Diffusion-based volume transmission in the striatum: not just for dopamine

Margaret E. Rice*
Department of Physiology and Neuroscience and Department of Neurosurgery
New York University School of Medicine, New York, NY 10016, USA.

(*margaret.rice@nyu.edu)

Introduction

Dopamine (DA) is an unconventional transmitter in many ways. The process underlying axonal DA release is one of its few conventional aspects: release occurs through vesicular exocytosis and is dependent on action potentials and Ca^{2+} entry through voltage-sensitive Ca^{2+} channels. But there conventionality ends. In addition to axonal release, DA neurons exhibit transmitter release from their somata and dendrites (somatodendritic release) by a still incompletely understood exocytotic mechanism that may involve non-vesicular storage organelles, as well as vesicles. Release sites in striatum are also a source of controversy, with anatomical evidence for DA release sites that lack postsynaptic specialization, as well as classical synapses. In addition, DA receptors are largely extrasynaptic, implying that DAergic communication is by volume transmission [1]. Consistent with this mode of communication, the DA transporter (DAT) is also extrasynaptic, with exclusive localization of DATs on DA axons in striatum (as well as DA somata and dendrites in midbrain), but not on other cells. This DAT localization allows DA spillover from release sites, which differs from regulation of conventional transmitters like glutamate, for which avid uptake by glia and neurons limits synaptic spillover. Regulation of DA release by other transmitters is also unconventional. Striatal DA release is regulated by glutamate, GABA, acetylcholine (ACh), and adenosine, despite anatomical evidence that none of these transmitter systems makes direct synaptic contact on DAergic axons in dorsal striatum. Such data indicate that *regulation* of DA release by these key transmitters must also involve diffusion-based volume transmission.

Methods

Striatal slices (400 μm) were prepared from adult, male guinea pigs anesthetized with 40 mg kg^{-1} pentobarbital (i.p.) in accordance with NIH guidelines and with IACUC approval. Methods for slice preparation and voltammetric monitoring of electrically evoked DA release have been described previously [2]. All measurements were made in bicarbonate-buffered ACSF at 32 °C containing (in mM): NaCl (124); KCl (3.7); $NaHCO_3$ (26); $CaCl_2$ (2.4); $MgSO_4$ (1.3); KH_2PO_4 (1.3); and glucose (10), saturated with 95% O_2/5% CO_2. DA release was evoked in dorsolateral striatum using a bipolar stimulating electrode with single pulses or brief pulse trains. Extracellular DA concentration ($[DA]_o$) was quantified at 100 ms intervals using 8 μm carbon-fiber microelectrodes and fast-scan cyclic

voltammetry. Regulation of evoked $[DA]_o$ by glutamate, GABA, ACh, and adenosine was assessed using selective receptor antagonists for each transmitter.

Results and Discussion

Local stimulation used to evoke DA release also causes the release of other transmitters. When the stimulus is a single pulse, monitored $[DA]_o$ is largely uninfluenced by concurrently released transmitters, whether or not DA axons have receptors for those transmitters, because the release of DA occurs before modulation by other transmitters can occur. For example, concurrently released glutamate does not regulate single-pulse evoked $[DA]_o$: blockade of ionotropic AMPA receptors (AMPARs) by GYKI-52466, has no effect on peak $[DA]_o$ [2]. Similarly, GABA acting at $GABA_A$ receptors ($GABA_ARs$) has no effect on single-pulse evoked $[DA]_o$, indicated by a lack of effect of the $GABA_AR$ antagonist picrotoxin (PTX) [2]. These data further indicate that glutamate and GABA do not provide tonic regulation of DA release.

When local pulse-train stimulation is used, however, peak $[DA]_o$ is sensitive to GYKI-52466 and to PTX [2-4]. Blockade of AMPARs causes a ~2-fold increase in evoked $[DA]_o$ (Figure 1A), whereas blockade of $GABA_ARs$ causes a ~50% decrease (Figure 1B). This indicates that under these conditions, glutamate acts at AMPARs to *inhibit* DA release, whereas GABA acts at $GABA_ARs$ to *enhance* it. Blockade of NMDA or $GABA_B$ receptors is without effect on either single-pulse or pulse-train evoked $[DA]_o$ in dorsolateral striatum [2]. The apparent absence of ionotropic glutamate and GABA receptors on DA axons in this region suggests that regulation by these transmitters might involve a second messenger. This is indeed the case: both glutamate and GABA act via diffusible hydrogen peroxide (H_2O_2), with prevention of the effects of GYKI-52466 and PTX on pulse-train evoked $[DA]_o$ by H_2O_2 scavenging enzymes [2]. The mechanism by which H_2O_2 inhibits DA release is the activation of ATP-sensitive K^+ (K_{ATP}) channels [2,5].

