Low-Dose Methylphenidate Actions on Tonic and Phasic Locus Coeruleus Discharge

David M. Devilbiss and Craig W. Berridge

Department of Psychology, University of Wisconsin, Madison, Wisconsin

Received June 27, 2006; accepted September 13, 2006

ABSTRACT

Methylphenidate (MPH) and other psychostimulants are highly effective in the treatment of attention deficit hyperactivity disorder (ADHD). Evidence indicates the therapeutic actions of stimulants in ADHD probably involve the locus coeruleus (LC)-norepinephrine system. LC neurons display different firing modes (tonic and phasic), each associated with distinct behavioral and cognitive processes. To date, the impact of low, clinically relevant doses of psychostimulants on LC discharge is unknown. The present study examined the effects of low-dose MPH on LC tonic and phasic discharge in the halothane-anesethetized rat. In these studies, MPH produced a dose-dependent suppression of tonic and phasic discharge that was relatively modest at the lower doses. Nonetheless, these lower doses of MPH suppressed the signal-to-noise ratio of excitatory phasic discharge and increased the signal-to-noise ratio of the inhibitory component of the phasic response. Largely comparable effects were observed with oral and intraperitoneal administration of MPH. Combined, these observations indicate relatively modest suppression of LC neuronal discharge activity by low-dose MPH and that evoked discharge may be more sensitive than tonic activity to the lowest doses of MPH. It is posited that the behavioral-calming and cognition-enhancing effects of low-dose psychostimulants probably involve modest alterations in LC discharge combined with increased catecholamine efflux within select forebrain regions (i.e., the prefrontal cortex).

Low doses of methylphenidate (MPH; Ritalin) and other psychostimulants are highly effective and widely prescribed for the treatment of attention deficit hyperactivity disorder (ADHD; for review, see Greenhill, 2001). Importantly, the majority of the documented cognitive-enhancing and behavioral-calming actions of low-dose MPH are not paradoxical or unique to ADHD. Instead, these actions are apparent in both normal human and animal subjects (Rapoport et al., 1980; Mehta et al., 2001; Kuczynski and Segal, 2002; Rapoport and Inoff-Germain, 1999; Kuczenski and Segal, 2001, 2002; Berridge et al., 2006). Moreover, these actions are in contrast to the cognition-imparing and locomotor-activating effects of higher doses of MPH and other stimulants (Segal, 1975; McCaughy and Sarter, 1995). Neurochemically, higher doses of psychostimulants increase catecholamine efflux widely throughout the brain (Kuczynski and Segal, 1994). In contrast, low, clinically relevant doses of MPH preferentially increase catecholamine efflux within the prefrontal cortex, with little effect on these neurotransmitters outside this region (Kuczynski and Segal, 2001, 2002; Berridge et al., 2006). Combined, these observations indicate that the majority of the behavioral and cognitive effects of low-dose psychostimulants are similar in normal and ADHD subjects and that the actions of low doses of these drugs seem qualitatively distinct from higher doses.

ADHD is associated with a dysregulation of a variety of cognitive and behavioral processes (Solanto, 1998). Evidence indicates the locus coeruleus (LC)-norepinephrine (NE) system exerts a critical modulatory influence on many of these processes (Biederman and Spencer, 1999; Berridge, 2001; for review, see Biederman and Waterhouse, 2003). LC neurons display two distinct firing modes: tonic and phasic. Tonic discharge refers to relatively slow and highly regular state-dependent activity that is associated with the regulation of arousal and a variety of state-dependent processes, including sensory information processing, attention, working memory, and motor processes (Foote et al., 1980; Aston-Jones and Bloom, 1981; Rajkowski et al., 1994; Devilbiss and Waterhouse, 2004; Arnsten and Dudley, 2005). Phasic discharge is driven by novel or salient sensory stimuli and is characterized by a brief burst of two to three action potentials followed by a sustained period of suppression of discharge activity (200–500 ms). Additionally, phasic discharge is associated

This work was supported by National Institutes of Health Grants DA10981 and MH14602.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.106.110015.

ABBREVIATIONS: MPH, methylphenidate; ADHD, attention deficit hyperactivity disorder; LC, locus coeruleus; NE, norepinephrine; EEG, electroencephalogram/electroencephalographic; DA dopamine.
with alerting/orienting reactions, sustained attention, and/or decision making and action (Rajkowski et al., 1994; Clayton et al., 2004).

