Comparison of Effects of Haloperidol Administration on Amphetamine-Stimulated Dopamine Release in the Rat Medial Prefrontal Cortex and Dorsal Striatum

ELIZABETH A. PEHEK

Department of Psychiatry, Case Western Reserve University, and Cleveland Veterans Affairs Medical Center, Brecksville, Ohio

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ABSTRACT

Research has shown that there are important neurochemical differences between the mesocortical and mesostriatal dopamine systems. The work reported in this paper has sought to compare the regulation of dopamine release in the medial prefrontal cortex and the anterior caudate-putamen. In vivo microdialysis was used to recover dialysate fluid for subsequent assay for dopamine concentrations. The responses to D2 antagonist (haloperidol) administration, which has been shown to increase impulse-dependent dopamine release, were compared. Results demonstrated a diminished effect of systemic haloperidol administration on dopamine efflux in the prefrontal cortex. The responses to systemic administration of a nonimpulse-dependent, transporter-mediated, dopamine releaser (d-amphetamine) were also contrasted. Results again demonstrated a diminished pharmacological effect in the cortex. The potential interaction of stimulation of these two types of dopamine release was examined by coadministration of these compounds. Haloperidol pretreatment dramatically potentiated the dopamine-releasing effect of amphetamine administration. This effect was observed in both the cortex and the striatum. Subsequent work demonstrated that this effect of haloperidol was mediated by D2-like receptors in the prefrontal cortex. These results are discussed in relation to other neurochemical and neuroanatomical studies demonstrating sparse densities of dopamine transporter sites and dopamine D2 receptors in the cortex compared with the striatum. They demonstrate a functional correlate to the recently reported, largely extrasynaptic localization of dopamine transporter sites in the prefrontal cortex. Furthermore, they demonstrate the existence of cortical D2-like autoreceptors that may normally be “silent” under basal conditions.

The prefrontal cortex has been implicated in a number of important functions, mental disorders, and behavioral states, including locomotion, working memory, schizophrenia, stress, and drug abuse. It is believed that these are regulated, in part, by dopamine (DA) release from the mesocortical dopaminergic pathway (Goeders and Smith, 1983; Weinberger, 1987; Abercrombie et al., 1989; Sawaguchi and Goldman-Rakic, 1991). This tract originates in the ventral tegmental area of the midbrain and projects to the medial prefrontal cortex (mPFC) in the rat (Emson and Koob, 1978). Several studies have shown that this pathway displays unique neurochemical characteristics relative to the other two major dopaminergic systems of the brain, the nigrostriatal and mesolimbic pathways (Bannon and Roth, 1983). For example, mesocortical neurons display faster rates of synthesis, turnover, and firing, exhibit more frequent and intense burst firing, and are more resistant to inhibition by DA D2 receptor subtype agonists (Cubeddu et al., 1990).

D2 receptor antagonists such as typical antipsychotic drugs increase DA turnover and dialysate DA concentrations in the dorsal (caudate-putamen: CP) and ventral (nucleus accumbens) striatum (Wolf et al., 1987; Moghaddam and Bunney, 1990). Studies have demonstrated that these effects result from the blockade of inhibitory DA autoreceptors, which, in turn, increase exocytotic, calcium-dependent, DA release. D2 antagonists produce relatively lesser effects on prefrontocortical DA release, which may be related to the rather unique neurochemistry of this brain region, including a relatively sparse density of DA D2 subtype receptors in the rat (Gaspar et al., 1995).

There are also differences between the mPFC and CP in the effects of agents that increase nonimpulse-dependent, carrier-mediated, DA release. Few studies have been performed in the mPFC, but uptake blockers such as cocaine have been reported to produce relatively lesser increases in...
mesocortical versus mesostriatal dialysate DA concentrations (Moghaddam and Bunney, 1989). In the striatum, when drugs that block the DA transporter (DAT) are combined with D2 antagonists, a pronounced synergistic increase in DA turnover and release is observed (Fuller et al., 1978; Waldmeier et al., 1985; Sharp et al., 1986; Westerink et al., 1987; Watanabe et al., 1989; Gudelsky et al., 1992; Tyler and Galloway, 1992). To our knowledge, this experiment has not been performed in the mPFC.