In contrast to glutamate and GABA, ACh and adenosine modulate DA release *directly* via receptors on DA axons, albeit without direct synaptic contacts. Consistent with localization of nicotinic ACh receptors (nAChRs) on DA axons, single-pulse evoked $[DA]_o$ is suppressed when nAChRs are blocked by mecamylamine (Figure 1C) [6], implying normal facilitation of DA release by ACh. Suppression of single-pulse evoked $[DA]_o$ when nAChRs are blocked also indicates that DA release is tonically regulated by ACh from striatal cholinergic interneurons. Intriguingly, $[DA]_o$ evoked by burst-like trains (25-100 Hz) are enhanced when nAChRs are blocked (Figure 1C), suggesting that ACh regulates DA release probability. Adenosine also facilitates DA release: blockade of A_{2A} receptors ($A_{2A}Rs$) by SCH-58621 leads to suppression of both single-pulse and pulse-train evoked $[DA]_o$ [7]. Suppression of single-pulse evoked release by $A_{2A}R$ blockade also implies that DA release is tonically regulated by adenosine.

In summary, DA readily spills over from release sites in dorsal striatum to activate extrasynaptic DA receptors. This release is modulated by synaptically

released glutamate and GABA, albeit *indirectly* via diffusible, H_2O_2. Although ACh and adenosine modulate DA release *directly* via receptors on DA axons, these extrasynaptic sites must be activated by diffusing ACh and adenosine. Thus, DA is far from the only unconventional transmitter in the striatum.

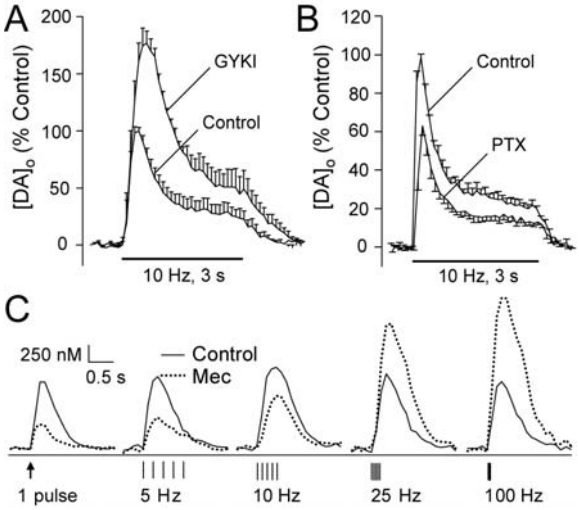

Figure 1. Regulation of striatal DA release by glutamate, GABA, and ACh. A) Blockade of AMPARs by GYKI-52466 (GYKI) enhances pulse-train evoked $[DA]_o$ [3]. B) Blockade $GABA_A$Rs by picrotoxin (PTX) inhibits evoked $[DA]_o$ [4]. C) Blockade of nAChRs by mecamylamine (Mec) inhibits single-pulse and low-frequency pulse-train evoked $[DA]_o$, but enhances $[DA]_o$ evoked by burst-like stimuli [6].

References

1. Rice ME, Cragg SJ (2008) Dopamine spillover after quantal release: rethinking dopamine transmission in the nigrostriatal pathway. *Brain Res Rev* (*in press*).
2. Avshalumov MV, Chen BT, Marshall SP, Peña DM, Rice ME (2003) Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H_2O_2. *J Neurosci* **23**:2744-2750.
3. Avshalumov MV, Patel JC, Rice ME (2008) AMPA receptor-dependent H_2O_2 generation in striatal spiny neurons, but not dopamine axons: one source of a retrograde signal that can inhibit dopamine release. *J Neurophysiol* (*in press*).
4. Sidló Z, Reggio PH, Rice ME (2008) Inhibition of striatal dopamine release by CB1 receptor activation requires nonsynaptic communication via GABA, H_2O_2, and K_{ATP} channels. *Neurochem Int* **52**: 80-88.
5. Avshalumov MV, Rice ME (2003) Activation of ATP-sensitive K^+ (K_{ATP}) channels by H_2O_2 underlies glutamate-dependent inhibition of striatal dopamine release. *Proc Natl Acad Sci (USA)* **100**:11729-11734.
6. Rice ME, Cragg SJ (2004) Nicotine amplifies reward-related dopamine signals in striatum. *Nature Neurosci* **7**:583-584.
7. Patel JC, Sideris A, Rice ME (2006) Caffeine enhances somatodendritic dopamine release: a mechanism involving mobilization of intracellular calcium stores? *2006 Abstract Viewer*: Soc for Neuroscience, Washington, DC, 337.5.

Spatiotemporal dynamics of evoked dopamine release in the rat striatum

Adrian C. Michael*, Keith F. Moquin, Yuexiang Wang,
Andrea Jaquins-Gerstl

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260 USA

(*amichael@pitt.edu)

Introduction

It has long been known that evoked dopamine release within the striatum is heterogeneous [1]. Several studies have shown that small shifts in the placement of carbon fiber microelectrodes can induce large changes in the amplitude of evoked DA release, which has been interpreted as a sign that electrodes can be placed at different proximities to active DA terminals. What remains vague, however, is the anatomical basis for this heterogeneity. DA-specific staining of striatal tissues, for example, reveals a diffuse distribution of DAergic elements without voids. A paradox exists, therefore, between the heterogeneity of voltammetric recordings and the apparent homogeneity of DAergic elements within the striatum.

We hypothesize instead that the heterogeneity of evoked release is derives from the status of DAergic terminals at the recording electrode. We further hypothesize that the instantaneous status of DA terminals might be related to the local basal level of dopamine in the extracellular space (ECS), leading to local variations in the DAergic tone at DA receptors, including terminal autoreceptors that regulate DA release (see also the abstract in this volume by Moquin and Michael). This hypothesis, however, raises the question at to the possible source of variations in basal ECS DA levels in the striatum.

