

Midbrain acetylcholine and glutamate receptors modulate accumbal dopamine release

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This study determined the role of ventral tegmental area acetylcholine and glutamate receptors in modulating laterodorsal tegmentum stimulation-evoked dopamine efflux in the nucleus accumbens. Rapid changes in dopamine oxidation current were measured at carbon fiber microelectrodes using fixed potential amperometry in urethane anesthetized male mice. Intraventricular infusions of the muscarinic acetylcholine receptor antagonist scopolamine, the nicotinic acetylcholine receptor antagonist mecamylamine, or the ionotropic glutamate receptor

antagonist kynureate significantly diminished dopamine efflux in the nucleus accumbens evoked by brief electrical stimulation of the laterodorsal tegmentum. These findings suggest that acetylcholine and ionotropic glutamate receptors influence rapid dopaminergic activity and thus the communication of behaviorally relevant information from ventral tegmental area dopamine cells to forebrain areas. *NeuroReport* 19:991–995 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

The mesoaccumbens dopamine pathway, comprising dopamine neuronal cells in the ventral tegmental area and their projections to the nucleus accumbens, is integral in reward-related behavior and addiction [1,2]. The laterodorsal tegmentum in the mesopontine brainstem provides a principal source of glutamatergic innervation to dopamine neurons in the ventral tegmental area, and together with the neighboring pedunculopontine tegmentum supplies the only known cholinergic excitatory input to this region [3–5]. Several lines of evidence indicate that the laterodorsal tegmentum may operate to facilitate dopamine-related behaviors [6,7], although a loss of coordinated balance between cholinergic/glutamatergic and dopaminergic systems may underlie deficits associated with psychiatric and neurological disorders [7–9].

In rats and mice electrical stimulation of the laterodorsal tegmentum evokes a three-component pattern of dopamine release in the nucleus accumbens, as measured by in-vivo chronoamperometry [10,11]. This includes an initial rapid and transient increase that reflects the stimulation of nicotinic acetylcholine receptors (nAChRs) and ionotropic glutamate receptors (iGluRs) in the ventral tegmental area, followed by a decrease below prestimulation levels that results from activation of autoinhibitory M2 muscarinic acetylcholine receptors (mAChRs) in the laterodorsal tegmentum, and finally a prolonged increase that is dependent on activation of M5 mAChRs in the ventral tegmental area. Together, this suggests different functional roles for these receptors, for example, iGluRs and nAChRs may underlie laterodorsal tegmentum-elicited dopamine cell burst firing in the ventral tegmental area and consequently

the transmission of motivational-related information to the forebrain. Activation of these receptors may therefore be important in the initiation of mesoaccumbens-driven behaviors, whereas M5 mAChRs in the ventral tegmental area may be necessary for the maintenance of these behaviors.

In light of evidence that excitatory inputs from the mesopontine influence mesoaccumbens dopamine transmission through activation of iGluRs, nAChRs, and mAChRs in the ventral tegmental area, this study aimed to elucidate the specific role of these receptors in modulating dopamine release (efflux) in the nucleus accumbens evoked by brief electrical stimulation of the laterodorsal tegmentum. We examined the effects of ventral tegmental area microinfusions of broad spectrum antagonists on evoked dopamine efflux in the nucleus accumbens, measured using in-vivo fixed potential amperometry coupled with carbon fiber microelectrodes that together permit the monitoring of transient synaptic or phasic increases in dopamine efflux in response to electrical stimulation [12].

Methods

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Memphis, and conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals. Efforts were made to reduce the number of animals used and to minimize animal pain and discomfort.

Animals and surgery

Twelve male C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine, USA), 9 weeks of age and weighing 20–30 g

at the time of surgery, were used. Animals were housed four per cage at $21 \pm 1^\circ\text{C}$ under a 12 h light:12 h dark cycle (0600 h). Food and water were available *ad libitum*.

