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Effects of haloperidol and apomorphine on the K^+-depolarized overflow of [^{3}H] dopamine from rat striatal slices*

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The therapeutic efficacy of antipsychotic drugs such as haloperidol has been attributed to the ability of these agents to inhibit central dopaminergic activity [1–4] by blockade of central dopamine (DA) receptors [5]. It is believed that, in the corpus striatum, this blockade produces an increased turnover [5–10] and presumably increased release of DA. The regulation of DA turnover [6] and release [11,12], in the striatum, is thought to be mediated by presynaptic receptors [11,12] as well as by a long-loop striato-nigral pathway involving postsynaptic DA receptors. Farnebo and Hamberger [11,12] proposed that presynaptic DA receptors might be involved in the modulation of DA release based on their observation that apomorphine, a DA agonist, inhibited stimulation-evoked release of [^{3}H]DA from striatal slices while the neuroleptics, pimozide and chlorpromazine, enhanced striatal [^{3}H]DA release. In contrast, other investigators [13,14] studied the effects of several antipsychotic drugs and found inhibition of electrically stimulated release of [^{3}H]DA from rat striatal slices.

In the present study, we investigated further the effects of haloperidol and apomorphine on basal and on stimulation-evoked overflow of [^{3}H]DA by K^+-depolarization of rat striatal slices.

Male Wistar rats were killed by decapitation, and the brains were immediately removed and placed on ice. The corpus striatum (caudate-putamen and globus pallidus) was dissected carefully and 300-μm slices (2 mm by 3 mm) were made, using a McIlwain tissue chopper cutting in a rostro-caudal plane. The striatal slices were radiolabeled with DA by incubating for 30 min at 37° in an atmosphere of 95% O_2 – 5% CO_2 in a Krebs-Ringer-bicarbonate buffer (KR–b) containing the following: NaCl, 119 mM; KCl, 4.75 mM; KH_2PO_4, 1.17 mM; $MgSO_4 \cdot 7H_2O$, 1.19 mM; $NaHCO_3$, 25.5 mM; $CaCl_2$, 1.27 mM; glucose, 5.6 mM; ascorbic acid, 0.1%; pargyline, 10 μM; and [^{3}H]DA, 100 nM (New England Nuclear, Boston, MA, sp. act. 10 Ci/mM). After the incubation, ten slices were transferred to each of two glass chambers designed for superfusion. The total volume in each chamber was 1.0 ml. The slices were superfused at a rate of 1.0 ml/min at 25° with KR–b for a period of 5 min to remove surface radioactivity from the slices. In the control chamber, KR–b superfusion alone was continued, while the experimental chamber was superfused with KR–b containing the drug to be tested. In most cases, after a 10 min period of superfusion, both chambers were then superfused for 2 min with KR–b in which the K^+ concentration was raised to 33 mM and the Ca^{2+} concentration was raised to 2.5 mM. Before, during and after K^+-depolarization, the superfused solution was collected continuously at 1 min intervals for a total period of 30 min and portions were taken for determination of total tritium by liquid scintillation spectrometry. The total ^{3}H remaining in the slices after superfusion was determined by counting a portion of a sonicated homogenate of the slices.

The amount of DA released (basal or stimulated, reflected as total ^{3}H overflow) was expressed as a percentage of ^{3}H in the slices at the onset of stimulation. The ^{3}H in the slices at the onset of K^+-depolarization was determined by counting the ^{3}H present in the slices at the end of the experiment and adding back the amount of ^{3}H which appeared in the effluent during the 2 min K^+-stimulation plus the amount of ^{3}H collected during an 18 min post-stimulation period. Basal efflux or spontaneous overflow of ^{3}H represented the average of the effluent radioactivity in the 1-ml superfused solution collected just before the K^+-stimulation and the radioactivity in the 1-ml, 30-min fraction, at which point the ^{3}H overflow had essentially returned to pre-stimulation levels. K^+stimulated ^{3}H overflow represented the total ^{3}H overflow during the 2-min stimulation plus 18-min post-stimulation period, minus basal efflux of total ^{3}H during that period.

Concentrated haloperidol solutions were made by dissolving the base (McNeil Labs) in 0.85% lactic acid. When apomorphine or DA was used, fresh KR–b containing the drug was introduced every 5 min.