Methods

Evoked dopamine release was monitored in the striatum of isoflorane-anesthetized rats using cylindrical carbon fiber microelectrodes and standard fast-scan cyclic voltammetry parameters (300 V/s, 0 to 1 to -.5 V vs Ag/AgCl, 100 ms scan intervals). Twisted bipolar stimulating electrodes were lowered into the MFB ipsilateral to the voltammetric recording sites. The MFB was stimulated with an optically-isolated, constant-current (125 μA peak), biphasic square waveform (the frequency, train length, and intra-train interval varied during these experiments).

Results and Discussion

The objective of this present study was to examine the range of dynamic responses recorded by voltammetry in the striatum during electrical stimulation

of the MFB (Fig 1). In some cases, electrical stimulation evokes an immediate voltammetric response indicating a rapid onset of DA release commensurate with the onset of the stimulation itself (Fig. 1, diamonds). In other cases, MFB stimulation evokes a slow voltammetric response, the release phase of which exhibits a curve shape that indicates signal induction, i.e. the rise in the response accelerates with time (Fig.1, squares). Diffusion has been presented as a possible explanation for slow-onset stimulus responses [1]. In this explanation, the electrode is supposed to be far from active DA terminals so that once released DA must diffuse though long distances in the extracellular space. On theoretical/mathematical grounds, such diffusional distortion is indeed expected to create an accelerating rising response, just as observed here. However, if a gap exists between the electrode and the terminals, then a delay in the voltammetric signal should also occur at the end of the stimulus. This is because the voltammetric signal returns to baseline through the actions of the dopamine transporter (DAT), which is selectively expressed by DA terminals. However, in the case of the Fig.1 (squares), there is no obvious delay in the response at the end of the stimulus. Instead, the signal returns rapidly towards the baseline, suggesting the presence of DA terminals near the electrode.

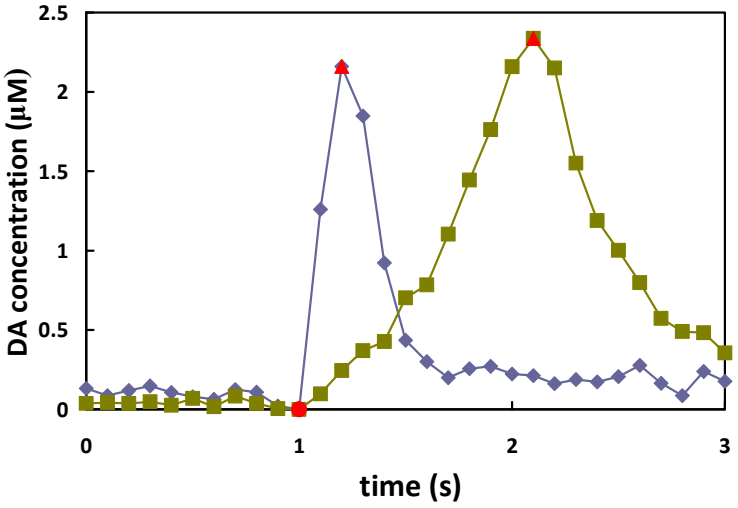

Figure 1. Immediate and Delayed Stimulus Responses in the Rat Striatum. These representative examples illustrate the immediate (diamonds) and delayed (squares) voltammetric response in the striatum during electrical stimulation of the MFB.

As explained in an accompanying abstract in this volume (Moquin and Michael), D2 autoreceptors cause the delay in the stimulus response. Thus, we now regard the range of evoked responses exhibited in Fig 1 to be a component of the heterogeneity of the status of DAergic terminals within the striatum, rather than a reflection of DAergic and non-DAergic sites. Hence, we use here the term 'spatiotemporal heterogeneity' of DA release to express the idea that dopamine dynamics are rapid at some striatal sites but slow at others. Furthermore, this

form of spatiotemporal heterogeneity leads in some cases to hybrid responses, reflective of both slow and rapid evoked DA release. An important feature of the hybrid responses is the demonstration that DA heterogeneity is a feature observed within a single animal, during a single MFB stimulus train. Thus, the range of dynamics is not attributable to animal-to-animal variations, variations in the placement of the stimulating electrode, depth of anesthesia, or other such experimental or technical details.

A question that arises from these observations is the origin of the spatiotemporal heterogeneity of evoked DA release. We hypothesize that this source is related to spatial variations in degree of terminal autoinhibition. For example, some voltammetric studies show that evoked release is initially unaffected by D2 antagonists such as raclopride [2]. In these cases, raclopride leads to an increase only in subsequent dopamine release during ongoing stimulation. The interpretation is that basal DA levels are too low to cause tonic autoinhibition and that the onset of autoinhibition occurs after stimulus evoked DA release. However, at sites where evoked release is initially delayed (Fig 1. squares), the effect of raclopride is immediate with the onset of the stimulus. We take this to mean that the delayed response occurs when basal DA levels (i.e. prestimulus) are sufficient to cause tonic autoinhibition. Thus, we conclude that the distinction between rapid and delayed evoked dynamics is related to the pre-existing basal ECS DA concentration at the site question and, more specifically, whether or not that concentration subjects the local terminals to autoinhibition. We mention that we have considered this possibility before on theoretical grounds [3].