Mice were anesthetized with urethane (1.5 g/kg, intraperitoneally), mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, California, USA) with the skull flat, and maintained at $36 \pm 0.5^\circ\text{C}$. A concentric bipolar stimulating electrode (SNE-100; Rhodes Medical Co., California, USA) was implanted into the left laterodorsal tegmentum (coordinates in mm: AP -1.0 from λ ; ML $+0.4$ from midline; DV -2.4 from dura) [13], and a 31 g stainless-steel guide cannula was implanted into the left ventral tegmental area for drug microinfusions with the cannula tip positioned 2 mm above site (coord. in mm: AP $+0.9$ from λ ; ML $+0.3$ from midline; DV -4.0 from dura) [13]. An Ag/AgCl reference and stainless steel auxiliary electrode combination was placed on contralateral cortical tissue -3.0 mm from bregma. A carbon fiber recording electrode (250 μm length \times 10 μm outer diameter; Thornel type P, Union Carbide, Pittsburgh, Pennsylvania, USA) was then implanted into the left nucleus accumbens core (coord. in mm: AP $+1.5$ from bregma; ML $+1.0$ from midline; -4.0 mm from dura) [13].

Fixed potential amperometry and electrical stimulation

Amperometric recordings in a Faraday cage consisted of applying a fixed potential ($+0.8\text{V}$) to the recording electrode and monitoring dopamine oxidation current (dopamine efflux) continuously (10 K samples/s) with an electrometer (e-corder 401 and Picostat, eDAQ Inc., Colorado Springs, Colorado, USA) filtered at 50 Hz [14]. Approximately 20 min after implantation of all electrodes, a series of 0.5 ms duration cathodal monophasic constant current pulses (15 pulses at 50 Hz applied every 30 s at 800 μA) was delivered to the stimulating electrode through an optical isolator and programmable pulse generator (Iso-Flex/Master-8; AMPI, Jerusalem, Israel).

Drug microinfusions

Separate 0.5 μl intraventricular tegmental area infusions of iGluR (kynurenate; 0.5 μg), nAChR (mecamylamine; 0.5 μg), and mAChR (scopolamine; 10 μg) antagonists were made through a fiberglass cannula (80 μm outer diameter, Polymicro Tech. Inc., Phoenix, Arizona, USA) connected to PE10 tubing and a 1.0 μl microsyringe (Scientific Glass Engineering, Inc., Austin, Texas, USA) mounted in a microinfusion pump (Stoelting, Wood Dale, Illinois, USA). After a 20-min recording period, the cannula was inserted into the guide 2 mm beyond the tip and infusions made at a rate of 0.25 $\mu\text{l}/\text{min}$. Changes in evoked dopamine efflux were monitored over the course of each drug's effect. Drugs were prepared immediately before use at doses determined by preliminary studies in this laboratory. Separate phosphate-buffered saline (PBS, pH ~ 7.4) infusions served as drug effect controls.

Data and statistical analysis

Prestimulation baseline currents were normalized to zero current values, with data points occurring 0.25 s before and 1.0 s after the onset of stimulation extracted from the continuous record at 30 s intervals for the first 2 min then 5 min intervals for 1 h. Peak increases in dopamine oxidation current evoked by laterodorsal tegmentum stimulation after

drug or PBS (control) infusion were expressed as percentage change with respect to preinfusion baseline responses (100%). Percentage changes were subsequently averaged across animals for each condition. Mean peak levels in oxidation currents following infusions were statistically compared with preinfusion baseline responses using paired two-tailed *t*-tests, and mean changes in oxidation currents were compared between control and drug conditions over the entire recording period using independent two-tailed *t*-tests ($\alpha=0.05$).

Histology and chemicals

At the end of each experiment direct anodic current (100 μA for 10 s) was applied to the stimulating electrode. Mice were then euthanized (intracardial urethane 0.345 g/ml) and their brains removed and prepared in 30%/10% sucrose/formalin and 0.1% potassium ferricyanide for cryostat sectioning. Placements of stimulating electrodes, recording electrodes, and drug infusion cannulae were determined under a light microscope and recorded on representative coronal diagrams [13].

Urethane, scopolamine hydrobromide, kynurenic acid, and mecamylamine hydrochloride were purchased from Sigma-Aldrich (St Louis, Missouri, USA). With the exception of urethane (distilled water), chemicals were dissolved in sterile PBS (pH ~ 7.4).

