

Voltammetric measurement of electrically evoked dopamine levels in the striatum of the anesthetized Syrian hamster

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Abstract

Microdialysis measurements in the Syrian hamster clearly demonstrate a role for accumbal dopamine (DA) in female sexual behavior. However, large probe size and slow sampling rate prevent associating specific behaviors with DA changes in subregions of the heterogeneous nucleus accumbens. Fast-scan cyclic voltammetry (FSCV) at a carbon-fiber microelectrode (CFM), which affords millisecond temporal resolution at a micron-sized probe, could address these important issues. Mostly used in other rodents, e.g. rats and mice, this technique has not been applied to hamsters. The goal of the present study was to establish the measurement of DA in the nucleus accumbens of the anesthetized male Syrian hamster using FSCV at a CFM. For comparison, DA was simultaneously measured in the caudate-putamen. Stimulation of the medial forebrain bundle was used to elicit DA. Electrically evoked DA levels in both striatal regions were sensitive to location of the stimulating electrode and CFM, stimulation frequency, inhibition of DA uptake by cocaine and DA autoreceptor blockade by raclopride. Regional differences were observed for DA release and uptake parameters, and the effects of cocaine. Taken together, these results establish the measurement of electrically evoked DA levels in the hamster striatum using FSCV at a CFM.

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1. Introduction

The Syrian hamster (*Mesocricetus auratus*) is an excellent model for investigating the neural substrates of sexual behavior. In particular, the female of this species exhibits an extended lordosis characterized by a relatively immobile posture for up to 15 min (Carter, 1973; Noble, 1979). A stationary female simplifies associating changes in female neurochemistry with mating behavior and minimizes movement artifact in recordings of neural activity. Microdialysis measurements in female Syrian hamsters additionally demonstrate that extracellular dopamine (DA) levels in the nucleus accumbens increase during copulation (Meisel et al., 1993). This increase in DA is blocked by vaginal occlusion (Kohlert et al., 1997) and is sensitized by sexual experience (Kohlert and Meisel, 1999). Moreover, results

from the hamster are in good agreement with those obtained from the rat (Becker et al., 2001; Jenkins and Becker, 2003) and support the hypothesis that accumbal DA plays an important role in female sexual behavior (Hull et al., 1999). However, the slow sampling rate (minutes) of microdialysis limits the utility of this technique for associating specific behaviors with DA changes. Furthermore, the large probe size (>250 μm) also hinders identifying subregions of the anatomically and functionally heterogeneous nucleus accumbens (Zahm, 2000) in which these changes occur.

Fast-scan cyclic voltammetry (FSCV) at a carbon-fiber microelectrode (CFM) affords millisecond temporal resolution at a micron-sized probe for monitoring brain DA (Adams, 1990). One unique feature of this real-time electroanalytical technique is the voltammogram, which serves as a chemical signature identifying the analyte detected (Baur et al., 1988; Michael et al., 1998). The voltammogram is critical for assessing neurochemistry during behavior, because several chemical species could be changing concurrently (Wightman and Robinson, 2002). Originally used in anesthetized rats to characterize dynamic changes

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in extracellular DA levels produced by electrical stimulation (Millar et al., 1985), FSCV at a CFM has more recently been extended to freely moving animals (Garris et al., 1997; Rebec et al., 1997). Further improvements in sensitivity now permit the monitoring of sub-second DA-concentration transients associated with sociosexual (Robinson et al., 2001, 2002), food-seeking (Roitman et al., 2004) and cocaine-seeking (Phillips et al., 2003) behavior. Used primarily in rats and mice, FSCV at a CFM has not been applied to hamsters, where its attractive measurement characteristics are advantageous for investigating the role of DA in sexual behavior.

The goal of the present study was to establish the measurement of electrically evoked DA levels in the anesthetized male Syrian hamster using FSCV at a CFM. Establishing these basic measurements in anesthetized males will be the first step towards the long-term objective of real-time DA monitoring in the female Syrian hamster during sexual behavior. Voltammetric recordings were taken in the caudate-putamen (CP) and core of the nucleus accumbens (NAcC). Short trains of electrical stimulation were applied to the medial forebrain bundle in order to elicit transient increases in extracellular DA levels in dorsal and ventral striata. Identification of DA as the measured species evoked by electrical stimulation was based on electrochemical, pharmacological and anatomical criteria. In general, the sensitivity of electrically evoked DA levels to location of the stimulating electrode and CFM, stimulation frequency, inhibition of DA uptake by cocaine and DA autoreceptor blockade by raclopride, was in good agreement with our previous work in the urethane-anesthetized male rat (Garris et al., 1993; Garris and Wightman, 1994; Wu et al., 2001a, 2002). Taken together, these results establish the measurement of electrically evoked DA levels in the Syrian hamster striatum using FSCV at a CFM.