Given the aforementioned information, dysfunction of the LC-NE system maybe a contributing factor in ADHD (Biederman and Spencer, 1999). Additionally or alternatively, alterations in LC discharge activity patterns may well contribute to the therapeutic actions of low-dose stimulants in the treatment of this disorder. Previous studies indicate that moderate-to-high doses of psychostimulants profoundly suppress tonic LC discharge activity via activation of $\alpha_2$-autoreceptors (Graham and Aghajanian, 1971; Ryan et al., 1985). However, our recent neurochemical studies indicate that clinically relevant doses of MPH exert minimal effects on NE (and dopamine) efflux within select forebrain regions (i.e., prefrontal cortex) and modest suppression of LC discharge activity contribute to the unique behavioral and cognitive actions of low-dose psychostimulants. A preliminary report of these findings has been published previously in abstract form (Devilbiss and Berridge, 2003).

Materials and Methods

Animals and Surgery. Male Sprague-Dawley rats (250–350 g; Charles River Laboratories, Inc., Wilmington, MA) were used in all studies. Animals were housed in pairs and provided with free access to food and water for approximately 1 week following arrival. Animals were anesthetized with halothane (1–4% in air; Halocarbon Laboratories, River Edge, NJ) and placed in a stereotaxic instrument. The stereotaxic incisor bar was set at 11.5 mm below zero. Body temperature was maintained at 36–38°C throughout the experiment.

The skull was exposed and a surface-to-depth bipolar electrode was implanted into the frontal cortex (A + 3.2, L ± 1.2, V-2.0) to record electroencephalographic (EEG) activity, as described previously (Berridge and Foote, 1991). The EEG electrode was secured to a skull screw with a cyanacrylate adhesive (Loctite 382; Henkel Loctite Co., Rocky Hill, CT). EEG signals were amplified and filtered (0.3–100.0 Hz) and recorded digitally with a 1401 A/D computer interface and Spike2 Software (CED Instruments, Cambridge, UK). Halothane concentrations were adjusted to maintain stable large-amplitude, slow-wave EEG activity while permitting a 2-s tail-pinch to elicit robust EEG desynchronization that persisted for 10 to 120 s beyond the pinch (Fig. 1A). A second hole was made in the skull above the LC (A, −12.6; L, ±1.2). A glass micropipette (impedance 5–12 MΩ) was lowered into the LC (V, from −6.0 to −6.5) to record electrophysiological activity of single LC neurons. Methods used for
LC recordings were similar to those used previously (Adams and Foote, 1988; Berridge and Foote, 1991). In brief, amplification and discrimination of LC neuronal discharge activity was made using an amplifier/window discriminator (Fintronics, New Haven, CT) and recorded simultaneously with EEG activity using a 1401 A/D computer interface and Spike2 software (CED Instruments). LC neurons were tentatively identified using previously described criteria (Foote et al., 1980; Fig. 1B). Sensory-evoked phasic discharge of LC neurons was elicited with 1.5-mA biphasic 50-ms pulses delivered to the hind paw contralateral to the LC recording electrode at a frequency of 0.1 Hz. For hind paw stimulation, a 30-gauge hypodermic needle was inserted into the skin above the joints between the tarsal, metatarsal, and phalanges of the first digit of the hind paw (Valentino and Foote, 1987). Under these conditions, LC phasic discharge does not habituate to repeated foot-shock (Valentino and Foote, 1987).

**Experimental Procedures.** The stability of single LC neuronal recordings and their responsiveness to foot-shock stimulation were confirmed. An increase in firing rate (3 S.D. above tonic rates) following sensory stimulation was used to verify the responsiveness of LC neurons to foot-shock. Following verification that the recording was sufficiently stable and that LC neurons were responsive to foot shock, the experimental session was begun. During control conditions, tonic and phasic LC discharge was determined over a 15-min interval. During this 15-min block, tonic discharge activity was recorded for the first 5 min, followed by 60 foot stimulations (0.1 Hz for 10 min). Vehicle (0.9% saline i.p.) or one of five doses of MPH (0.25, 0.5, or 1.0 mg/kg i.p. or 2.0 or 4.0 mg/kg oral gavage) was administered following this control period. Tonic and phasic LC activity was further assessed following vehicle or drug administration in 15-min blocks for the duration of the experiment.

