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Region and Domain Dependent Action of Nomifensine

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Abstract

The dopamine (DA) terminal fields in the rat dorsal striatum (DS) and nucleus accumbens core (NAcc) are organized as patchworks of domains that exhibit distinct kinetics of DA release and clearance. The present study used fast-scan cyclic voltammetry recordings of electrically evoked DA overflow to test the hypothesis that nomifensine might exhibit domain-dependent actions within the NAcc, as we previously found to be the case within the DS. Within the NAcc, nomifensine preferentially enhanced evoked dopamine overflow in the slow compared to the fast domains. To seek a kinetic explanation for nomifensine's selective actions, we quantified the apparent K_M of DA clearance by numerically evaluating the derivative of the descending phase of the DA signal after the end of the stimulus. For comparison, we likewise quantified apparent K_M in the domains of the DS. As expected because it is a competitive inhibitor, nomifensine significantly increased the apparent K_M in both the fast and slow domains of both the NAcc and DS. However, our analysis also leads to the novel finding that nomifensine preferentially increases the apparent K_M in the NAcc compared to the DS: apparent K_M increased by ~500% in the NAcc and ~200% in the DS.

Keywords

Dopamine; Nomifensine; Preferential Action; Apparent K_M ; Voltammetry

INTRODUCTION

Dopamine (DA) is crucial to the motor, motivation, and reward related functions of the central nervous system (Johnson & Kenny, 2010; Jenkinson & Brown, 2011; Volkow *et al.*, 2011). DA neurotransmission is initiated by the release of DA molecules and terminated by their reuptake via the dopamine transporter (DAT). The spatiotemporal dynamics of DA's extracellular concentration, and therefore the extent and duration of DA receptor stimulation, are intricately determined by the kinetics of DA release and clearance as well as the mass transport of DA through the extracellular space (Wightman *et al.*, 1988; Garris *et al.*, 1994; Cragg & Rice, 2004; Taylor *et al.*, 2012; Taylor *et al.*, 2013). Because numerous drugs that affect DA release and clearance have therapeutic value (Gottwald & Aminoff, 2011; Volkow *et al.*, 2012) and/or abuse potential (Phillips *et al.*, 2003; Hollander & Carelli,

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2007), it is highly significant to know how such drugs alter the spatiotemporal dynamics of DA concentrations in the extracellular space.

The psychostimulants cocaine and nomifensine are competitive DAT inhibitors and thus alter DA's extracellular dynamics (Church *et al.*, 1987; Koob & Bloom, 1988; Kuhar *et al.*, 1991). That the actions of such drugs vary between brain regions has been a subject of intense interest. For example, their tendency to preferentially increase measures of extracellular DA in the nucleus accumbens (NAc) compared to the dorsal striatum (DS) conforms well with the central importance of the NAc in the mechanisms of substance abuse (Dichiaro & Imperato, 1988; Carboni *et al.*, 1989; Cass *et al.*, 1992; Cass *et al.*, 1993; Wu *et al.*, 2001a). But, the present understanding of this preferential action remains somewhat incomplete because, first, the DS receives the densest dopaminergic innervation in the brain (Bjorklund & Dunnett, 2007), second, as yet there is no clear sign that DAT inhibitors exhibit greater potency in the NAc than DS (Boja & Kuhar, 1989; Izenwasser *et al.*, 1990; Jones *et al.*, 1995b) and, third, as yet there is no clear sign that competitive DAT inhibitors, such as cocaine and nomifensine, preferentially increase the DAT's apparent K_M in the NAc: rather, prior reports suggest that nomifensine acts preferentially in the DS and that cocaine acts non-preferentially (Wu *et al.*, 2001a; Wu *et al.*, 2001b).

We (Shu *et al.*, 2013) have reported that the core of the NAc (NAcc), similarly to the DS (Moquin & Michael, 2009; Wang *et al.*, 2010; Moquin & Michael, 2011), contains a patchwork of DA kinetic domains that respond in unique fashion to electrical stimulation of the medial forebrain bundle. The discovery of these domains leads us to hypothesize that the actions of nomifensine might be domain-dependent within the NAcc, as we previously found to be the case in the DS (Moquin & Michael, 2009; Taylor *et al.*, 2012). So, the objectives of the present study were, first, to test the hypothesis that nomifensine exhibits domain-dependent actions in the NAcc and, second, to compare nomifensine's actions in the NAcc with those in the DS (Taylor *et al.*, 2013). We report herein for the first time a) that, within the NAcc, nomifensine preferentially promotes evoked DA release in the slow compared to the fast domains and b) that nomifensine preferentially increases the apparent K_M of DA clearance in the NAcc compared to the DS.