The present study was designed to compare the effects of administration of the DA releaser/uptake blocker d-amphetamine (AMPH) on dialysate DA concentrations in the mPFC and the anterolateral CP and to compare the effects of DA autoreceptor blockade, produced by haloperidol (HAL) administration, on AMPH-stimulated DA release in these two structures. Additionally, whether these combined effects were the result of DA D2-like receptor blockade was tested in the prefrontal cortex.

Materials and Methods

Animal Preparation. All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee. Experimentally naive, male Sprague-Dawley rats were used. Rats ranged in weight from 200–400 g at the time of the surgery. Rats were anesthetized with a mixture of ketamine (70 mg/kg) and xylazine (6 mg/kg) administered i.m. Subsequently, the animals were mounted in a stereotaxic frame and dura was carefully removed. Stainless steel guide cannulas (21 gauge) were implanted on the brain surface above the prefrontal cortex (anterior-posterior 3.2, medial-lateral 0.8) or anterior CP (anterior-posterior 1.2, medial-lateral 3.4) (–3.2 head angle; Paxinos and Watson, 1982) 2 to 4 days before the experiments. On the days of the experiments, dialysis probes were lowered through these guide cannulas in awake animals, terminating in the structures of interest. Rats were used once and, after each experiment, probe placements were verified. This was accomplished by perfusing the intact microdialysis probes with cresyl violet dye. Brains were removed and frozen. They were then dissected manually (sliced with a razor blade) and drawn freehand on appropriate sections copied from a rat brain atlas (Paxinos and Watson, 1982). Data from rats with probe placements outside the brain regions of interest were not used.

Microdialysis. The microdialysis probes used a concentric flow design and were constructed as described previously (Yamamoto and Pehek, 1990). The membranes were 5.5 and 4 mm long for probes placed in the prefrontal cortex and striatum, respectively. Animals were housed in cylindrical buckets and tethered to liquid swivels placed in the prefrontal cortex and striatum, respectively. Animals were anesthetized with a mixture of ketamine (70 mg/kg) and xylazine (6 mg/kg) administered i.m. Subsequently, the animals were mounted in a stereotaxic frame and dura was carefully removed. Stainless steel guide cannulas (21 gauge) were implanted on the brain surface above the prefrontal cortex (anterior-posterior 3.2, medial-lateral 0.8) or anterior CP (anterior-posterior 1.2, medial-lateral 3.4) (–3.2 head angle; Paxinos and Watson, 1982) 2 to 4 days before the experiments. On the days of the experiments, dialysis probes were lowered through these guide cannulas in awake animals, terminating in the structures of interest. Rats were used once and, after each experiment, probe placements were verified. This was accomplished by perfusing the intact microdialysis probes with cresyl violet dye. Brains were removed and frozen. They were then dissected manually (sliced with a razor blade) and drawn freehand on appropriate sections copied from a rat brain atlas (Paxinos and Watson, 1982). Data from rats with probe placements outside the brain regions of interest were not used.

Results

Concentrations of dialysate DA per HPLC injection (20 μl) were 0.59 ± 0.04 pg in the mPFC (equivalent to 0.13 fmol/μl; n = 80). Concentrations in the anterolateral CP were approximately ten times higher: 3.81 ± 0.10 pg/20 μl (1.25 fmol/μl; n = 49).

Effect of HAL Alone on DA Efflux in the CP and mPFC. Injections of HAL + vehicleAMPH caused a moderate increase in dialysate DA concentrations in the CP [F(7,35) = 9.09, p < .0001]. The maximal effect of HAL alone occurred 90 min after administration (Fig. 1). HAL administration did not significantly affect dialysate DA concentrations in the mPFC (these rats were part of an earlier study and did not receive vehicleAMPH injections).