Finally, we consider the possible sources of the variation in basal DA levels that are apparently involved in the determining the localized states of terminal autoinhibition. We hypothesize that this is related to the source of high basal dopamine concentrations we recently reported within the rat striatum [4], i.e. glutamate-driven DA release as revealed by the voltammetric response to intrastriatal infusion the glutamate antagonist, kynurenate.

References

1. Peters JL, Michael AC (2000) Changes in the kinetics of dopamine release and uptake have differential effect on the spatial distribution of extracellular dopamine concentration in rat striatum. *J Neurochem* **74**:1563-1573.
2. Benoit-Marand M, Borrelli E, Gonon F (2001) Inhibition of dopamine release via presynaptic D2 receptors: time course and functional characteristics in vivo. *J Neurosci* **21**:9134-9141.
3. Michael AC, Borland LM, Mitala JJ Jr, Willoughby BM, Motzko CM (2005) Theory for the impact of basal turnover on dopamine clearance kinetics in the rat striatum after medial forebrain bundle simulation and pressure ejection. *J Neurochem* **94**:1202-1222.
4. Borland LM, Michael AC (2004) Votlammetric study of the control of striatal dopamine release by glutamate. *J Neurochem* **91**:220-229.

Voltammetric and amperometric real-time measurements of chemical transmission in murine adrenal slices

J. Petrović and R.M. Wightman*

Department of Chemistry and Neuroscience Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3290 USA

(*rmw@unc.edu)

Introduction

The chromaffin cell has been extensively studied as a neuronal cell model and has revealed critical information about the fundamental mechanisms underlying exocytosis. While the majority of these studies have been conducted in cell cultures, isolated chromaffin cells exhibit a number of phenotypic modifications that render them different from their physiological counterparts [1]. Furthermore, they lack endogenous cholinergic innervations, which regulate chromaffin cell excitability *in vivo*. Lastly, there are only a few literature reports addressing the fate of catecholamines after exocytosis [2]. In this work we report, for the first time, a comprehensive study of factors that control the secretion and clearance of released catecholamines following exocytosis in an *in situ* murine adrenal slice preparation which mimics physiological conditions more closely. We have investigated the control of release by acetylcholine, uptake by adjacent tissue, and spontaneous release activity by employing electroanalytical methods.

Chromaffin cells secrete electroactive catecholamines epinephrine and norepinephrine which can be measured electrochemically via fast-scan cyclic voltammetry (FSCV) and constant-potential amperometry (CPA). CPA offers sub-millisecond temporal resolution which is complimented by FSCV's sensitivity and its selectivity based on the analyte's signature oxidation and reduction potentials. The electrochemical sensor employed in this work was a carbon-fiber microelectrode with a tip diameter of <10 μm, which provides the spatial resolution suited for probing adrenal slice architectural microenvironments. The electrochemical techniques in conjunction with microelectrode sensors offer temporal and spatial resolution necessary to probe real-time chemical transmission at chromaffin cells within the adrenal gland.

Methods

Adrenal slice preparation: Wild-type C57/BL6 female mice (3-8 weeks of age, Jackson Laboratories) were anesthetized by ether inhalation, decapitated, and subjected to midline abdominal incision which allowed for access to adrenal glands. Glands were placed into cold and oxygen saturated bicarbonate-buffered saline, BBS2, containing (in mM): 0.1 $CaCl_2$, 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 3 $MgCl_2$, and 10 glucose at pH=7.4 and subjected to a thorough fat tissue removal procedure under a dissecting microscope. Each gland was

placed into 3 % agarose in BBS2 (Promega, gelling point of 24-28 °C) and secured onto the Teflon specimen holder using instant glue. The specimen holder containing agarose block was mounted into a vibroslicer filled with cold and oxygenated BBS2 (Campden for World Precision Instruments). Adrenal glands were sliced into 200-300 μm sections. The experimental slice was immediately placed into a perfusion chamber (open diamond bath heated chamber, Warner Instruments, LLC) and secured with a stainless steel Lycra® thread anchor. The chamber was continually superfused with oxygen saturated bicarbonate-buffered saline, BBS1, containing (in mM): 2 $CaCl_2$, 125 NaCl, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgCl_2$, and 10 glucose at pH=7.4. To ensure physiological conditions and minimize slice perturbations, both the perfusion chamber and BBS1 were heated to 37 °C (dual automatic temperature controller, Warner Instruments, LLC). All of the experiments were completed within 10 hour *post mortem* period [3].

Electrode fabrication and electrochemical measurements: Elliptical T650 carbon-fiber microelectrodes were fabricated as described previously [4]. Catecholamine release was monitored with the Axopatch 200B (Axon Instruments, Molecular Devices, Union City, CA). For amperometric studies the applied potential at the working electrode was held at +650 mV vs. Ag/AgCl and the data was collected at 30 kHz and low-pass filtered at 5 kHz. The recorded data was then filtered using a 300 Hz Butterworth filter. For voltammetric recordings the potential was scanned from -400 mV to +1000 mV and back to -400 mV at a scan rate of 600 V/s with the repetition rate of 10 Hz. In between scans, the electrode potential was held at -400 mV. The instrumentation was controlled via custom written LABVIEW software. Amperometric spikes were analyzed using Mini Analysis (Synaptosoft, Inc.). Voltammetric data was examined in custom written LABVIEW software, statistical analysis was performed in Microsoft Office 2007 Excel package, and the data was displayed using GraphPad Prism 4.0 (GraphPad Software, Inc.).