METHODS AND MATERIALS

Chemicals were used as-received from the indicated suppliers and solutions were prepared with ultrapure water (NANOPure, Barnstead, Dubuque, IA, USA). Nomifensine maleate (Sigma-Aldrich, St Louis, MO) was dissolved in phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 1.47 mM KH_2PO_4 , 10 mM Na_2HPO_4 , pH 7.4). Dopamine hydrochloride (Sigma-Aldrich) was dissolved just before use in artificial cerebrospinal fluid (aCSF, 144 mM NaCl, 1.2 mM CaCl_2 , 2.7 mM KCl, 1.0 mM MgCl_2 , 2.0 mM NaH_2PO_4 , pH 7.4) and stored under N_2 to prevent DA oxidation. Paraformaldehyde (PFA, 4% in phosphate buffered saline) was prepared from resin (Sigma-Aldrich) and stored at 4°C before use. Isoflurane was from Baxter Healthcare (Deerfield, IL, USA).

Carbon fiber microelectrodes were constructed with 7- μm diameter carbon fibers (T650, Cytec Carbon Fibers, LLC, Piedmont, SC, USA). The fibers were trimmed to a length of

200 μm and immersed in reagent grade isopropyl alcohol (Sigma) for 20 min before use (Bath *et al.*, 2000).

Background subtracted fast-scan cyclic voltammetry (FSCV) was performed with a potentiostat (University of Pittsburgh, Department of Chemistry Electronics Shop), a current amplifier (Keithley 428, Keithley Instruments, Inc., Cleveland, OH, USA), and software (TarHeel CV v4, courtesy of Prof. Michael Heien, Department of Chemistry and Biochemistry, University of Arizona, Tucson, USA (Heien *et al.*, 2003). Between scans the applied potential was held at 0 V vs. Ag/AgCl. During the scans the voltage was swept linearly to 1.0 V, then to -0.5 V and back to 0 V at 400 V/s: the scan repetition frequency was 10 Hz.

Electrode calibration was performed using freshly prepared DA solutions before and after *in vivo* recordings. In vivo dopamine concentrations were determined by post-calibration.

Subjects were male Sprague-Dawley rats ($n = 37$, 250-275 g, Hilltop Laboratories, Scottsdale, PA, USA). All procedures involving animals were in accordance with NIH guidelines (publication 86-23) and approved by The Institutional Animal Care and Use Committee of the University of Pittsburgh.

Surgery was performed as described previously (Shu *et al.*, 2013) under isoflurane anesthesia (5% by vol. initially and 2.5% for maintenance): isoflurane was vaporized (Cyprane Fortec, Fraser Harlake, Inc., Orchard Park, NY) in oxygen and delivered through a ventilator (Harvard Apparatus, Holliston, MA, USA). The anesthetized rats were maintained at 37°C with a homoeothermic blanket (Harvard Apparatus). Carbon fiber electrodes were first placed into the striatum (A/P +1.2 mm, M/L +1.4 mm, D/V 5.0 mm below dura) (Paxinos & Watson, 1998) and bipolar, stainless-steel stimulating electrodes (MS303/B, Plastics One, Inc., Roanoke, VA, USA) were placed as described before (Ewing *et al.*, 1983; Kuhr *et al.*, 1984; Michael *et al.*, 1987) in the medial forebrain bundle (A/P -4.3 mm, M/L +1.2 mm, D/V from 7.5 below dura: the vertical coordinate was adjusted until evoked release was observed). Finally, without moving the stimulating electrode, the carbon fiber electrodes were slowly lowered into the NAcc (D/V 6.6-7.6 mm below dura).

Histological examination was performed as described previously (Peters *et al.*, 2004; Shu *et al.*, 2013) to verify that microelectrodes were correctly placed in the NAcc.

Electrical stimulation (constant current, biphasic square wave, pulse intensity 250 μA , pulse width 2 ms) was supplied by a pair of stimulus isolators (NL800A, Digitimer, Ltd., Hertfordshire, England). Stimulation was performed at 60, 30 and 15 Hz: details of the train duration are provided in the Results section.