Effect of AMPH on DA Efflux in CP and mPFC. Administration of AMPH dose dependently increased dialysate concentrations of DA in the CP and mPFC. This effect was significantly greater in the CP at all doses of AMPH (Fig. 2); significant time × brain area interaction for all three doses: 1.25 mg/kg: F(7,56) = 18.89, p < .001; 2.50 mg/kg: F(7,63) = 18.75, p < .001; 5.0 mg/kg: F(7,98) = 82.38, p < .00001 (all rats received vehicleHAL injections 30 min before AMPH; in addition, the 5.0 mg/kg AMPH rats received vehicleAPO 60 min before AMPH). The time courses of action were also different for the two brain areas. In the CP, the peak effect occurred at 30 min after AMPH administration (the 60-min
Effect of QUIN on HAL Potentiation of AMPH-Stimulated DA Efflux in the mPFC. QUIN administration dose dependently attenuated the potentiation by HAL administration of AMPH-stimulated cortical DA release (Fig. 7). This attenuation was significant at the 10.0 mg/kg quinpirole dose [significant drug × time interaction for the VEH/HAL/AMPH versus QUIN 10.0/HAL/AMPH groups: F(7,70) = 2.46, p < .026].

Discussion

The results of the present study demonstrate that, administered individually, both the DA D2 antagonist HAL and the DA uptake blocker/releaser AMPH increase dialysate DA concentrations to a greater degree in the anterolateral CP than the mPFC. When these agents were combined, a potentiation was observed in both structures. This potentiation was dependent on the dose of AMPH that was employed. In both structures, this effect was attenuated by earlier administration of the nonspecific DA agonist APO. This effect was characterized in the mPFC to be D2-like as the D2, D3, and D4 agonist QUIN dose dependently attenuated it. These results are in agreement with previous findings that the mesocortical DA system possesses D2-like inhibitory release-regulating autoreceptors (Wolf and Roth, 1987). However, the present work indicates that these mesocortical DA receptors may normally be “silent” under basal conditions, and thus their presence may not be revealed experimentally until DA release is stimulated.

Comparison of AMPH Effects in CP and mPFC

The primary mechanism of inactivation of synaptic DA is the process of uptake back into the presynaptic terminal through the DAT. Previous research has shown that d-AMPH acts on the DAT protein to reverse its direction so that DA is released instead of being taken up through this site into the presynapse (Liang and Rutledge, 1982). Uptake blockers like cocaine may also increase dialysate DA concentrations, but to a lesser degree than AMPH (Moghaddam and Bunney, 1989). The majority of this work has been done in the striatum. However, several in vitro and in vivo studies of the mPFC have demonstrated diminished effects of uptake inhibitors on DA uptake and release in the prefrontal cortex (Hadfield and Nugent, 1983; Izenwasser et al., 1990; Elsworth et al., 1993; Wheeler et al., 1993; Cass and Gerhardt, 1995). One study that compared the ventral striatum (nucleus accumbens) with the mPFC demonstrated that cocaine administration produced less of an increase in dialysate DA in the mPFC (Moghaddam and Bunney, 1989). These structures did not differ in the magnitude of response to AMPH. However, this latter finding does not conflict with the present results, because the dorsal striatum (CP) was studied here. Other work has shown that DA efflux is more sensitive to AMPH administration in the dorsal, relative to the ventral, striatum (Pehek et al., 1990). The present study, employing three doses of AMPH in the unanesthetized rat, clearly demonstrates that, over the dose range employed, anterolateral dorsal striatal dialysate DA concentrations are more sensitive to AMPH administration than prefrontocortical DA.