**MPH Dose Selection and Administration Procedures.** MPH HCl [d-threo-methyl α-phenyl-α-(2-piperidyl)acetate hydrochloride; M2892; Sigma-Aldrich, St. Louis, MO] was dissolved in 0.9% saline. Clinically, psychostimulants are administered orally and exert therapeutic actions at low doses that result in peak plasma concentrations within the range of 8 to 40 ng/ml (Swanson and Volkow, 2002). Although MPH is administered orally for the treatment of ADHD, the majority of studies that have examined the behavioral, neurochemical, and electrophysiological actions of moderate- and high-dose stimulants in rodents used i.p. or subcutaneous administration. Therefore, in the current study it was of interest to examine the effects of both i.p. and oral MPH on LC discharge activity. In the rat, MPH has been estimated to yield clinically relevant peak plasma concentrations when administered i.p. at a dose of 0.25 to 0.5 mg/kg and orally at a dose of 1.0 to 3.0 mg/kg (Kuczenski and Segal, 2002; Berridge et al., 2006). Importantly, these low doses of MPH enhance working memory and sustained attention in rats, similar to that observed in humans (Arnst and Dudley, 2005; Berridge et al., 2006). Moreover, when administered in the rat at these low doses, MPH is devoid of arousal-increasing and locomotor-activating effects, and, under conditions associated with elevated locomotion, suppresses locomotor activity (Kuczenski and Segal, 2002; Berridge et al., 2006). Thus, within this dose range, MPH produces several behavioral/cognitive actions that are similar to that observed in both normal and ADHD human subjects (Solanto, 1998). Based on these observations, the current study examined the effects of 0.25, 0.5, and 1.0 mg/kg MPH administered i.p. and 2.0 and 4.0 mg/kg MPH administered by oral gavage. MPH was administered orally via a length of PE50 tubing inserted into the stomach before placing the animal into the stereotaxic instrument.

The half-life of MPH in the rat is approximately 1 h (Wargin et al., 1983) with peak plasma levels observed approximately at 5 to 15 min following oral or i.p. administration, respectively (Berridge et al., 2006). Consistent with this, low doses of MPH induced increases in DA and NE reach a maximum within 15 to 30 min following drug administration and return to baseline over the subsequent 30 to 60 min (depending on dose). Thus, based on these observations, LC activity was determined for 60 min post-MPH administration.

**Data Analysis.** Spike trains from recorded LC neurons were used to construct rate histograms of spontaneous activity and peri-stimulus time histograms of foot-shock-evoked phasic discharge. Excitatory and inhibitory components of the phasic response were calculated using procedures similar to those described previously (Aston-Jones and Bloom, 1981b). In brief, tonic LC firing rates were calculated as the mean discharge rate during the 5-min periods between periods of foot-shock stimulation. In addition, the incidence of burst activity within periods of tonic discharge was calculated. Burst discharge was defined as two or more action potentials occurring at a rate faster than 10 Hz with an interspike interval of less than 125 ms (Page and Abercrombie, 1999). An additional measure of spontaneous discharge rate was calculated during the 10-min period (n = 60) of stimulus presentation. In these analyses, average LC discharge was calculated during the 2.5 s immediately before foot-shock. This second metric of spontaneous firing rate was used to (1) control for possible changes in tonic firing rates during trains of foot-shock and (2) provide a baseline discharge rate for phasic response signal-to-noise calculations (see below).