Transmural electrical slice stimulation: The slices were electrically stimulated via two tungsten wires glued together at a distance of approximately 150 μm. The stimulating electrodes were positioned on top of slices under a 20X water immersion objective on an upright microscope (Nikon Eclipse E600FN). After positioning between the stimulating electrodes, the working elliptical microelectrode was lowered to a distance of approximately 64 μm into the tissue. The slices were electrically stimulated via delivery of 10 biphasic pulses at a frequency of 10 Hz and a magnitude of 350 μA with a pulse width of 2 ms/phase.

Results and Discussion

Adrenal slices were found to be electrically excitable and the release was stimulation pulse, frequency, and calcium dependent. Upon stimulation there was a rapid increase in extracellular catecholamine concentration followed by a slower decay that suggested uptake (Figure 1.A, t=0 s). To characterize uptake,

adrenal slices were incubated with varying concentrations of the nonselective uptake inhibitor cocaine. Cocaine slowed the rate of uptake in a concentration dependent manner. The electrically evoked release was completely and reversibly blocked with tetrodotoxin (TTX) and hexamethonium (HEX). In some instances a second signal (Figure 1.A, t >5 s), termed slow phase, was observed and found to be insensitive to both TTX and HEX. The slow phase remained when slices were electrically stimulated in low calcium buffer (0.1 mM).

Not only are adrenal slices electrically excitable, but catecholaminergic secretory events were also observed occurring independently of electrical stimulation (Figure 1.B). CPA revealed spontaneous events whose amperometric spike characteristics such as half-width and area closely resemble those observed for vesicular release in isolated chromaffin cells. Spontaneous events that occurred at a frequency of 0.32±0.04 Hz were reversibly blocked by the nicotinic acetylcholine receptor antagonist, HEX, implying mediation of spontaneous release via nicotinic receptors. CPA data suggests vesicular nature of spontaneous release in adrenal slices.

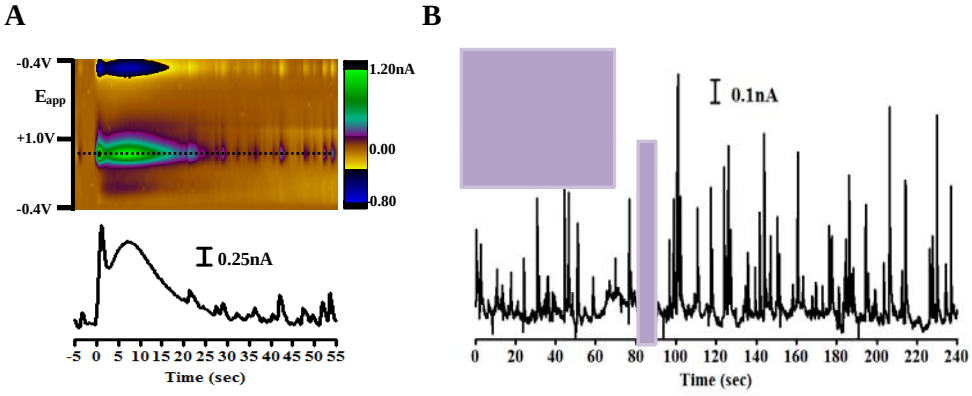

Figure 1. Adrenal slices exhibit electrical stimulation excitability (A) and spontaneous secretory activity (B). The top panel in **Figure 1.A** shows a representative color plot of the current changes (color encoded with the potential plotted on the ordinate and time on the abscissa) observed during the delivery of an electrical stimulus to an adrenal slice. In the bottom panel shown is the current trace extracted from the color plot at the catecholamine oxidation potential (dotted line, 0.6 V vs. Ag/AgCl) and plotted vs. time. **Figure 1.B** shows a current vs. time trace of spontaneous release events which were observed in the absence of electrical stimulation. FSCV confirms their catecholaminergic nature (inset cyclic voltammogram extracted from the spike at 85 s).

The authors would like to acknowledge Charles Miller for technical assistance and advice on data analysis. This work was supported by NIH NS-38879.

References

1. Benavides A, Calvo S, Tornero D, Gonzalez-Garcia C, Cena V (2004) Adrenal medulla calcium channel population is not conserved in bovine chromaffin cells in culture. *Neuroscience* **128**:99-109.
2. Wakade AR, Wakade TD (1984) Absence of catecholamine uptake mechanism in the isolated perfused adrenal gland of the rat. *Neurosci Lett* **50(1-3)**:139-143.
3. Chan SA, Polo-Parada L, Landmesser LT, Smith C (2005) Adrenal chromaffin cells exhibit impaired granule trafficking in NCAM knockout mice. *J Neurophysiol* **94**:1037-1047.
4. Cahill PS, Walker QD, Finnegan JM, Mickelson GE, Travis ER, Wightman RM (1996) Microelectrodes for the measurement of catecholamines in biological systems. *Anal Chem* **68**:3180-3186.