Results

Stereotaxic placements of infusion cannulae, recording and stimulating electrodes

Recording electrode placements ($n=12$) were confined to the nucleus accumbens core (range in mm: 1.42–1.7 anterior to bregma; 0.8–1.2 lateral to midline; 3.9–4.2 ventral to dura) (Fig. 1a). Cannula tip placements ($n=12$) were localized within the ventral tegmental area (range in mm: 0.4–0.64 anterior to interaural zero; 0.2–0.4 lateral to midline; 1.3–1.7

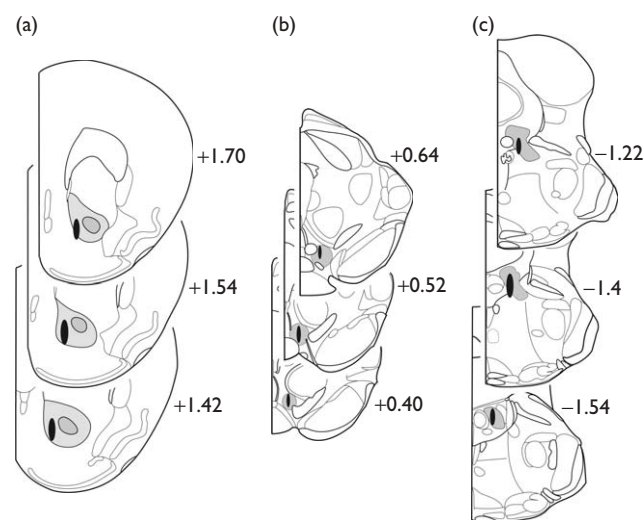


Fig. 1 Representative coronal sections of the mouse brain (adapted from the atlas of Paxinos and Franklin [13]), with black shaded areas indicating the placements of (a) amperometric recording electrodes in the nucleus accumbens, (b) drug infusion cannulae in the ventral tegmental area and (c) stimulating electrodes in the laterodorsal tegmentum. Numbers correspond to mm from (a) bregma and (b and c) interaural zero.