The excitatory component of the phasic response was calculated as the mean frequency of discharge during the epoch where the response exceeded 3 S.D. above spontaneous rates (Fig. 1C, indicated between a and b). The magnitude of the inhibitory component was calculated as the firing rate within the period between 100 and 500 ms following stimulus presentation (Fig. 1C, e–f) subtracted from the average spontaneous firing rate. The duration of the inhibitory component was calculated as the time that the mean frequency of discharge was 1 S.D. below spontaneous firing rates (Aston-Jones and Bloom, 1981b). Furthermore, signal-to-noise ratios of the magnitude of the excitatory or inhibitory component of the phasic response were calculated. Signal-to-noise ratios for LC neurons can be calculated by several methods. Z-score calculations (excitatory response – background/S.D. background) or (background – inhibitory response)/S.D. background were used for these studies over the more simple measure of signal-to-noise (excitatory response – background) due to the additional sensitivity of this measure to potential changes in the variability of the underlying tonic firing rate that could result from periodic foot-shock. For these analyses, tonic discharge was calculated from the 2.5 s immediately preceding foot-shock rather than from the 5-min block of tonic discharge preceding foot-shock trials. This was done to avoid potential changes in the underlying tonic firing rate that could result from periodic foot-shock. Despite concerns that foot-shock might alter tonic discharge rates, in the end no substantial differences were observed between measures of tonic LC activity and background discharge rates during foot-shock. Data collected in the current study were expressed as a percentage of baseline discharge rates (prevehicle or pre-MPH) or percentage of change from saline, which permits comparisons between cells with different tonic or phasic discharge rates. A mixed two-way analysis of variance with both repeated (time) and between (treatment)-measures was used to determine statistical significance of MPH-induced changes in LC discharge as a function of dose and time. Post hoc comparisons were performed using the Fisher least significant difference test.

**Histology.** Following each experiment, Chicago blue dye was iontophoretically ejected into the recording site (glass electrodes contain 3 M NaCl + 2% Chicago blue). The animal was deeply anesthetized and perfused with 50 ml of 4% formaldehyde. The brain was placed in 50 ml of the perfusion solution for a minimum of 24 h. Frozen 40-μm coronal sections were collected through the LC and counterstained with Neutral Red.

**Results**

**Overview**

Extracellular activity of individual LC neurons was recorded from 88 animals (one cell/animal) before and after i.p.
or oral gavage vehicle or MPH administration. LC activity is sensitive to alterations in anesthetic plane as indicated by EEG activity patterns in the halothane-anesthetized rat (Berridge et al., 1993; Berridge and Abercrombie, 1999). Therefore, to avoid alterations in LC activity that might result from drug-induced changes in arousal, EEG was monitored throughout the experimental session (see Materials and Methods; Fig. 1). Data from LC recordings were eliminated from further analysis if EEG desynchronization was observed. Single-cell recordings were obtained largely from the central one-third of the LC nucleus, as confirmed by histological analysis (Fig. 1D). Tonic firing rate and phasic responses to sensory stimuli were similar to those previously reported for LC neurons (Akaike, 1982). Under baseline conditions, tonic LC responses averaged 1.5 ± 0.17 Hz (±S.E.M.), excitable phasic discharge averaged 22 ± 3.04 Hz, and activity during inhibitory phasic discharge averaged 0.5 ± 0.24 Hz for 572 ± 98 ms. In addition, under baseline conditions an average of 0.1 ± 0.01 spontaneous bursts (more than two spikes/burst) was observed across all animals tested. Vehicle administration (i.p. or oral) did not alter tonic or phasic firing rates (Fig. 2). Moreover, there was no difference in LC discharge activity following i.p. or oral administration of vehicle. Thus, data from oral and i.p. vehicle-treated animals were combined for all analyses.

**Effects of MPH on Tonic Activity of LC Neurons**

**Tonic LC Activity.** The i.p. administration of MPH dose-dependently suppressed tonic firing rates of LC neurons [Fig. 3; dose, \( F_{(3,46)} = 7.14, p < 0.001 \); time, \( F_{(3,138)} = 54.03, p < 0.001 \); and dose \( \times \) time, \( F_{(9,138)} = 11.06, p < 0.001 \)]. At the lower doses, estimated to produce peak plasma concentrations within the range associated with clinical efficacy (0.25 and 0.5 mg/kg), MPH produced a relatively modest suppression of tonic discharge (i.e., from −37 to −45%). In contrast, following 1.0 mg/kg, estimated to yield peak plasma concentrations at the high end or above the range associated with clinical efficacy, the suppression of tonic activity was more pronounced (−58%). Oral administration of low-dose MPH also elicited a modest suppression of tonic LC discharge [from −27 to −38%; dose, \( F_{(2,46)} = 2.92, p = 0.066 \); time, \( F_{(3,108)} = 15.70, p < 0.001 \); and dose \( \times \) time, \( F_{(6,108)} = 4.94, p < 0.001 \)].