2. Methods

2.1. Animals

Male Syrian hamsters were obtained from Harlan Industries (Indianapolis, IN) at 8–10 weeks of age (90–100 g). Experiments were typically performed on animals weighing approximately 110–130 g. Animals were housed under controlled lighting, temperature and humidity. Food and water were available ad libitum. Care was in accordance with NIH guidelines (publication 86–23) and approved by the Institutional Animal Use and Care Committee of Illinois State University.

2.2. Surgery

Hamsters were anesthetized with urethane (1.5 g/kg, i.p.) and immobilized in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) as previously described for the rat (Wu et al., 2001a, 2002). After exposing the skull, holes were drilled for placement of stimulating, sensing and reference electrodes. The stimulating electrode was initially placed just dorsal of the medial forebrain bundle in the lateral hypothalamus of the right brain. In most experiments, the stimulating electrode was incrementally lowered until a robust signal, voltammetrically

identified as DA, was observed in both the CP and NAcC and not changed thereafter for the duration of measurements. In one experiment, the stimulating electrode was incrementally lowered through the medial forebrain bundle. In most animals, a CFM was positioned in the dorsomedial CP and NAcC for simultaneous voltammetric monitoring of DA in both striatal regions, although only a single region was examined in some animals. In all experiments, the CFM was located ipsilateral to the stimulating electrode, and the reference electrode was placed contralaterally in superficial cortex. Stereotaxic coordinates were obtained from *The Golden Hamster Atlas* (Morin and Wood, 2001). The anteroposterior (AP) and mediolateral (ML) reference was bregma, and the dorsoventral (DV) reference was dura. The following coordinates (mm) were used: CP, AP = +2.9, ML = +1.5–2.5, DV = –3.5 to 5.0; NAcC, AP = +2.9, ML = +1.5–2.5, DV = –5.0 to 6.5; medial forebrain bundle, AP = –0.8, ML = +1.2, DV = –7.0–7.8.

2.3. Voltammetry

Cylindrical carbon-fiber microelectrodes were constructed as previously described (Cahill et al., 1996). The carbon fiber ($d = 5 \mu\text{m}$, T650, Amoco Greenville, SC) extended beyond the glass insulation for approximately 100–200 μm . Electrochemistry was performed using an EI 400 bipotentiostat (Cypress Systems, Lawrence, KS) and computer controlled (Michael et al., 1999). The potential of the CFM was linearly scanned from a resting potential of –0.4 to 1.0 V and back again every 100 ms at a rate of 300 V/s; a chloridized silver wire (Ag/AgCl) was used as the reference electrode. Temporal responses were determined by monitoring current over the peak oxidation potential for DA at each voltammogram, typically 600 mV, and converting to concentration based on post-calibration (Wu et al., 2001a) using flow injection analysis (Kristensen et al., 1986). The calibration buffer consisted of 150 mM sodium chloride adjusted to a pH of 7.4. Because divalent cations are found in high concentrations in brain extracellular fluid and decrease the sensitivity of the CFM to DA by a factor of 2 (Kume-Kick and Rice, 1998), our divalent cation-free calibration buffer underestimates DA concentration by a factor of 2. However, post-calibration also overestimates DA concentration by a similar amount, due to loss of CFM sensitivity to DA during brain implantation (Logman et al., 2000). Consequently, no adjustment was made for either divalent cations or brain implantation, whose opposing effects on calibration factor cancel each other (Wu et al., 2001a). For identification of DA, background subtracted cyclic voltammograms were calculated by subtracting voltammograms recorded during baseline from those recorded during electrical stimulation and visually compared to background subtracted voltammograms taken during post-calibration. A chloridized silver wire was used as the reference electrode (Ag/AgCl).

2.4. Electrical stimulation

Biphasic stimulation pulses, 300 μA and 2 ms each phase, were computer generated. The duration of the pulse train was 2 s unless otherwise noted. Frequency of the pulse train varied

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