Experiment design: Once the recording and stimulating electrodes were in position, stimulus responses were recorded before and 30 min after a single dose of nomifensine (20 mg/kg i.p.) with no alterations in the electrodes, recording location, stimulating location, stimulation parameters, etc. As during our prior study in the DS (Taylor *et al.*, 2012), we compared pre- and post-nomifensine responses in the same group of animals to assure the effect of the drug was established under the same recording and stimulating conditions.

Data Analysis: Overshoot duration was defined by the time needed after the end of the stimulus for the evoked response to reach its maximum amplitude. The overshoot concentration was the amount by which the dopamine concentration continued to increase after the end of the stimulus. We quantified the apparent K_M of DA clearance by numerically evaluating the derivative of the descending phase of the response and finding the point at which the slope was one-half the corresponding apparent V_{max} reported by Shu *et al.*, 2013. Statistical analyses were by the t-test, two-way analysis of variance (ANOVA) and two-way ANOVA with a repeated measures design (IBM SPSS Statistics 20).

RESULTS

Evoked responses reported recorded within the NAcc were objectively identified as fast or slow domains according to the criterion established by Shu *et al.*, 2013 (see Fig 1 of Shu *et al.*, 2013: fast sites produce evoked DA within 200 ms of the start of a stimulus (60 Hz, 250 μ A) but slow sites do not). The fast and slow profiles recorded during this study exhibit no statistically significant differences from those recorded in different animals and reported in Fig. 2 of Shu *et al.*, 2013 (Supplementary Information, Fig. S1). The sites of all NAcc recordings reported herein were confirmed by histology (Supplementary Information, Fig. S2).

Format of the presentation of evoked responses

Evoked responses are reported in Figs. 1–4, Fig. 6, and Supplementary Figs. S1 and S3 in a consistent format (Fig. 7 is in a different format, which is explained below). The solid and dash lines are the averages of responses recorded in groups of rats: the n values, the number of rats per group, are stated in the figure legends. In the main text, solid lines are pre-nomifensine responses from fast and slow domains, and dash lines are post-nomifensine responses: the dotted lines are the $\pm$ SEM intervals: black symbols denote when the stimulus begins and ends. The stimulus frequency and duration are stated in the text and in the figure legends: the stimulus current was fixed at 250 μ A.

Domain-dependent effects of nomifensine: fast domains

Nomifensine affects evoked responses in the NAcc fast domains (Fig. 1, stimulus = 0.2 s, 60 Hz; Fig. 2, stimulus = 1 s, 60 Hz). Nomifensine did not significantly affect the response during the 0.2-s stimulus (Fig. 1b, two-way ANOVA, repeated measures design: nomifensine $F(1,12) = 0.220$, $p > 0.5$) but significantly increased both the duration (Fig. 1c, paired-samples t-test: $p < 0.00005$) and amplitude (Fig. 1d, paired-samples t-test: $p < 0.005$) of the overshoot. Nomifensine did not affect the response during the 1-s stimulus (Fig. 2b, time 0.1 to 1.0 s, two-way ANOVA, repeated measures design: nomifensine $F(1,12) = 2.190$, $p > 0.1$) but significantly increased both the duration (Fig. 2c, paired-samples t-test: $p < 0.0005$) and amplitude (Fig. 2d paired-samples t-test: $p < 0.005$) of the overshoot. Thus, in the NAcc fast domains, nomifensine primarily acts on the overshoot portion of the response to brief stimuli.

Domain-dependent effects of nomifensine: slow domains

Nomifensine affects evoked responses in the NAcc slow domains (Fig. 3, stimulus = 0.2 s, 60 Hz; Fig. 4, stimulus = 1 s, 60 Hz). Before nomifensine, the 0.2-s stimulus evoked no detectable DA overflow. However, after nomifensine the response exhibited a maximal overflow of $\sim 1 \mu\text{M}$ DA and a duration of ~ 10 s. The onset of these responses was delayed with respect to the start of the stimulus: in four out of eight cases, the onset of the overflow began after the end of the stimulus. Nomifensine significantly increased the responses during the 1-s stimulus (Fig. 4b, time 0.1 to 1.0 s, two-way ANOVA, repeated measures design: nomifensine $F(1,14) = 6.738$, $p < 0.05$; time & nomifensine interaction $F(9,126) = 8.451$, $p < 0.01$) and significantly increased both the duration (Fig. 4c, paired-samples t-test: $p < 0.0005$) and amplitude (Fig. 4d, paired-samples t-test: $p < 0.005$) of the response overshoot. Thus, nomifensine acts on both DA release during the stimulus and the overshoot of the response in NAcc slow domains.