**LC Burst Activity.** Burst discharge of tonic LC activity is associated with more efficient NE release (Page and Abercrombie, 1999). In general, both i.p. [dose, \( F_{(3,38)} = 5.56, p = 0.003 \); time, \( F_{(3,114)} = 11.82, p < 0.001 \); and dose \( \times \) time, \( F_{(9,114)} = 1.69, p = 0.099 \)] and oral MPH [dose, \( F_{(2,25)} = 1.39, p = 0.269 \); time, \( F_{(3,75)} = 1.26, p = 0.296 \); and dose \( \times \) time, \( F_{(6,75)} = 1.42, p = 0.22 \)] had relatively mild effects on burst discharge (Fig. 4). The only exception to this was observed with the highest dose of i.p.-administered MPH, which significantly suppressed burst activity to a maximum of −41%.

**Effects of MPH on Phasic Activity of LC Neurons**

Additional analyses examined the effects of MPH on the excitatory and inhibitory components of phasic discharge. Analysis of phasic activation examined 1) stimulus-evoked phasic discharge (evoked-discharge) and 2) excitatory dis-
charge normalized to spontaneous firing rates (signal to noise). Analyses of the inhibitory component examined: 1) suppression of spontaneous discharge, 2) percentage of suppression relative to baseline discharge rates, and 3) the duration of this component.

Evoked Discharge. The i.p. and oral MPH produced a dose-dependent suppression of evoked LC discharge (Fig. 5, A and B). Following i.p. administration, MPH-induced suppression of evoked-discharge was significant at all doses [dose, $F_{(3,45)} = 16.94, p < 0.001$; time, $F_{(3,135)} = 4.59, p = 0.004$; and dose $\times$ time, $F_{(9,135)} = 1.16, p = 0.327$]. At the lowest doses tested (0.25 and 0.5 mg/kg), MPH produced a relatively small suppression of evoked discharge (i.e., from $-28$ to $-41\%$). In contrast, 1.0 mg/kg i.p. produced a more pronounced suppression of evoked discharge ($-58\%$). Despite the robust suppressive effects of 1.0 mg/kg on evoked discharge, only the lowest dose of i.p. MPH produced a significant suppression of the signal to noise of evoked discharge [Fig. 5C; dose, $F_{(3,40)} = 5.71, p = 0.022$; time, $F_{(3,120)} = 2.44, p = 0.068$; and dose $\times$ time, $F_{(9,120)} = 1.85, p = 0.066$], indicating a larger effect on evoked discharge than tonic discharge at this dose.

Similar to that observed with i.p. administration, all doses of oral MPH significantly suppressed evoked-discharge [Fig. 5B; dose, $F_{(2,25)} = 22.46, p < 0.001$; time, $F_{(3,75)} = 2.89, p = 0.04$; and dose $\times$ time, $F_{(6,75)} = 1.26, p = 0.286$]. In addition, similar to i.p. administration, only the lowest dose of oral MPH elicited a significant suppression of the signal to noise of evoked discharge [Fig. 5D; dose, $F_{(2,28)} = 18.10, p < 0.001$; time, $F_{(3,84)} = 3.67, p = 0.015$; and dose $\times$ time, $F_{(6,84)} = 1.22, p = 0.302$]. Combined, these observations indicate that evoked discharge may be more sensitive than tonic activity to lowest doses of MPH.

Inhibitory Discharge. The i.p. MPH dose-dependently increased the magnitude of the inhibitory component of the phasic response (phasic inhibition) [Fig. 6A; dose, $F_{(3,33)} = 5.83, p = 0.003$; time, $F_{(3,99)} = 7.51, p < 0.001$; and dose $\times$ time, $F_{(9,99)} = 1.56, p = 0.138$]. At the lowest dose (0.25 mg/kg), this effect was relatively mild (i.e., $-112\%$ of saline). Following the 0.5-mg/kg dose, a more substantial and signif-

![Fig. 4. Reduction in the incidence of burst firing by MPH. A, i.p. administration of MPH results in a moderate dose-dependent reduction of the number of incidences of burst firing. Reductions in burst firing 15 to 60 min postdrug were statistically different from vehicle only for the 1.0-mg/kg dose. B, oral MPH did not significantly alter burst firing of LC neurons. *p < 0.05; **p < 0.01.](image)

