adjacent midbrain extrapyramidal area in the albino rat. I. Retrograde tracing studies. J. Comp. Neurol. 321:515–543; 1992.
41. Stellar, J. R.; Corbett, D.: Regional neuroleptic microinjections indicate a role for nucleus accumbens in lateral hypothalamic self-stimulation reward. Brain Res. 477:126–143; 1989.
42. Vilaro, M. T.; Palacios, J. M.; Mengod, G.: Localization of m5 muscarinic receptor mRNA in rat brain examined by in situ hybridization histochemistry. Neurosci. Lett. 114:154–159; 1990.
43. Wada, E.; Wada, K.; Boulter, J.; Deneris, E.; Heinemann, S.; Patrick J.; Swanson, L. W.: Distribution of $\alpha 2$, $\alpha 3$, $\alpha 4$, and $\beta 2$ neuronal nicotinic receptor subunit mRNAs in the central nervous system: A hybridization study in the rat. J. Comp. Neurol. 284: 314–335; 1989.
44. Weiner, D. M.; Levey, A. I.; Brann, M. R.: Expression of muscarinic acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proc. Natl. Acad. Sci. USA 87:7050–7054; 1990.
45. Williams, M.; Robinson, J. L.: Binding of the nicotinic cholinergic antagonist dihydro-beta-erythroidine, to rat brain tissue. J. Neurosci. 4:2906–2911; 1984.
46. Wise, R. A.; Rompre, P.-P.: Brain dopamine and reward. Annu. Rev. Psychol. 40:191–225; 1989.
47. Yeomans, J. S.: Quantitative measurement of neural post-stimulation excitability with behavioral methods. Physiol. Behav. 15: 593–602; 1975.
48. Yeomans, J. S.: Absolute refractory periods of self-stimulation neurons. Physiol. Behav. 22:911–919; 1979.
49. Yeomans, J. S.: Mechanisms of brain-stimulation reward. In: Epstein, A. N.; Morrison, A. R., eds. Progress in psychobiology and physiological psychology, vol. 13. New York: Academic Press; 1988:227–266.
50. Yeomans, J. S.: Principles of brain stimulation. New York: Oxford University Press; 1990.
51. Yeomans, J. S.: Role of tegmental cholinergic neurons in dopaminergic activation, antimuscarinic psychosis and schizophrenia. Neuropsychopharmacology 12:3–16; 1995.
52. Yeomans, J. S.; Baptista, M.; Takeuchi, J.; Kofman, O.; Ralph, M. R.: The role of nicotinic and muscarinic receptors in the ventral tegmental area in intracranial self-stimulation. Soc. Neurosci. Abstr. 21:732; 1995.
53. Yeomans, J. S.; Kofman, O.; McFarlane, V.: Cholinergic involvement in lateral hypothalamic rewarding brain stimulation. Brain Res. 329:19–26; 1985.
54. Yeomans, J. S.; Maidment, N. S.; Bunney, B. S.: Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells. Brain Res. 450:86–93; 1988.
55. Yeomans, J. S.; Mathur, A.; Tampakeras, M.: Rewarding brain stimulation: Role of tegmental cholinergic neurons that activate dopamine neurons. Behav. Neurosci. 107:1077–1087; 1993.
56. Yeomans, J. S.; Mercouris, N.; Ellard, C.: Behaviorally measured refractory periods are lengthened by reducing electrode tip exposure or raising current. Behav. Neurosci. 99:913–928; 1985.