In a separate set of experiments without added drugs, superfused solutions collected during the 10-min pre-stimulation or during the 2-min stimulation and 8-min post-stimulation periods were pooled and the amount of ^{3}H recovered as [^{3}H]DA and [^{3}H]DA metabolites was determined using a modification of the method of Graeffe *et al.* [15].

Approximately 88 per cent of the applied ^{3}H activity from either basal or K^+-stimulated overflow fractions was recovered as [^{3}H]DA or [^{3}H]DA metabolites from alumina and Dowex 50 column chromatography. Approximately 46 per cent of the recovered ^{3}H from samples taken during the pre-stimulation period was found to be unchanged DA, the remainder being predominantly 3-*O*-methyldopamine. [^{3}H]dopamine represented 80 per cent of the recovered ^{3}H in samples pooled from K^+-stimulation onset to the end of the peak of stimulation, while the remaining ^{3}H was found as 3-*O*-methyldopamine (18.6%); acid and neutral metabolites, presumably dihydroxyphenylacetic acid (DOPAC) and DOPET, were less then 2%. The recovered ^{3}H remaining in the slices post-superfusion was found to be 93% dopamine. Authentic radiolabeled [^{3}H]DA and [^{14}C]-3-*O*-methyldopamine (both purified by thin-layer chromatography) were used to determine recoveries. The recovery of DA through both columns was approximately 65 per cent while the recovery of 3-*O*-methyldopamine from the alumina column was 92 per cent. These experiments indicate that the increase in ^{3}H in the superfused solution due to K^+-stimulation appears to be a valid measure of the release of prelabeled DA. The effect of haloperidol, apomorphine and DA re-uptake blockers on the ratio of [^{3}H]DA and [^{3}H]DA metabolites was not determined.

Haloperidol was observed to have a biphasic effect on the total ^{3}H overflow after K^+-depolarization (see Fig. 1). Low concentrations (10 – 100 nM) increased the stimulated overflow of ^{3}H while higher concentrations (1 – 100 μM) reduced the K^+-evoked overflow. Basal ^{3}H overflow was unaffected by haloperidol at low concentrations but was enhanced markedly by high concentrations.

Apomorphine (10 – 100 μM) was not very effective in inhibiting stimulation-induced overflow of ^{3}H (see Table 1), although 0.1 μM did produce a marginally significant reduction. Similarly, basal efflux of ^{3}H was not altered by apomorphine. These findings are in accordance with the observations made by Farnebo and Hamberger [11,12], who also found a significant inhibitory effect by 0.1 μM apomorphine while neither higher nor lower concentrations altered the electrically stimulated overflow of [^{3}H]DA. Dismukes and Mulder [14] tested 1 and 10 μM apomorphine on the electrically stimulated release of [^{3}H]DA from striatal slices and also were unable to show any significant inhibition.

The enhanced ^{3}H overflow produced by 10 nM haloperidol

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was reversed significantly by the introduction of either 0.1 μM apomorphine or 1 μM DA, 5 min prior to stimulation (see Table 2), although this concentration of DA did not significantly reduce the K^+-evoked ^3H overflow when tested alone. Dopamine (1 μM) slightly increased basal ^3H efflux but this increase was not significant nor was this increase sufficiently great enough to explain the reversal of the haloperidol-induced enhancement of the K^+-stimulated ^3H overflow.

Drug-induced changes in ^3H neurotransmitter overflow during stimulation (as a measure of stimulated release) may occur by several mechanisms. An increase in overflow could be due to inhibition of the re-uptake mechanism without an actual increase in transmitter release or, if re-uptake of the neurotransmitter is unaffected, the increase could be due to a real increase in release. When the re-uptake of [^3H]DA was blocked by superfusing striatal slices in the presence of

Table 1. Effect of apomorphine on K^+-depolarized [^3H]dopamine overflow from rat striatal slices

Apomorphine concn (μM)	K^+-depolarized ^3H overflow (%)*	
	Control	With Apo
0.01	12.7 ± 3.2	12.5 ± 1.6
0.10	12.9 ± 1.7	10.3 ± 1.6†
1.00	17.6 ± 4.7	15.1 ± 3.2
10.00	16.4 ± 7.8	15.2 ± 5.9
100.00	12.4 ± 4.6	12.1 ± 4.8

*Mean ± S. D.; each concentration represents four experiments (four rats) except 0.1 μM, where N = 5.