![Fig. 5. Effects of MPH on the excitatory component of phasic discharge of LC neurons. A, i.p. administration of MPH yielded a dose-dependent suppression of excitatory phasic discharge. B, oral MPH suppressed the excitatory component of phasic discharge by LC neurons similar to that observed with i.p. administration. C, only the lowest dose of i.p. MPH produced a small, but significant, decrease in the signal-to-noise ratio. D, oral MPH decreased the signal-to-noise ratio at the 2.0-mg/kg and not the 4.0-mg/kg dose. *p < 0.05; **p < 0.01.](image)
icant increase in inhibition was observed (−173%). The effect of the highest dose of MPH (1.0 mg/kg) was only somewhat larger than that observed with 0.5 mg/kg i.p. (−196%). The apparent lack of dose dependence between 0.5 and 1.0 mg/kg on phasic inhibition probably reflects a floor effect (i.e., the near-complete suppression of discharge activity apparent at the 0.5 mg/kg). A significant facilitation of the signal-to-noise ratio of the inhibitory response was observed only at the 0.5-mg/kg dose [Fig. 6C; dose, $F_{3,44} = 5.66, p = 0.002$; time, $F_{(3,132)} = 6.05, p < 0.001$; and dose $\times$ time, $F_{(9,132)} = 1.64, p = 0.11$].

Oral administration of low-dose MPH tended to increase phasic inhibition, although these effects did not reach statistical significance [dose, $F_{2,27} = 2.42, p = 0.108$; time, $F_{(3,81)} = 5.16, p = 0.003$; and dose $\times$ time, $F_{(6,81)} = 1.91, p = 0.09$]. Similar to that observed with i.p. administration, the lowest oral dose produced a moderate suppression of LC discharge (−63%) during the inhibitory component, whereas the highest dose had a larger effect on phasic inhibition. Also similar to that observed with i.p. administration, the signal-to-noise ratio of the inhibitory response was significantly facilitated at the lowest but not the highest dose of MPH [Fig. 6D; dose, $F_{1,30} = 8.11, p = 0.008$; time, $F_{(3,90)} = 9.15, p < 0.001$; and dose $\times$ time, $F_{(6,90)} = 3.24, p = 0.006$].

**Phasic Inhibition Duration.** In general, low-dose MPH had minimal effects on the duration of the inhibitory component (Fig. 7). Only the highest doses of MPH administered i.p. [dose, $F_{3,30} = 10.32, p < 0.0001$; time, $F_{(3,90)} = 2.11, p = 0.104$; and dose $\times$ time, $F_{(9,90)} = 3.71, p < 0.0005$] or orally [dose, $F_{2,27} = 7.92, p = 0.002$; time, $F_{(3,81)} = 0.78, p = 0.513$; and dose $\times$ time, $F_{(6,81)} = 2.58, p < 0.05$] significantly increased the duration of the inhibitory component. Increases in the duration of the inhibitory component following the highest doses of i.p. or oral MPH reached a maximum of 145 and 69% above saline, respectively.

**Discussion**

At low doses, MPH and other psychostimulants exert largely similar behavioral-calming and cognition-enhancing actions in normal individuals and ADHD patients (Solanto, 1998). These actions are in contrast to those of higher doses.
of stimulants that impair cognitive function and exert pronounced reinforcing, locomotor-activating, and/or stereotypy-inducing actions (Segal, 1975; McGaughy and Sarter, 1995). Moreover, in contrast, to that observed with moderate-to-high doses of MPH and other psychostimulants (Kuczenski and Segal, 1994), low, clinically relevant doses of MPH increase NE and DA efflux primarily within the prefrontal cortex, having minimal effects on NE/DA efflux outside this region (Berridge et al., 2006).