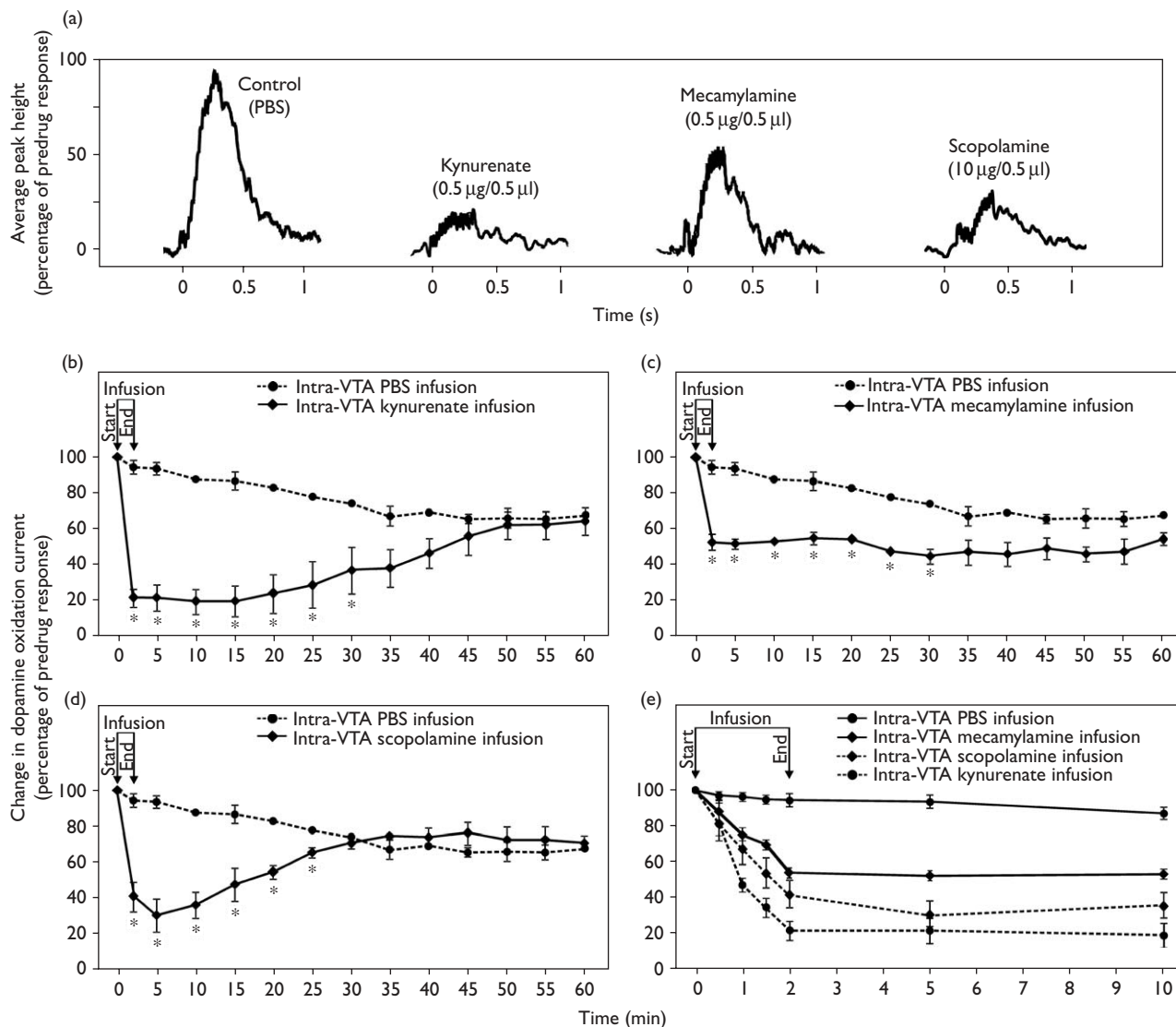


Fig. 2 Amperometric recordings of dopamine oxidation current within the nucleus accumbens evoked by electrical stimulation of the laterodorsal tegmentum. Profiles (a) illustrate mean peak effects in response to ventral tegmental area (VTA) microinfusions of phosphate-buffered saline (PBS) (control), kynureate (ionotropic glutamate receptor antagonist), mecamylamine (nicotinic acetylcholine receptor antagonist) or scopolamine (muscarinic acetylcholine receptor antagonist), with respect to predrug baseline responses (100%). Time zero indicates the start of the train of 15 pulses at 50 Hz. Time courses of the effects of (b) kynureate, (c) mecamylamine, and (d) scopolamine with respect to PBS. The first 10 min of postdrug recordings was extracted (e), illustrating the relative time taken for each drug to inhibit evoked dopamine responses. Lines represent mean changes in amperometric responses over time with standard error bars. *Significantly lower dopamine response after drug infusion compared with PBS.

dorsal from interaural zero) (Fig. 1b). Stimulating electrode tips ($n=12$) were positioned within the laterodorsal tegmentum (range in mm: 1.22–1.54 posterior to interaural zero; 0.4–0.8 lateral to midline; 2.2–2.5 dorsal from interaural zero) (Fig. 1c).

Effects of ionotropic glutamate receptor, nicotinic acetylcholine receptor, or muscarinic acetylcholine receptor blockade on stimulation-evoked dopamine efflux

Intraventricular tegmental area infusion of PBS ($n=3$) did not significantly alter laterodorsal tegmentum stimulation-evoked dopamine efflux in the nucleus accumbens from preinfusion baseline levels ($94.2\% \pm 3.6$ at the end of the 2 min infusion, $P=0.185$) (Fig. 2a and e). Microinfusion of

the iGluR antagonist kynureate ($0.5 \mu\text{g}/0.5 \mu\text{l}$; $n=3$) or the nAChR antagonist mecamylamine ($0.5 \mu\text{g}/0.5 \mu\text{l}$; $n=3$) significantly decreased evoked dopamine efflux, reaching a maximum of $22.2\% \pm 5.3$ and $54.3\% \pm 2.8$, respectively ($P < 0.05$), immediately after completion of the 2 min infusion (Fig. 2a and e). For each of these drugs, evoked dopamine efflux returned to levels equivalent to those observed in control animals approximately 35 min after infusion (Fig. 2b and c). Microinfusion of the mAChR antagonist scopolamine ($10 \mu\text{g}/0.5 \mu\text{l}$; $n=3$) significantly attenuated evoked dopamine efflux, reaching a maximum of $30.6\% \pm 8.9$ ($P < 0.05$) 3 min after completion of the 2 min infusion (Fig. 2a and e). Evoked dopamine efflux returned to levels equivalent to those observed in control animals approximately 30 min after infusion (Fig. 2d).