Neurochemical modulation of anesthetic traits

R. Lydic* and H.A. Baghdoyan
Department of Anesthesiology, University of Michigan, Ann Arbor, MI, USA
(*rlydic@umich.edu)

Introduction

Volatile anesthetics and opioids provide the standard of anesthesia care, yet in no case is it understood how these molecules cause the anesthetic traits of analgesia, immobility, amnesia, blunted autonomic control, and loss of consciousness. The correlation of anesthetic potency with lipid solubility, and the suggestion that anesthesia resulted from a generalized distortion of the neuronal lipid bilayer, have been supplanted by evidence that at clinically relevant concentrations anesthetic molecules bind to hydrophobic sites on cell surface proteins [1]. We postulated in 1994 that brain regions which evolved to regulate states of sleep are preferentially involved in generating anesthetic states [2]. Support for such a postulate requires data demonstrating that the endogenous neurochemicals regulating sleep and wakefulness are significantly altered by anesthetics. This research program is using in vivo microdialysis to test the hypothesis that release of neurotransmitters and neuromodulators regulating states of sleep and wakefulness is altered by opioids and volatile anesthetics.

Methods

Brain regions studied include those areas known to contribute to the regulation of sleep (prefrontal cortex, basal forebrain, pontine reticular formation) and nuclei related to the respiratory depression caused by sleep, volatile anesthetics, and opioids (e.g., hypoglossal and trigeminal). Studies use anesthetized and behaving animals. Neurotransmitter and neuromodulator systems investigated to date include acetylcholine (ACh), opioid, nitric oxide (NO), hypocretin/orexin, and adenosine. Microdialysis samples are quantified during electrographically confirmed states of wakefulness, non-rapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Studies using anesthetized animals monitor and maintain levels of isoflurane or halothane. At the completion of experiments, brains are processed for histological confirmation of dialysis sites.

Results

Cholinergic neurotransmission in the medial pontine reticular formation increases cortical activation and REM sleep. ACh release in the pontine reticular formation is significantly decreased by isoflurane, halothane, enflurane, morphine, and fentanyl [3]. The basal forebrain provides cholinergic input to the cortex, and morphine decreases ACh release in prefrontal cortex, in part, by actions at the levels of the basal forebrain [4]. NO modulates neuronal excitability and dialysis delivery of the NO donor NOC-12 to mouse pontine

reticular formation increases ACh release, and the soluble guanylate cyclase inhibitor ODQ decreases pontine reticular formation ACh release [5]. The hypothalamic peptide hypocretin/orexin promotes wakefulness. The resumption of wakefulness from isoflurane anesthesia is delayed by a hypocretin receptor antagonist and by genetic ablation of hypocretin neurons [6]. Dialysis delivery of hypocretin/orexin to rat pontine reticular formation causes a concentration dependent increase in ACh release [7], consistent with the possibility that delayed wake-up in hypocretin/orexin deficient mice is modulated by pontine reticular formation ACh. Adenosine promotes sleep and microdialysis delivery of an adenosine agonist into the pontine reticular formation decreases ACh release and delays recovery time from halothane anesthesia [8]. Dialysis deliver of opioids to the pontine reticular formation decreases local adenosine levels [9], consistent with evidence that adenosine promotes sleep whereas opioids disrupt sleep [10]. Dialysis delivery of opioids increases ACh release in hypoglossal [11] and trigeminal [12] nuclei.

Discussion

No single brain region and no single molecule causes sleep or anesthesia. The confluence of evidence, however, suggests that ACh may be a unifying molecule through which volatile anesthetics and opioids alter traits characteristic of both anesthesia [3] and sleep [13].

References

1. Franks NP, Lieb WR (1994) Molecular and cellular mechanisms of general anesthesia. *Nature* **367**:607-614.
2. Lydic R, Biebuyck JF (1994) Sleep neurobiology: Relevance for mechanistic studies of anaesthesia. *Br J Anaesth* **72**:506-508.
3. Lydic R, Baghdoyan HA (2005) Sleep, anesthesiology, and the neurobiology of arousal state control. *Anesthesiology* **103**:1268-12695.
4. Osman NI, Baghdoyan HA, Lydic R (2005) Morphine inhibits acetylcholine release in rat prefrontal cortex when delivered systemically or by microdialysis to basal forebrain. *Anesthesiology* **103**:779-787.
5. Lydic R, Garza-Grande R, Struthers R, Baghdoyan HA (2006) Nitric oxide in B6 mouse and nitric oxide-sensitive soluble guanylate cyclase in cat modulate acetylcholine release in pontine reticular formation. *J Appl Physiol* **100**:1666-1673.
6. Kelz MB, Sun Y, Chen J, Cheng Meng Q, Moore JT, Veasey SC, Dixon S, Thorton M, Funato H, Yanagisawa M (2008) An essential role for orexins in emergence from general anesthesia. *Proc Natl Acad Sci U S A* **105**:1309-1314.
7. Bernard R, Lydic R, Baghdoyan HA (2003) Hypocretin-1 causes G protein activation and increases ACh release in rat pons. *Eur J Neurosci* **18**:1775-1785.
8. Tanase D, Baghdoyan HA, Lydic R (2003) Dialysis delivery of an adenosine A_1 receptor agonist to the pontine reticular formation decreases acetylcholine release and increases anesthesia recovery time. *Anesthesiology* **98**:912-920.