Domain-dependent effects of nomifensine: fast and slow comparisons

Within the NAcc, nomifensine preferentially increased three measures of evoked DA release in the slow compared to the fast domains (Fig. 5: the post-nomifensine values of initial rate of DA release and the duration and amplitude of the overshoot are normalized with respect to their pre-nomifensine values). Nomifensine preferentially increased the initial rate of DA release and the duration and amplitude of the overshoot in the slow (Fig. 5, white bars) compared to the fast domains (Fig. 5, black bars) (Fig. 5a-c, independent-samples t-test: $p < 0.05$). Thus, nomifensine preferentially enhances evoked DA release in the slow NAcc domains.

The effects of nomifensine in slow domains during low frequency stimulation

We examined the effect of nomifensine on evoked responses in NAcc slow domains at lower stimulus frequency (Fig. 6). Upon stimulation at 15 Hz, a response was observed only after nomifensine administration (Fig. 6a). Hence, the NAcc slow domain exhibits the same “timing mismatch” phenomenon that we described previously in the slow domains of the DS (Taylor *et al.*, 2013). Upon stimulation at 30 Hz, the pre-nomifensine response exhibited an initial delay of 1-2 s followed by short-term facilitation of evoked release, i.e. the response curved-upward as the stimulation continued (Fig. 6b, solid). Nomifensine eliminated the initial delay and the short-term facilitation but also increased the duration of the overshoot (Fig. 6b, dash). Thus, nomifensine exhibits an asymmetric effect on the temporal features of the DA response: nomifensine decreases the initial delay in the response when the stimulus begins but increases the overshoot when the stimulus ends.

Comparison of DA's NAcc dynamics after nomifensine: 0.2 s responses

It is relevant (see Discussion) to compare the dynamics of the post-nomifensine fast and slow responses (Fig. 7). Fig. 7 compares the 0.2 s slow post-nomifensine response (dash) to the 0.2 s fast post-nomifensine response after subtraction of the fast pre-nomifensine response (solid), both normalized to their respective maximum amplitude. The intention of subtracting the fast pre-response from the fast-post response is to isolate the nomifensine-induced overshoot component: this subtraction was not necessary in the case of the slow

response because the preresponse was non-detectable. The nomifensine-induced overshoots observed in fast and slow domains are essentially identical (Fig. 7).

Nomifensine's impact on the apparent K_M of the DAT

We quantified the apparent K_M of DA clearance by numerically evaluating the derivative of the descending phase of the responses. For this purpose, we found it necessary to extend the stimulus duration to 3 s to ensure that the rate of clearance exceeded the one-half V_{max} values (see Supplementary Information: the 3-s responses are reported in Fig. S3 and the individual apparent K_M values are reported in Fig. S4). To gauge the effects of nomifensine, we normalized the post-nomifensine apparent K_M values with respect to their pre-nomifensine magnitude (Fig. 8). Nomifensine significantly increased all four apparent K_M values. The increase in apparent K_M was significantly larger in the NAcc (~500%) than in the DS (~200%) but was not domain-dependent (two-way ANOVA: regions $F(1,20) = 13.213$, $p < 0.002$, domains $F(1,20) = 0.932$, $p > 0.3$). To our knowledge, this is the first report of a preferential kinetic action of a DAT inhibitor in the NAcc compared to the DS.

Color plots

In order to acquiesce to the demands of two reviewers of this report, we provide 3-dimensional color plots of the FSCV data for the 1-s stimulus responses recorded before and after administration of nomifensine in the fast domains of the NAcc (Fig. 9). These color plots, which provide no additional information over that which has already been stated above, show the FSCV data in a voltage-versus-time coordinate with current represented by a color scale. To prepare these plots, we normalized the background-subtracted FSCV currents with respect to each electrode's post-calibration sensitivity factor and averaged the results across the group of animals ($n=7$). The plots show the dopamine oxidation peak, the quinone reduction peak, and background noise.