†Significantly different from slices without drug, $P < 0.05$, 1-tail.

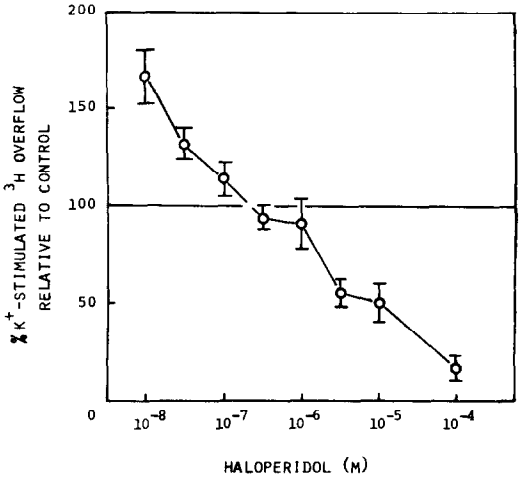

Fig. 1. Effect of haloperidol on K^+-depolarized [^3H]DA overflow from rat striatal slices. The per cent standard deviation for experiments without haloperidol (N = 8) was ± 5.2. Each point in the figure represents the mean ± S. D. as per cent of control slices of four experiments (four rats) except for 0.01 μM, where N = 5. The per cent of K^+-stimulated ^3H overflow from slices in the absence of haloperidol was 12.1 ± 2.3 (N = 34). The per cent of K^+-stimulated ^3H overflow from slices in the presence of haloperidol was as follows (with significant difference from control slices in parentheses): 0.01 μM, 21.5 ± 7.2 ($P < 0.02$); 0.05 μM, 19.9 ± 2.4 ($P < 0.02$); 0.1 μM 13.9 ± 4.6; 0.5 μM, 12.4 ± 2.0; 1.0 μM, 8.6 ± 4.9; 5.0 μM, 5.1 ± 0.8 ($P < 0.001$); 10.0 μM, 7.3 ± 1.9 ($P < 0.05$); and 100.0 μM, 1.5 ± 0.3 ($P < 0.02$).

Table 2. Effects of dopamine and apomorphine on basal and on K^+-depolarized [^3H]dopamine release (as total ^3H overflow) from rat striatal slices superfused with haloperidol or cocaine

Drug	K^+-depolarized ^3H overflow (%)*			Basal ^3H overflow (%)*		
	Without DA or Apo	With DA (1 μM)	With Apo (0.1 μM)	With Apo (1 μM)	Without DA or Apo	With DA (1 μM)	With Apo (0.1 μM)	With Apo (1 μM)
None	13.1 ± 4.0 N = 64	11.4 ± 3.6 N = 3	10.3 ± 1.6† N = 5	15.1 ± 3.2 N = 4	4.9 ± 1.7 N = 64	7.8 ± 3.0 N = 3	5.2 ± 0.7 N = 5	5.7 ± 1.2 N = 4
Haloperidol (0.01 μM)	19.9 ± 4.8‡ N = 12	12.3 ± 2.9§ N = 4	14.3 ± 3.1¶ N = 4	16.6 ± 2.0 N = 3	5.1 ± 1.0 N = 12	6.2 ± 2.0 N = 4	4.8 ± 0.8 N = 4	4.8 ± 0.6 N = 3
Cocaine (0.1 mM)	22.0 ± 5.2‡ N = 6				6.2 ± 1.6 N = 6			
Haloperidol (0.01 μM) + cocaine (0.1 mM)	23.2 ± 3.0‡ N = 4				5.4 ± 1.1 N = 4			

* Mean ± S. D.
† Significantly different from slices superfused without added drug, $P < 0.05$.
‡ Significantly different from slices superfused without added drug, $P < 0.02$.
§ Significantly different from slices superfused with 0.01 μM haloperidol alone, $P < 0.02$.
¶ Significantly different from slices superfused with 0.01 μM haloperidol alone, $P < 0.05$.

