Rapid sampling microdialysis and fast-scan cyclic voltammetry reveal that morphine and oxycodone differentially alter dopamine transmission in the nucleus accumbens

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Introduction

While practically all drugs of abuse increase dopamine (DA) transmission in the mesolimbic pathway [1, 2], various pharmacological classes of drugs affect DA transmission via different mechanisms [3]. Traditionally, microdialysis has been used to measure changes in neurotransmission following drug administration. While these studies have laid the groundwork for our understanding of the pharmacology of drugs of abuse, the precise nature of in vivo DA transmission resulting from drug administration and how this differs across drugs is an ongoing area of investigation. Since traditional microdialysis measures analytes in dialysate extracted from the brain, and a certain volume of dialysate is required for analysis, the technique has generally been limited to sampling changes in DA concentration [DA] every 10-20 min [4]. Techniques with improved temporal resolution facilitate better understanding of the precise nature of DA transmission.

Recent advances in microdialysis have dramatically improved the technique’s temporal resolution; indeed, the temporal resolution for certain neurotransmitters in vivo has increased to less than 1 min [5, 6]. While these advances are excellent, they do not allow phasic neurotransmission to be measured, and transmitter concentrations are averaged over a large area (generally 1-2 mm). Thus, with this technique alone, we are unable to determine subsecond drug-induced changes in neurotransmission. For this, voltammetric measures are more appropriate.

The electrochemical technique fast-scan cyclic voltammetry (FSCV) can capture sub-second changes in extracellular [DA] in highly specific terminal fields (i.e. within distinct mesolimbic projection systems [7, 8]); therefore, this technique is a valuable tool for examining drug-induced phasic changes in DA transmission [9, 10]. In addition to good temporal resolution, the carbon-fiber microelectrodes used in FSCV facilitate excellent spatial resolution with little to no brain damage [11-13]. But while FSCV facilitates the analysis of sub-second changes in DA transmission, comparisons across time exceeding 90 s is difficult to obtain in freely-moving animals because of changes in the background and electrode drift [14, 15]. Additionally, since FSCV is a differential technique, absolute concentrations of DA cannot be quantified. Therefore, we propose that the
convergent use of both techniques provides the optimal situation in which to elucidate the precise nature of drug-induced changes in neurotransmission.

Here, we use rapid-sampling microdialysis coupled with novel liquid chromatography – mass spectrometry (LC-MS) technology for dialysate analysis, and, in separate subjects, FSCV to examine the effects of two different opiates (morphine and oxycodone) on DA neurotransmission in the nucleus accumbens (NAc). While morphine has been shown to increase DA transmission in the NAc [16, 17], and oxycodone has been shown to increase striatal DA [18], we know very little about the nature of these respective increases or how these opiates’ effects on DA transmission may differ. Additionally, while multiple studies have examined the effects of subcutaneous morphine on DA transmission [17, 19, 20], rarely have the effects of intravenous (i.v.) morphine on DA neurotransmission been examined [16] although i.v. self-administration is often seen in opiate abuse [21, 22]. Therefore, we use both FSCV and rapid-sampling microdialysis to examine sub-second and minute-by-minute changes in DA transmission in the NAc following i.v. morphine and oxycodone administration.

Methods

Subjects: Male rats (~300 g) with i.v. jugular catheters were purchased from Charles River Laboratories (Winington, MA) and single-housed with free access to rat chow and water. For FSCV experiments, a guide cannula was implanted over the NAc, a stimulating reference in the VTA, and a reference electrode in contralateral cortex as described elsewhere [23]. Subjects for microdialysis experiments were implanted with a guide cannula over the NAc as described previously [24].

FSCV: A carbon-fiber microelectrode was lowered the day of testing. Baseline DA transmission was recorded for 15 min followed by a control saline injection and increasing cumulative doses of morphine or oxycodone (0.1, 0.5, 1.0 mg/kg). Finally, 1 mg/kg raclopride was infused for a positive control. [DA] was recorded for 15 min following each i.v. infusion with an intervening electrical stimulation to ensure electrode stability [9].

Microdialysis: On the day of testing, a microdialysis probe (extending 1 mm below the cannula) was lowered into the guide cannula, and aCSF was perfused at 2 µL/min for 2 hrs. Then, 60 sec samples of dialysate were collected continuously. After 15 min of baseline sampling, each subject received a control saline infusion followed by 0.5 mg/kg i.v. of either morphine or oxycodone. Borate buffer, benzoyl chloride, and internal standards were added to each sample. HPLC-MS was used to analyze the dialysate (described in detail elsewhere [25]).

Results and Discussion

While both morphine and oxycodone were found to increase DA transmission in the NAc immediately following infusion, the nature of the increases by the two drugs was quite different. First, using FSCV, we showed that oxycodone caused a dose-dependent increase in [DA]. Medium and high doses of oxycodone caused a robust increase in [DA], transient frequency, and
transient amplitude for the duration of the 15 min recording period. In contrast, all morphine doses caused an increase in [DA] in the NAc core and shell within ~ 5 seconds of the infusion (similar to oxycodone) and remained high for the first minute; however, after this initial increase, [DA] decreased to baseline levels (or below) soon after the infusion. Furthermore, [DA] remained at or below basal levels for the duration of the recording period.

The very brief increase following morphine was unexpected since previous studies have reported a long-lasting increase in [DA] following morphine administration [16, 20, 26]; however, these microdialysis studies used long sampling periods with low temporal resolution. Therefore, we conducted rapid-sampling microdialysis which would detect the rapid spike in [DA] revealed by our FSCV study. Since the magnitude of drug-evoked DA after infusion of 0.5 mg/kg of morphine and 0.5 mg/kg oxycodone was similar to our FSCV results, these doses were chosen for the microdialysis experiments. Our microdialysis results similarly revealed that i.v. morphine initially caused a brief increase in DA transmission in the NAc and quickly returned to baseline levels, confirming the FSCV results. In addition to this initial increase, the microdialysis data showed a second increase in [DA] approximately 20 min after the morphine infusion relative to baseline, which likely explains why traditional microdialysis studies report long-lasting increases. Oxycodone administration, on the other hand, resulted in a long-lasting steady increase in [DA]. This effect was also consistent with the FSCV results, following a more traditional opiate profile.

Our morphine results revealed a brief increase in [DA] followed by a second increase in [DA] later. Although previous microdialysis studies have shown one long-lasting increase in [DA] following morphine administration [16, 20, 26]; the significantly longer sampling periods of these studies likely averaged across the two separate increases in [DA] masking the dynamic nature of morphine's effects on DA transmission in the NAc. Our identification of the initial peak in [DA] with both fast techniques demonstrates the importance of rapid temporal sampling. Importantly, both techniques reveal complimentary results: oxycodone causes a robust, long-lasting increase in DA transmission whereas morphine causes an initially brief increase followed by second increase approximately 20 min later. This study demonstrates that FSCV and rapid-sampling microdialysis are both important techniques for gaining a comprehensive understanding of DA dynamics resulting from drugs of abuse.

References