Responsivity to AMPH has been shown to correlate with density of dopaminergic innervation in the striatum (Yamamoto and Pehek, 1990). The dopaminergic innervation of the rat cortex is less dense than that of the striatum. This
Fig. 2. Comparison of effects of AMPH administration on dialysate concentrations of DA in mPFC and dorsal CP of rat brain. Values are expressed as percentage of baseline DA and are mean ± S.E.M. A, effects of 1.25 mg/kg AMPH. Vehicle for HAL was injected at time 0, and AMPH was injected at time 30 min (CP: n = 5; mPFC: n = 5). B, effects of 2.50 mg/kg AMPH. Vehicle for HAL was injected at time 0, and AMPH was injected at time 30 min (CP: n = 7; mPFC: n = 7). C, effects of 5.0 mg/kg AMPH. Vehicle for APO was injected at time −30, vehicle for HAL at time 0, and AMPH time 30 min (CP: n = 7; mPFC: n = 9).
Fig. 3. Effects of HAL (1.0 mg/kg) and AMPH administration on dialysate concentrations of DA in dorsal CP of rat brain. Values are expressed as percentage of baseline DA and are mean ± S.E.M. A, effects of 1.25 mg/kg AMPH alone or with HAL pretreatment. Vehicle or HAL were injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 5; HAL + AMPH: n = 6). B, effects of 2.5 mg/kg AMPH alone or with HAL pretreatment. Vehicle or HAL were injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 4; HAL + AMPH: n = 6). C, effects of 5.0 mg/kg AMPH alone or with HAL pretreatment. Vehicle for APO was injected at time −30, HAL was injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 7; HAL + AMPH: n = 8).
Fig. 4. Effects of HAL (1.0 mg/kg) and AMPH administration on dialysate concentrations of DA in mPFC of rat brain. Values are expressed as percentage of baseline DA and are mean ± S.E.M. A, effects of 1.25 mg/kg AMPH alone or with HAL pretreatment. Vehicle or HAL were injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 5; HAL + AMPH: n = 4). B, effects of 2.5 mg/kg AMPH alone or with HAL pretreatment. Vehicle or HAL were injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 7; HAL + AMPH: n = 7). C, effects of 5.0 mg/kg AMPH alone or with HAL pretreatment. Vehicle for APO was injected at time -30, HAL was injected at time 0, and AMPH was injected at time 30 min (AMPH alone: n = 9; HAL + AMPH: n = 10).
diminished density of cortical DA axon terminals is correlated with reductions in intracellular and dialysate DA concentrations relative to the striatum (Hemby et al., 1992; Garris et al., 1993). Corresponding to this, receptor autoradiographic studies have demonstrated fewer DAT sites (Javitch et al., 1985; Mennicken et al., 1992). Thus, the present reduced responsivity of the mPFC to AMPH may, in part, simply reflect this diminished dopaminergic innervation. However, recent work by Sesack and colleagues suggests that this reduced responsivity to a DAT ligand may also reflect reduced numbers of DAT sites per neuron in the mPFC (Sesack et al., 1998). This latter study examined the cellular distribution of DAT antibodies in the rat and monkey. They determined that prefrontocortical DAT sites were largely extrasynaptic, both in absolute terms and relative to the anterior cingulate cortex and the striatum. Quantitatively, there were less DAT sites/neuron in the mPFC. Thus, the low DA innervation density of the mPFC, in combination with sparse and mostly extrasynaptic DAT sites, may result in a diminished capacity of this structure to respond to drugs.
Mesocortical DA autoreceptors are thought to be lacking, or less sensitive to, DA agonists (Bannon and Roth, 1983). Specifically, it has been proposed that the mesocortical system only possesses nerve terminal D2-like release regulating autoreceptors. This autoreceptor subsensitivity, coupled with the relative paucity of prefrontocortical D2 receptors (Gaspar et al., 1995), may explain the present finding that basal DA is not augmented significantly in the mPFC after systemic HAL administration. Thus, under basal conditions, these cortical autoreceptors may be "silent".

HAL administration did stimulate striatal DA release. These findings are in agreement with previous studies demonstrating a greater increase in basal dialysate DA concentrations after D2 antagonist administration in the striatum relative to the mPFC (Moghaddam et al., 1990; Yamamoto et al., 1994).