DISCUSSION

The domain-dependent evoked DA responses (pre-nomifensine) recorded in the NAcc are not statistically different from those reported previously by Shu *et al.* (2013, see Supplementary Fig. S1), confirming that the NAcc domains are preserved across groups of animals. As in the DS (Taylor *et al.*, 2012), nomifensine exerts robust and domain-dependent actions on evoked responses in the NAcc (Figs. 1–6). Together with our previous DS results (Taylor *et al.*, 2012), the present study provides a comprehensive evaluation of both the region-dependent and domain-independent actions of a DAT inhibitor, for the first time.

Summary of nomifensine's actions in the NAcc

When stimulation is delivered at 60 Hz in relatively brief trains (0.2 or 1 s, Figs. 1–4), nomifensine increases both the duration and amplitude of the response overshoot in both domains. In the case of the 0.2 s stimulus trains, the post-nomifensine maximum DA signal in the fast domain was more than 400% of the pre-nomifensine maximum amplitude and the overshoot duration was ~500% longer than the stimulus. In the slow domain, the pre-nomifensine response to the 0.2 s stimulus train was non-detectable (Fig. 3), so the post-

nomifensine response is predominantly overshoot. Interestingly, the temporal features of the overshoots in the fast and slow domains are essentially identical (Fig. 7). Nomifensine significantly increased the DA concentrations observed during the 1-s stimulus trains in the slow domains, but not in the fast domains.

Nomifensine preferentially acts on NAcc slow domains compared to NAcc fast domains

With a 1-s stimulus, it was possible to quantify the initial rate of evoked DA release as well as the duration and amplitude of the overshoots in fast and slow domains under consistent experimental conditions. We normalized the post-nomifensine values with respect to their pre-nomifensine values (Fig. 5). All three measures of evoked DA release were significantly larger in the slow domain. We therefore conclude that nomifensine acts preferentially on evoked DA release in the NAcc slow domains compared to the NAcc fast domains. We report this phenomenon here for the first time.

Taylor *et al.* (2012) reported the domain-dependent actions of nomifensine on evoked DA release in the DS. There are notable differences between the NAcc and DS. First, nomifensine had a much larger effect on the initial rate of DA release in the DS slow domains (~800%) than the NAcc slow domains (~200%). In contrast with the NAcc, nomifensine's actions on the duration and amplitude of the DS overshoot were largest in the fast domains. Thus, we report here for the first time that the domain-dependent actions of nomifensine are also region-dependent. These findings add to the view that the domains of the NAcc and DS are truly distinct from one other, reinforcing the concept that DA kinetics are both region and domain dependent.

Nomifensine's actions on the apparent K_M of DA uptake: considerations

As a competitive DAT inhibitor, nomifensine's primary pharmacological action is to change the apparent K_M of DA uptake. However, DAT inhibitors have additional secondary actions. For example, in anesthetized animals, DAT inhibition decreases the firing rate of DA neurons via indirect agonism of the D2 receptors (Studer & Schultz, 1987; Einhorn *et al.*, 1988; Mercuri *et al.*, 1991), while in non-anesthetized animals DAT inhibitors induce phasic DA events (Aragona *et al.*, 2008) and burst firing of DA neurons (Koulchitsky *et al.*, 2012). Here, however, we wish to evaluate nomifensine's primary action by determining the apparent K_M of DA clearance. To do so, however, requires consideration of how to go about extracting apparent K_M information from evoked responses.

In several previous reports, K_M values were quantified by means of a numerical model that simulates the evoked responses (Wightman *et al.*, 1988; Jones *et al.*, 1995a; Wu *et al.*, 2001a; Wu *et al.*, 2001b). The model assumes that a gap, or a physical space, is interposed between the recording electrode and nearby DA terminals: such a gap is expected to cause diffusional distortion of the evoked responses. However, as we have explained before (Moquin & Michael, 2009; Taylor *et al.*, 2012), the observed features of evoked responses in the DS cast doubt on the diffusion gap distortion model, which in turn, casts doubt on the kinetic values obtained via simulation. We now report that the same issue arises in the NAcc.