Substantial evidence suggests LC discharge mode and rate are critical variables for a variety of behavioral/cognitive processes (for review, see Berridge and Waterhouse, 2003). Moreover, it is well documented that higher doses of psychostimulants profoundly suppress tonic LC discharge activity, probably contributing to the cognition-impairing actions of high-dose psychostimulants (Graham and Aghajanian, 1971; Ryan et al., 1985; Lacroix and Ferron, 1988; Ishimatsu et al., 2002). The absence of information regarding the actions of low, clinically relevant doses of psychostimulants on LC signaling limits our ability to formulate hypotheses regarding the role of the LC in the therapeutic actions of these drugs. The current studies characterized the actions of low-dose MPH on tonic and phasic LC discharge. These studies demonstrate that when administered at low, clinically relevant doses (see below), MPH produces a relatively modest dose-dependent suppression of both tonic and phasic LC discharge. At the lowest doses, MPH also suppressed the signal-to-noise ratio of evoked phasic discharge. Moreover, the lower doses of MPH increased phasic inhibition and the signal-to-noise ratio of phasic inhibition. Given that moderate alterations in LC discharge are associated with changes in forebrain signal processing and cognition (Rajkowski et al., 1994; Devilbiss and Waterhouse, 2004; Arnsten and Li, 2005), relatively modest effects of low-dose MPH on LC discharge activity could nonetheless have a significant affect on cognition and behavior.

**Dose and Route of Administration.** The behavioral and cognitive actions of psychostimulants are highly dependent on dose. Low-dose psychostimulants produce behavioral-calming and cognitive-enhancing actions that are in distinct contrast to the behavioral actions of higher doses of these drugs. Importantly, these behavioral and cognitive actions are not limited to ADHD but are also observed in normal human and animal subjects (Rapoport et al., 1980; Solanto, 1998; Mehta et al., 2001; Kuczenski and Segal, 2002; Rapoport and Inoff-Germain, 2002; Wilens et al., 2004; Arnsten and Dudley, 2005). Combined, these and other observations indicate that the majority of the documented behavioral actions of low-dose stimulants are not unique to ADHD. Moreover, these observations indicate that when administered at low doses in both humans and rats, MPH and other psychostimulants are, in fact, not stimulatory but instead exert behavioral-calming and cognition-enhancing actions.

Clinically, MPH is routinely administered by an oral route. Nonetheless, the majority of information on the neural actions of psychostimulants has involved the use of i.p. or subcutaneous injection. Thus, to better compare with the existing literature, the current studies used both oral and i.p. MPH administration at doses that have been estimated to produce peak plasma levels in rats that fall within the range associated with clinical efficacy (Kuczenski and Segal, 2002; Berridge et al., 2006). Importantly, at these doses, MPH improves working memory and sustained attention while suppressing elevated baseline levels of locomotor activity in rats and mice (Kuczenski and Segal, 2002; Arnsten and Dudley, 2005; Berridge et al., 2006). In the current studies, we observed similar actions of oral and i.p. MPH on LC neuronal activity, when dose is adjusted to produce similar plasma levels. This is consistent with previous microdialysis studies demonstrating a largely similar time course and magnitude of action of oral and i.p. MPH on NE and dopamine efflux when dose is adjusted appropriately (Kuczenski and Segal, 2001, 2002; Berridge et al., 2006).

**Behavioral Relevance of MPH-Induced Alterations in LC Discharge.** The neural substrates underlying the behavioral and cognitive actions of low-dose stimulants are currently unknown. However, evidence suggests an involvement of the LC-NE system in these actions (Biederman and Spencer, 1999; for review, see Arnsten and Li, 2005). Moreover, substantial evidence indicates that optimal performance on a variety of cognitive tasks depends on a relatively narrow range of tonic and phasic LC discharge (Rajkowski et al., 1994; Clayton et al., 2004). For example, performance in a vigilance task is associated with an intermediate level of tonic LC discharge that in turn promotes maximal phasic evoked discharge (Aston-Jones and Bloom, 1981a; Rajkowski et al., 1994). On this task, both high and low LC tonic discharges are associated with attentional impairment (Rajkowski et al., 1994; Clayton et al., 2004). This inverted-U dose-response curve is similar to that observed for the relationship between NE neurotransmission and working memory (Arnsten, 2001).

The current results demonstrate that in contrast to that observed with higher doses (Lacroix and Ferron, 1988; Ishimatsu et al., 2002), low doses of MPH documented to improve cognitive performance in rats (Berridge et al., 2006) produce only a moderate suppression of tonic LC discharge activity. This moderate suppression might help maintain phasic activity within a range that is associated with sustained and focused attention (Clayton et al., 2004). Importantly, a majority of phasic discharge was preserved following low doses of MPH. Thus, at these doses MPH preserves the transfer of temporally locked sensory information to LC terminal fields. This probably contrasts with that of higher doses associated with cognitive impairment. At the lower doses tested, MPH produced a significant, although relatively modest, suppression of the signal-to-noise ratio of phasic discharge. It is possible that this action facilitates suppression of attending to and/or responding to distracting, nonsalient environmental stimuli.