cocaine (0.1 mM, see Table 2) or benztropine (1 μM), the K^+-stimulated overflow of ^3H was increased significantly as compared to slices superfused in the absence of either of these re-uptake blockers. Neuroleptics have been reported to inhibit [^3H]DA re-uptake and also to act as releasers of [^3H]DA basal efflux from striatal synaptosomes [16,17]; however, we found that low concentrations of haloperidol (10 – 100 nM) had no effect on the uptake of [^3H]DA in striatal slices or on the basal efflux of ^3H from slices prelabeled with [^3H]DA, although high concentrations did increase basal efflux. We also found that the increase in stimulation-evoked ^3H overflow produced by cocaine was not diminished significantly by DA while DA antagonized significantly the increase in K^+-stimulated ^3H overflow produced by 10 nM haloperidol. These data demonstrate that the enhancement of stimulation-evoked [^3H]DA overflow by low concentrations of haloperidol is not due to a blockade of neuronal uptake. However, we were unable to demonstrate an additive effect of cocaine or benztropine and haloperidol, as would be expected if the blockade of re-uptake and the haloperidol effect were independent. Dismukes and Mulder [14] also found that, in the presence of cocaine, 1 μM haloperidol did not alter basal or electrically stimulated ^3H overflow from striatal slices prelabeled with [^3H]DA. Westfall *et al.* [18], using a different experimental design, have shown an additive effect of benztropine and the neuroleptic, fluphenazine, on the K^+-stimulated overflow of [^3H]DA from striatal slices. In their study, striatal slices were continuously superfused with L-3,5-[^3H]tyrosine until a steady state of [^3H]DA synthesis and release was achieved. Under these conditions, it was shown that the enhancing effect by fluphenazine on K^+-evoked [^3H]DA overflow probably occurs through a mechanism different from that produced by benztropine. It is interesting that the expected additive effect of a DA receptor blocker and a neuronal re-uptake blocker could be demonstrated if the releasable pool derived from the vesicular or newly synthesized DA pool. In our study, slices were prelabeled with [^3H]DA; thus any newly synthesized store of DA was unlabeled. It appears, therefore, that newly synthesized DA constitutes a different pool from that formed by preloading slices with [^3H]DA. The findings of Westfall *et al.* [18], when combined with our findings and those of Dismukes and Mulder [14], indicate that it is the newly synthesized DA pool which may be regulated by presynaptic receptors.

The lack of a dose-dependent inhibitory effect by apomorphine is puzzling. It may be that the [^3H]DA and/or endogenous newly synthesized DA released by K^+ depolarization [18] might reach threshold concentrations for inhibition of release by interacting with presynaptic DA receptors, in which case the addition of exogenous DA or apomorphine would have little effect.

Our observation of a biphasic effect of haloperidol on K^+-stimulated overflow of [^3H]DA from rat striatal slices appears to reconcile the discrepancies between the studies of Farnebo and Hamberger [11,12] and those of Seeman and Lee [13]. The marked reduction in stimulation-evoked overflow of [^3H]DA by haloperidol at high concentrations is probably an apparent effect resulting from (1) a marked increase in basal efflux due to inhibition of the DA re-uptake mechanism by high concentrations [17], and (2) non-specific membrane actions related to the local anesthetic properties of neuroleptics [16,19,20]. The marked enhancement of K^+-stimulated overflow of [^3H]DA from striatal slices by low concentrations of haloperidol and the antagonism of this effect by DA or apomorphine are consistent with a presynaptic and/or postsynaptic receptor effect.

Our findings do not resolve the controversy about the involvement of presynaptic DA receptors in modulating DA release. Dopamine release might also be regulated via postsynaptic receptor activation of short intra-striato-pallidal loops [21], some of which are probably intact in the striatal slice preparation. Indeed, it has been suggested that intra-striato-pallidal γ-aminobutyric acid (GABA) inhibitory systems [21–23] and/or cholinergic systems [24,25] may regulate DA release. Postsynaptic receptor blockade of DA receptors on intra-striatal GABA or cholinergic neurons by low concentrations of haloperidol could either release DA nerve terminals from an inhibitory effect of GABA or disinhibit cholinergic input to DA nerve endings.

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