A diffusion gap is predicted to cause two types of temporal distortion of the evoked responses; an initial lag in the DA signal when the stimulus begins and an overshoot of the DA signal after the stimulus ends. A diffusion gap would necessarily cause these distortions to go hand-in-hand: if there is an initial lag, there must also be an overshoot, and vice versa. However, in both the DS and NAcc, responses with overshoot but without initial lag are entirely commonplace. In fast domains, for example, DAT inhibition leads to prominent overshoot but no obvious lag. According to the diffusion gap model, this should not be.

A modified diffusion gap model described by Venton *et al.*, (2003) hypothesized that DAT inhibition increases the apparent dimension of the diffusion gap, invoking the concept that DAT inhibition extends DA's lifetime in the extracellular space and so enables it to diffuse further. The evoked responses do not support this hypothesis:

First, both in the DS and the NAcc, DAT inhibition diminishes the initial lag in slow domains, this effect being most obvious at low stimulus frequencies (Fig. 6: see also Fig. 5 of Taylor *et al.*, 2013). This observation directly contradicts the hypothesis that DAT inhibition increases the apparent diffusion gap. Moreover, the rapid onset of the post-nomifensine responses confirms the presence of DA terminals in close proximity to the recording electrode, which shows that there is no diffusion gap to cause diffusional distortion. Rather than diffusional distortion, we conclude that the temporal features of the evoked responses reflect the local activity of DA terminals in the immediate vicinity of the recording site.

Second, our findings do not provide clear evidence that DA's diffusion distance post-nomifensine is domain dependent (Fig. 7). Originally, responses with prominent initial lag, were attributed to a poor choice of recording site with a large diffusion gap interposed between the electrode and its nearest-neighbor DA terminals (Kuhr *et al.*, 1987; Engstrom *et al.*, 1988; Wightman *et al.*, 1988). Likewise, responses without lag were attributed to small gaps and minimal diffusional distortion. In that case, however, then the different gap sizes would necessarily produce distinct temporal profiles, regardless of experimental conditions. After nomifensine, however, this is simply not what is observed (see Fig. 7, above, and Fig. 9 of Taylor *et al.*, 2012): the essentially identical appearance of the post-nomifensine overshoots would be impossible if the fast and slow responses were caused by different gaps.

Nomifensine's actions on the apparent K_M of DA uptake: the analysis

Because our findings do not confirm the presence of a diffusion gap, we chose to quantify apparent K_M directly from the slope of the descending phase of the response. The advantage of slope analysis is that it makes no mass transfer assumptions. However, the kinetic parameters obtained by slope analysis are clearly not the intrinsic, biophysical parameters of the DAT *per se*. Nevertheless, if it is to be assumed, as is generally the case, that the DA signal at the electrode is a useful analog of DA neurotransmission, then the apparent values are of great interest for the clear, simple, and logical reason that mass transport delivers DA molecules not only to the recording electrodes but also to their pre- and post-synaptic targets.

Nomifensine significantly increases the apparent K_M in both the fast and slow domains of both the NAcc and DS: this is an expected result because nomifensine is a competitive inhibitor. However, nomifensine preferentially increases the apparent K_M in the NAcc compared to the DS (Fig. 8), a phenomenon we report here for the first time. Interestingly, although the effect of nomifensine on apparent K_M is clearly region-dependent, it is not domain-dependent: the proportional increase in apparent K_M was similar in the fast and slow domains of each region (Fig. 8). Thus, the change in apparent K_M may not by itself explain all the region and domain dependent actions of nomifensine on DA release. But, as mentioned above, DAT inhibitors have secondary pharmacological effects.

CONCLUSION

This study reaffirms the presence of a patchwork of DA kinetic domains within the NAcc and establishes that nomifensine's actions within the NAcc are domain dependent. Nomifensine preferentially enhances three measures of DA release in the NAcc slow domains compared to the NAcc fast domains: the rate of initial release as well as the duration and amplitude of the overshoot. In this respect, the domain-dependent actions of nomifensine are distinct from those in the DS. Moreover, nomifensine preferentially increases the apparent K_M of DA clearance in the NAcc compared to the DS, and this preferential effect was domain-independent. This first report of a preferential kinetic effect of a DAT inhibitor in the NAcc is in good accord with prior literature showing that DAT inhibitors preferentially affect the NAcc compared to the DS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

DA	dopamine
NAc	nucleus accumbens
NAcc	nucleus accumbens core
DS	dorsal striatum
DAT	dopamine transporter
FSCV	fast-scan cyclic voltammetry

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Figure 1.