9. Nelson AM, Ignasiak DI, Baghdoyan HA, Lydic R (2008) Microdialysis delivery of morphine sulfate to rat pontine reticular formation (PRF) decreases PRF adenosine levels. *Exp Biol* :945.11.
10. Lydic R, Baghdoyan HA (2007) Neurochemical mechanisms mediating opioid-induced REM sleep disruption. In: Lavigne G, Sessle B, Choinière M, Soja P (eds) Sleep and Pain. International Association for the Study of Pain (IASP) Press, Seattle, pp 99-122.
11. Skulsky EM, Osman NI, Baghdoyan HA, Lydic R (2007) Microdialysis delivery of morphine to the hypoglossal nucleus of Wistar rat increases hypoglossal acetylcholine release. *Sleep* **30**:566-573.
12. Bowman H, Zhu Z, Baghdoyan HA, Lydic R (2008) Acetylcholine release in rat trigeminal motor nucleus is increased by microdialysis delivery of morphine. *Exp Biol*: LB148.
13. Lydic R, Baghdoyan HA (2007) Acetylcholine modulates sleep and wakefulness: A synaptic perspective. In: Monti JM, Pandi-Perumal SR, Sinton CM (eds) The Neurochemistry of Sleep and Wakefulness. Cambridge University Press, Cambridge, pp 109-143.

Cortical and hippocampal-evoked accumbens dopamine release: Neuronal plasticity mediated by glucocorticoid receptors in the midbrain

Susannah J. Tye[1]*, Kendall H. Lee[1], Charles D. Blaha[2]
[1]Dept. Neurosurg., Mayo Clin., Rochester, MN; [2]Dept. Psych., Univ. Memphis, Memphis, TN

(*tye.susannah@mayo.edu)

Introduction

Synaptic transmission can vary enormously depending on the recent activity history of the neuronal pathway, with modifications lasting from milliseconds to months, or longer. Short-term and long-term plasticity underlie dysregulation of the mesocorticolimbic dopamine system, with important behavioral and psychiatric consequences [1, 2]. Glucocorticoid receptor (GR) actions in the VTA are critically implicated in enhancement of glutamate-dopamine synaptic strength in the ventral tegmental area (VTA) [3]. This GR-mediated plasticity is functionally similar to that resulting from a single exposure to drugs of abuse [3]. Furthermore, GR activation in the VTA is implicated in modulation of glutamate-evoked dopamine cell burst firing activity and NMDA-mediated responses [4]. However, due to the highly transient nature of DA neuronal activity and consequent phasic nucleus accumbens (NAc) dopamine release, little is known about the role of midbrain GRs in mediating this important signal. We have explored the role of VTA GRs in the modulation of phasic dopamine efflux in the NAc evoked by stimulation of the medial prefrontal cortex (mPFC) and ventral subiculum of the hippocampus (vHIP). In particular, we have assessed how the responsiveness of the NAc dopamine signal to these inputs is modulated over the short and long-term by GR actions in the VTA. Such short and long-term shifts in phasic NAc dopamine neurotransmission have important implications for information processing in hippocampal and cortical circuitries dysregulated in pathologies of schizophrenia and addiction.

Methods

Male hooded-Wistar rats were urethane (1.5 g/kg, i.p.) anesthetized and mounted in a stereotaxic frame in accordance with [5]. A single bipolar stimulating electrode was implanted into the left mPFC at 35° along the ML plane (final bregma coordinates: AP +3.2 mm, ML +0.6 mm, DV –3.2 mm) or left vHIP (AP -6.1 mm from bregma, ML +5.3 mm from midline, DV -6.0 mm from dura) of each rat. A drug infusion cannula (80 µm o.d.) was inserted into the VTA (interaural coordinates: AP +3.0 mm, ML +0.5 mm, DV +2.2 mm). Carbon-fiber recording electrodes (~500x10 µm) were implanted into the left NAc core (bregma coordinates: AP 1.7 mm, ML +1.2 mm, DV -7.0 mm). An Ag/AgCl reference/auxiliary electrode was placed onto contralateral cortical tissue.

Fixed potential amperometric recordings were conducted as previously described [6]. In one series of experiments burst-like (10 pulses at 100 Hz) stimulation was applied to either the mPFC or vHIP and a one-off tetanic stimulation (TES) (1000 pulses at 100 Hz) was delivered. The TES was either preceded or followed by intra-VTA GR activation with corticosterone (1 µg/µl). In a second series of experiments, paired trains of burst-like stimulation (10 pulses at 100 Hz) were applied to either the mPFC or vHIP. Changes in the first and second phasic dopamine signal in response to intra-VTA corticosterone (1 µg/µl) were assessed.

Results and Discussion

Application of a TES to the mPFC induced a significant attenuation ($p<0.01$) of mPFC-evoked phasic dopamine efflux (data not shown). This long-term depression (LTD) lasted for over 2 hrs, with a maximal decrease of 33±15% at 30 mins. Intra-VTA infusion of corticosterone (1 µg/µl) 2 mins prior to TES reversed the direction of plasticity, blocking LTD and permitting the expression of long-term potentiation (LTP) for approximately 2 hrs. Intra-VTA infusion of corticosterone 2 mins post-TES resulted in a similar potentiation of the evoked response, but extended the duration of LTP for >4 hrs (Fig 1A). In contrast, application of a TES to the vHIP did not significantly alter the transient vHIP-evoked NAc dopamine signal (data not shown). Intra-VTA infusion of corticosterone 2 mins prior to TES significantly attenuated vHIP-evoked dopamine release for ~2 hrs. Interestingly, when intra-VTA corticosterone infusion was delivered 2 mins post-TES a significant potentiation of the evoked dopamine response was observed for approximately 2 hrs (Fig 1B).