HAL + AMPH in CP. In the striatum, there are a number of studies demonstrating D2 antagonist potentiation of the effects of DA uptake blockers on DA neurochemistry. Studies of tissue content have shown that HAL administration increases DA metabolism (dihydroxyphenylacetic acid concentrations) in both the striatum and frontal cortex (McMillen, 1981). This effect was potentiated by administration of the DA uptake blocker amfonelic acid.

AMPH increased DA synthesis, measured by rate of dopa accumulation after NSD-1015 administration, in the striatum but not the mPFC (Tyler and Galloway, 1992). In this study, the additional presence of the D2 blocker eticlopride further potentiated DA synthesis in the striatum. Thus, augmentation of DA synthesis may at least partially explain the present potentiation of DA release by the coadministration of HAL and AMPH in the CP.

In vivo microdialysis studies in the striatum have also demonstrated a potentiation of dialysate DA levels by combined administration of a D2 antagonist and a DA uptake blocker. These studies include systemic coadministration of sulpiride with AMPH (Sharp et al., 1986), HAL with amfonelic acid (Gudelsky et al., 1992), and HAL with amfonelic acid or GBR-12909 (Westerink et al., 1987). The results of the present study are similar to those of Westerink and colleagues, who reported "a strong synergistic rise of DA release". Tetrodotoxin administration blocked this synergism, indicating a role for impulse-dependent DA release (Westerink et al., 1987). Intrastriatal administration of methamphetamine was also potentiated by local application of sulpiride (Watanabe et al., 1989). The present results demonstrate that this potentiation is also observed with the combination of HAL and AMPH. Furthermore, DA receptor blockade was implicated, as pretreatment with the nonspecific DA agonist APO reversed the potentiation.

HAL + AMPH in mPFC. Despite the present lack of effect of HAL on basal mPFC DA, pretreatment before AMPH administration dramatically potentiated the AMPH-induced release of cortical DA. This result was similar to those previously reported in the striatum. Previous electrophysiological reports have indicated that the mesocortical system lacks both cell body impulse-regulating and nerve terminal synthesis-regulating autoreceptors (Bannon and Roth, 1983). Thus, it is unlikely that the HAL-induced potentiation in the mPFC resulted from actions at the cell body level or on synthesis-regulating autoreceptors per se. Rather, research has indicated the presence of nerve terminal release-regulating au-
to receptors in the mesocortical system (Cubeddu et al., 1990; Gobert et al., 1996). Thus, it is likely that the present results with HAL and AMPH resulted from actions on D2-like release-regulating autoreceptors. However, the present work considered alone cannot differentiate between these autoreceptor subtypes.

Tyler and Galloway (1992) suggested that, because the mPFC lacks synthesis-regulating autoreceptors, regulation of synthesis might be achieved solely by end-product inhibition of tyrosine hydroxylase. Thus, intracellular DA stores may normally act to decrease cortical DA synthesis. However, blockade of D2-like release regulating inhibitory autoreceptors would produce an increase in release and subsequent decrease in intracellular stores of DA. This, in turn, may decrease end-product inhibition and thereby increase the synthesis of DA, which would provide additional substrate for AMPH to act upon. Thus, the present HAL + AMPH potentiation in the mPFC may have resulted from effects of release-regulating autoreceptors on AMPH-stimulated, but not basal, cortical DA efflux. It is possible that these autoreceptors only play an active role in the regulation of mesocortical DA release under conditions of activation. Alternatively, these autoreceptors may function tonically, but may fail to affect basal DA efflux as measured by microdialysis. Because the density of cortical D2 receptors is low, blockade by HAL may slightly increase basal release, but below the detection limits for microdialysis sampling.

Earlier administration of 2.0 mg/kg of the nonspecific DA agonist APO reversed the present HAL-induced potentiation in the mPFC. This suggests that the augmentation of DA release is mediated by DA receptors, but does not indicate what type. Surprisingly, a higher dose (20 mg/kg) did not reverse the effect of HAL in the mPFC (but did in the CP). DA D1 receptors are more abundant than the D2 subtype in the mPFC. This situation is reversed in the CP. Perhaps differential effects of 20 mg/kg APO on contrasting D1/D2 receptor ratios explains the contrasting effects of this dose in the two brain areas. However, the present finding that pretreatment with QUIN attenuated the HAL-induced potentiation in the mPFC implies that functional D2-like autoreceptors are present in this structure and mediate the effects of HAL pretreatment on AMPH-induced DA release. This is consistent with earlier studies (Wolf et al., 1987).