The effects of nomifensine on evoked DA overflow in fast NAcc domains (stimulus = 0.2 s, 60 Hz, 250 μA). In this and subsequent figures, solid and dash lines show the average of responses recorded in a group of rats and dotted lines show the SEM. Black symbols denote when the stimulus starts and stops. (A) Evoked responses ($n=7$) recorded before (solid) and after (dash) nomifensine administration. (B) Nomifensine did not significantly (two-way ANOVA, repeated measures design: nomifensine $F(1,12) = 0.220$, $p > 0.5$) affect the DA overflow during the 0.2 s stimulus. (C & D) Nomifensine significantly increased the duration and amplitude (paired-samples t-tests: ** $p < 0.005$, **** $p < 0.00005$) of the stimulus overshoot.

Figure 2.

The effects of nomifensine on evoked DA overflow in fast NAcc domains (stimulus = 1 s, 60 Hz, 250 μA). (A) Evoked responses ($n = 7$) recorded before (solid) and after (dash) nomifensine administration. (B) Nomifensine did not significantly (time 0.1 to 1.0 s, two-way ANOVA, repeated measures design: nomifensine $F(1,12) = 2.190$, $p > 0.1$) affect the rate of evoked DA overflow during the stimulus. (C & D) Nomifensine significantly increased the duration and amplitude (paired-samples t-tests: ** $p < 0.005$, *** $p < 0.0005$) of the stimulus overshoot.

Figure 3.
Evoked responses (n=8) recorded in slow NAcc domains (stimulus = 0.2 s, 60 Hz, 250 μ A)
before (solid) and after (dash) nomifensine administration.

Figure 4.

Effect of nomifensine on evoked DA overflow in slow NAcc domains (stimulus = 1 s, 60 Hz, 250 μA). (A) Evoked responses ($n = 8$) recorded before (solid) and after (dash) nomifensine administration. (B) Nomifensine significantly (time 0.1 to 1.0 s, Θ two-way ANOVA, repeated measures design: nomifensine $F(1,14) = 6.738$, $p < 0.05$; time & nomifensine interaction $F(9,126) = 8.451$, $p < 0.01$) increased the rate of DA overflow during the stimulus. (C & D) Nomifensine significantly increased the duration and amplitude (paired-samples t-tests: ** $p < 0.005$, *** $p < 0.0005$) of the stimulus overshoot.

Figure 5.

The normalized effects of nomifensine on the evoked NAcc responses are domain-dependent (stimulus = 1 s, 60 Hz, 250 μ A). (A) The initial rate of DA overflow (fast: 0-0.3s, slow: 0.3-0.5s), (B) the overshoot duration, and (C) the overshoot amplitude are reported here normalized with respect to their pre-nomifensine values. (A-C) Nomifensine preferentially increased the initial rate of DA release and the duration and amplitude of the overshoot in the slow (white bars) compared to the fast domains (black bars) (independent-samples t-tests: $*p < 0.05$).

Figure 6. Evoked responses (n=5) recorded in NAcc slow domains before (solid) and after (dash) nomifensine administration (A, stimulus = 180 pulses, 15 Hz, 250 μ A; B, stimulus = 180 pulses, 30 Hz, 250 μ A).

Figure 7.

Comparison of “pure overshoots” observed in fast (solid) and slow (dash) NAcc domains after nomifensine administration (stimulus = 0.2 s, 60 Hz, 250 μ A). The blue line was obtained from Fig. 1a by subtracting the pre-drug response from the post-nomifensine response (see also Fig. 9 of Taylor *et al.*, 2012). The dash line is from Fig. 3. The two responses are normalized to their maximum amplitudes to enable comparison of their temporal features (SEMs omitted for clarity).

Figure 8.

Comparison of apparent K_M values obtained in fast and slow domains in the NAcc (white) and DS (black). The post-nomifensine apparent K_M values are normalized with respect to the pre-drug values. The normalized values are region-dependent and domain-independent ($\dagger$ two-way ANOVA: regions $F(1,20) = 13.213$, $p < 0.002$; domains $F(1,20) = 0.932$, $p > 0.3$).

Figure 9. Color plot (see text for explanation) of the FSCV data recorded during a 1-s stimulus (A) before and (B) after administration of nomifensine in fast NAcc domains.