The current study demonstrates that low-dose MPH enhances the magnitude and signal-to-noise ratio of LC phasic inhibition. Few studies have addressed the role of the phasic inhibitory component of LC discharge in cognitive function. However, previous studies demonstrate that cortical responses are enhanced when coincident with the LC phasic inhibitory component (Waterhouse et al., 1998). Thus, MPH-induced facilitation of phasic inhibition may facilitate signal processing within cortical regions associated with attention and impulsivity (e.g., prefrontal cortex). Therefore, actions of MPH on phasic inhibition could well be an important component of the unique behavioral actions of low doses of this drug.
Methodological Considerations. These data were obtained from lightly halothane-anesthetized rats, as determined by EEG recordings. This preparation provides certain advantages over the unanesthetized animal, including the facilitation of obtaining stable and high-quality single-unit recordings over prolonged periods while avoiding state-dependent alterations in LC discharge. Importantly, tonic discharge rates of LC neurons under these anesthetic conditions (1.5 Hz) are similar to those observed in awake animals (Foote et al., 1980; Aston-Jones and Bloom, 1981a). Moreover, the use of this preparation permits direct comparison with results obtained in previous studies that examined the effects of higher doses of psychostimulants on tonic LC neuronal activity. In both anesthetized and unanesthetized animals, tonic and phasic LC firing are dependent on cortical activity state (Berridge and Foote, 1991; Berridge et al., 1993, Berridge and Abercrombie, 1999). Given that stimulants can activate forebrain EEG (Berridge and Morris, 2000), cortical EEG activity was monitored in all animals. Under these experimental conditions, these doses of MPH did not produce obvious cortical activation, consistent with minimal wake-promoting actions observed with these doses in unanesthetized animals (Berridge et al., 2006). Thus, MPH-induced alterations in LC discharge are not due to indirect actions of MPH on forebrain neuronal activity state. Anesthesia can reduce tonic and phasic LC activity, particularly at deeper levels of anesthesia (Aston-Jones and Bloom, 1981b). In the current study, a relatively low level of anesthesia was administered, as indicated by a robust tail-pinch-induced EEG activation. Under these conditions, we observed robust sensory-evoked phasic responses and tonic discharge rates, similar to that observed in unanesthetized animals (Aston-Jones and Bloom, 1981a). Combined, these observations indicate it is likely that the actions of MPH observed in the current study reflect actions that occur in the absence of anesthesia, although future studies should verify this hypothesis.

Summary. The current observations indicate that, when administered at doses that yield clinically relevant plasma levels, MPH produces a moderate suppression of both tonic and evoked phasic discharge. The signal-to-noise ratio of the evoked phasic discharge was suppressed following the lowest dose of MPH. In contrast, phasic inhibition and the signal-to-noise ratio of this component were increased by low-dose MPH. Evidence suggests that combined, these actions may well contribute to the ability of low-dose stimulants to suppress attention and/or responsivity to distracting, nonsalient, environmental stimuli. The actions of low-dose MPH on LC discharge activity occur in conjunction with the relatively selective increase in catecholamine neurotransmission within the prefrontal cortex. It is posited that the behavioral-calming and cognition-enhancing actions of low-dose MPH involve moderate actions on LC discharge combined with alterations in catecholamine neurotransmission within the prefrontal cortex.

Acknowledgments

We thank Kate Reis for help in collecting these data.

References


Arnsten AF and Dudley AG (2005) Methylphenidate improves prefrontal cortical cognitive function through alpha2 adrenoceptor and dopamine D1 receptor actions: relevance to therapeutic effects in Attention Deficit Hyperactivity Disorder. Behav Brain Funct 1:2.


Rapport JL, Buchshauss MA, Weingartner H, Zahn TP, Ludlow C, and Mikkelson

Downloaded from jpet.aspetjournals.org on July 9, 2017.


Address correspondence to. Dr. David M. Devilbiss, Department of Psychology, W. J. Brogden Psychology Bldg., University of Wisconsin-Madison, 1202 W. Johnson St., Madison, WI 53706. E-mail: ddevilbiss@wisc.edu