Gobert and colleagues (Gobert et al., 1995; Gobert et al., 1996) have provided neurochemical evidence that the mesocortical system contains inhibitory D3 autoreceptors that are normally “silent” but are activated under conditions of stimulated DA release. However, recent findings with D3-knockout mice indicate that, while D3 receptors modulate mesolimbic DA release, they do not serve as autoreceptors in this DA system (Koeltzow et al., 1998). Rather, they may be part of a short-loop feedback system. Whether or not the mesocortical system is regulated in a similar manner remains to be determined. Because both HAL and QUIN have appreciable affinities for D2, D3, and D4 receptors (Seeman and Van Tol, 1994), the present findings can only suggest that D2-like receptors modulate mPFC DA release.

**Summary and Conclusions.** The present paper demonstrates a diminished functional neurochemical effect of the DA releaser d-AMPH on in vivo DA release in the rat mPFC relative to the CP. This may be related to previous reports demonstrating decreased DA innervation and numbers of DAT sites as well as a predominant extrasynaptic localization of these sites. Confirming previous reports, there was also a diminished neurochemical effect of the D2 antagonist HAL on dialysate DA concentrations. This may be related to the relatively low D2-receptor density in the mPFC. However, the present results support the existence of D2-like autoreceptors that may normally be “silent” under basal conditions. Based on previous work by others, it is likely that these autoreceptors regulate release of mesocortical DA (Cubeddu et al., 1990, Gobert et al., 1996, Wolf et al., 1987). When DA release was stimulated by AMPH, earlier blockade of these autoreceptors by HAL administration resulted in a dramatic potentiation of AMPH-stimulated DA release in the mPFC and CP. In both structures, blockade of release-inhibition may decrease the intracellular pool of DA and thus decrease end-product inhibition of DA synthesis. DA synthesis may then be augmented, resulting in increases in newly synthesized DA, which have been shown to be released preferentially by AMPH (Chiveh and Moore, 1975). This augmentation of intracellular DA, the substrate for AMPH, may explain the greater than additive effects of a D2 antagonist and a drug acting on DAT sites. As suggested by Cass and Gerhardt (1995), synaptic concentrations of prefrontocortical DA may be regulated more by modulation of release than uptake.

In the striatum, HAL-induced blockade of synthesis-regulating and impulse-regulating DA autoreceptors may have contributed to the potentiation of carrier-mediated DA release. Presumably, this did not contribute to the present results in the mPFC as previous work has indicated an absence of these autoreceptors in the mesocortical system. Thus, one might have expected a greater potentiation of the AMPH response by HAL in the CP relative to the mPFC. This was generally not observed. The decreased numbers of cortical DAT sites may also explain this. In the presence of high doses of AMPH, synaptic DA may be reduced significantly in the striatum by re-uptake. This may not be true in the mPFC, where blockade of the existing D2-like autoreceptors may render the mesocortical system relatively unable to self-regulate. This suggests that, in general, the mesocortical system may be less able to self-regulate, particularly as it relates to the termination of DA action in the synapse. Because many cortical DAT sites are extrasynaptic, it has been suggested that the activity of DA may be prolonged in prefrontocortical synapses (Sesack et al., 1998). Collectively, these findings may have important functional implications in the mPFC, where dopaminergic activity is thought to regulate emotion, cognition, and responses to stress and psycho-stimulants.

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**References**


Send reprint requests to: Elizabeth A. Pehek, Ph.D., Psychiatry Service 116A(B), Veterans’ Affairs Medical Center, 10000 Brecksville Rd., Brecksville, OH 44141. E-mail: eap6@po.cwru.edu.