Discussion

Laterodorsal tegmentum stimulation-evoked dopamine efflux in the nucleus accumbens was significantly attenuated by kynurenate, mecamylamine, and scopolamine independently microinfused into the ventral tegmental area. Together with evidence that pharmacological activation of mAChRs, nAChRs, and iGluRs in the ventral tegmental area stimulates dopamine neuronal activity [15–17] and dopamine release in the nucleus accumbens [3,18,19], our results suggest that these receptors are involved in modulating excitatory cholinergic and glutamatergic inputs arising from the laterodorsal tegmentum [4]. Importantly, the brief, excitatory first component of a triphasic pattern of change in laterodorsal tegmentum stimulation-evoked dopamine efflux in the nucleus accumbens, measured by in-vivo chronoamperometry, is mediated by iGluRs and nAChRs localized in the ventral tegmental area [10]. This suggests that rapid and transient increases in continuous amperometric dopamine oxidation current seen, presently appear to be analogous with this first component. This outcome is similar to findings from research investigating the role of cholinergic and glutamatergic receptors in mediating components of pedunclopontine tegmentum stimulation-evoked dopamine efflux in the striatum [14].

Input from the laterodorsal tegmentum is required for burst firing of dopaminergic cells in the ventral tegmental area [20], which is thought to convey motivationally relevant information to forebrain areas involved in the induction of reward and related processes [21]. The finding that iGluRs and nAChRs in the ventral tegmental area are involved in mediating rapid dopaminergic activity suggests that these receptors may influence laterodorsal tegmentum-induced dopamine cell burst firing [22]. Although the use of broad spectrum antagonists in this study precludes identification of specific receptor subtypes, *N*-methyl-*D*-aspartic acid iGluRs [23] and $\alpha 7$ type nAChRs [24] in the ventral tegmental area are known to be predominant in mediating burst firing of dopamine neurons. These receptors may thus be involved in the initiation of incentive-related behaviors; for example, rats will self-administer the mixed cholinergic agonist carbachol or the acetylcholinesterase inhibitor neostigmine into the posterior ventral tegmental area suggesting that brief phasic increases in cholinergic neurotransmission in this region trigger a rewarding state [25].

M5 mAChRs on dopamine cells in the ventral tegmental area are important in mediating delayed and prolonged mesoaccumbens dopaminergic activity [10,11]. The previous finding that scopolamine blockade of these receptors does not diminish the first excitatory component in laterodorsal tegmentum stimulation-evoked dopamine efflux in the nucleus accumbens observed by in-vivo chronoamperometry [10,11] seems inconsistent with the present results which suggest that mAChRs in the ventral tegmental area are in part responsible for rapid changes in dopamine cell activity. This discrepancy, however, may be explained, in part, by methodological differences between these two studies. First, in the present experiments dopamine efflux was monitored in response to 15 pulse stimulations repeated every 30 s, whereas previous chronoamperometry studies monitored dopamine efflux in response to and following a single bout of 1050 pulse stimulations [10,11]. Second, this study measured stimulation-evoked dopamine efflux during and immediately after scopolamine infusion into the ventral

tegmental area. Here, the inhibitory effect of scopolamine on evoked dopamine efflux diminished 30 min after infusion (Fig. 2d). This suggests that any influence of this antagonist on the first excitatory component may not have been observed by chronoamperometry given that in those experiments evoked changes in dopamine were measured 30–60 min after systemic injection or microinfusion of scopolamine [10,11]. However, as scopolamine is a non-subtype selective mAChR antagonist, and the first excitatory component in laterodorsal tegmentum stimulation-evoked dopamine efflux is unaffected by genetic inactivation of the M5 subtype [11], this suggests that other mAChRs (e.g. the M1 subtype) may potentially be involved in mediating rapid increases in dopamine cell activity in the ventral tegmental area.

Conclusion

Findings from this study implicate iGluRs, nAChRs, and mAChRs within the ventral tegmental area in mediating rapid and transient increases in laterodorsal tegmentum-evoked forebrain dopamine efflux. Given that disruption in the functional connectivity between mesopontine and midbrain dopamine systems may in part be responsible for the manifestation of symptoms characteristic of several psychiatric and neurological disorders [7–9], this outcome suggests that cholinergic and glutamatergic receptors may serve as effective targets in the pharmacotherapy of these disorders.

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