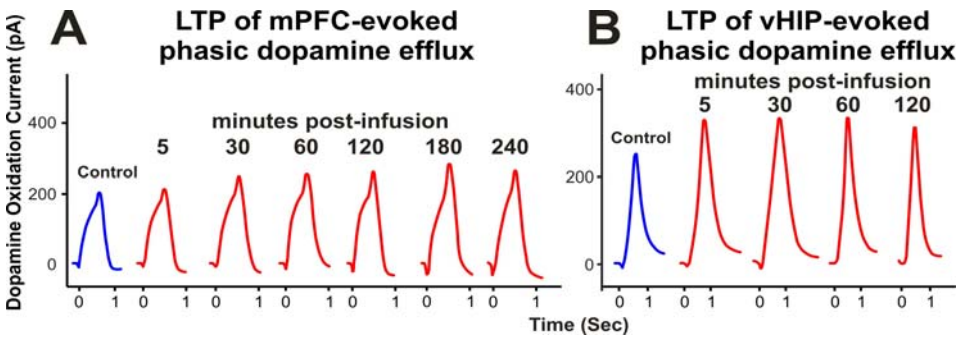

Figure 1. Long-term potentiation of mPFC and vHIP-evoked NAc dopamine efflux. Intra-VTA corticosterone (1 µg/1 µl) infusion 2 min post-TES (1000 pulses at 100 Hz) induces LTP of mPFC **(A)** and vHIP **(B)** evoked (10 pulses at 100 Hz) phasic NAc dopamine efflux. Each response represents the average of six burst-like stimulation-evoked responses pre (control) and post (in mins) infusion.

Application of paired burst-like stimulation to the mPFC or vHIP significantly attenuated the second evoked NAc dopamine response relative to the first in each instance (Fig. 2A and 2C). Intra-VTA corticosterone (1 µg/µl) reversed the direction of this plasticity from short-term depression (STD) to short-term

potentiation (STP) of phasic NAc dopamine efflux (Fig. 2B and 2D). Interestingly, corticosterone treatment also enhanced the first dopamine response evoked by mPFC stimulation, but not that of vHIP stimulation.

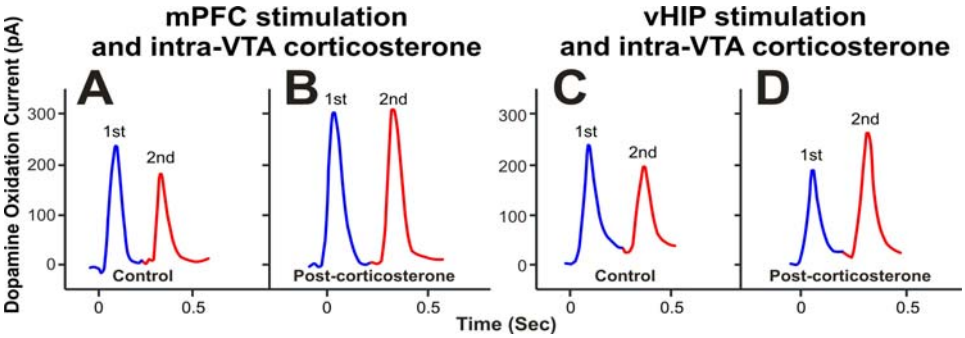

Figure 2. Short-term plasticity of mPFC and vHIP-evoked NAc dopamine efflux. Intra-VTA corticosterone (1 µg/1 µl) infusion reverses STD **(A & C)** of the second (red lines) of the paired mPFC **(B)** and vHIP **(D)** evoked (10 pulses at 100 Hz) phasic NAc dopamine signals, while differentially affecting the first. Each response represents the average of six burst-like stimulation-evoked responses pre (control, A & C) and 30 mins post-infusion (B & D).

These data highlight the important role for intra-VTA GRs in the modulation of both short and long-term plasticity of phasic mesoaccumbens dopamine neurotransmission. Thus, GRs appear to mediate the computational processing capacity of the mesocorticolimbic system, as well as contribute to the establishment of long-term neuroadaptations. This capacity may underlie the established role of GRs in dysregulation of mesoaccumbens dopamine neurotransmission observed in pathologies of schizophrenia and addiction [3].

References

1. Grace AA (2000) Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res Rev* **31**:330-341.
2. Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* **162**:1403-1413.
3. Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron* **37**:577-582.
4. Marinelli M (2007) Dopaminergic reward pathways and effects of stress. In: al'Absi M, (ed) Stress and addiction: biological and psychological mechanisms. Elsevier Ltd. San Diego, CA, pp 41-83.
5. Paxinos G, Watson C (1997) The Rat Brain in Stereotaxic Coordinates, 3rd ed. New York, Academic Press.
6. Forster GL, Blaha CD (2003) Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. *Eur J Neurosci* **17**:751-762.