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EFFECTS OF HALOPERIDOL AND APOMORPHINE ON CATECHOLAMINE METABOLISM IN BRAIN SLICES

RESERPINE-LIKE EFFECTS OF HALOPERIDOL

RICHARD L. DELANOY* and ADRIAN J. DUNN
Department of Neuroscience, University of Florida, College of Medicine, Gainesville, FL 32610, U.S.A.

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Abstract—The accumulation, release and catabolism of [^{3}H]dopamine (DA) and [^{3}H]norepinephrine (NE) synthesized from [^{3}H]tyrosine were measured in mouse striatal and substantia nigral slices. Apomorphine inhibited both [^{3}H]NE and [^{3}H]DA accumulation ($IC_{50} < 10^{-6}$ M), presumably by acting on a presynaptic receptor. Haloperidol (10^{-8} M) caused a small, but significant increase in [^{3}H]DA accumulation from [^{3}H]tyrosine in the presence of 26 mM K^+, possibly reflecting blockade of presynaptic receptors activated by released DA. However, at higher concentrations (10^{-6} to 10^{-5} M), haloperidol inhibited [^{3}H]DA and [^{3}H]NE accumulation. Reserpine also potently inhibited catecholamine synthesis; chlorpromazine had only a weak effect, and fluphenazine was ineffective. Both haloperidol (10^{-5} M) and reserpine (10^{-7} M), but not chlorpromazine and fluphenazine, markedly increased the formation of labeled dihydroxyphenylacetic acid (DOPAC) and increased the spontaneous release of labeled DA from striatal slices preloaded with [^{3}H]tyrosine or [^{14}C]DA. These data suggest that haloperidol has some direct effects on DA metabolism that are unrelated to DA-receptor blockade. Because the effects of haloperidol are apparently independent of DA release, haloperidol may elevate cytoplasmic DA by altering its vesicular storage. This would, in turn, increase the spontaneous release of labeled DA by diffusion, the oxidation of DA to DOPAC by monoamine oxidase, and the end-product inhibition of tyrosine hydroxylase.

In noradrenergic neurons, dendritic receptors inhibit cellular firing in response to adrenergic agonists [1]. Also, exogenous norepinephrine (NE)† inhibits the K^+-stimulated release of [^{3}H]NE from noradrenergic terminals *in vitro* [2]. These effects are presumed to be mediated by autoreceptors, i.e. receptors that recognize the neurotransmitter released by the cells themselves.

Parallel experiments have investigated the potential existence of dopaminergic autoreceptors. Iontophoretic administration of DA or apomorphine into the substantia nigra inhibits the firing of dopaminergic neurons in the pars compacta of the substantia nigra [3, 4]. The existence of dendrodentritic synapses between dopaminergic dendrites in the pars reticulata of the substantia nigra [5] also supports the suggestion of Aghajanian and Bunney [6] that autoreceptors exist on the dendrites and/or the soma of dopaminergic neurons.

The existence of a presynaptic autoreceptor on DA terminals is supported by the finding that [^{3}H]apomorphine binding is reduced following lesions of the nigrostriatal tract made 6-hydroxydopamine [7]. However, in contrast to the results with NE, studies contemporary with these did not reveal an effect of dopamine agonists on DA release [8–10]. Also, DA-receptor blockers inhibited the simulated release of [^{3}H]DA [8, 10], an effect that would not be predicted by an action on presynaptic DA receptors. More recent studies have reported an autoreceptor-mediated inhibition of DA release [11, 12].

An alternative mechanism by which autoreceptors might regulate synaptic efficacy is by inhibiting DA synthesis. At micromolar concentrations, apomorphine strongly inhibited DA synthesis in striatal slices and synaptosomes, an effect reported to be reversed by neuroleptics [13, 14]. However, haloperidol also inhibited DA synthesis in striatal slices at micromolar concentrations [13], a result that contradicts both the hypothesized regulation of DA synthesis by an autoreceptor, and the consistent finding that haloperidol activates DA synthesis *in vivo* [15–17]. The paradox that both a DA agonist and a DA antagonist have the same effect on synthesis prompted us to examine further the effects of these two agents. In a single incubation system, we compared the effects of haloperidol and apomorphine on catecholamine (CA) synthesis, and DA release, metabolism and uptake.

MATERIALS AND METHODS

Materials. Male CD-1 mice (Charles River Laboratories, Wilmington, MA, 25–30 g) were used in all experiments; they were individually housed for

* Present address to which correspondence should be sent: Dr. Richard Delanoy, Department of Psychology, Gilmer Hall, University of Virginia, Charlottesville, VA 22901, U.S.A.

† Abbreviations: CA, catecholamine; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EGTA, ethyleneglycolbis(amino-ethylether)tetra-acetate; MAO, monoamine oxidase; NE, norepinephrine; and TH, tyrosine hydroxylase.

the 3 days immediately prior to killing. Animals were maintained on a 7:00 a.m. to 7:00 p.m. lighting schedule.

Drugs used in these experiments were: apomorphine hydrochloride (Sigma Chemical Co., St. Louis, MO), chlorpromazine hydrochloride (Sigma), fluphenazine hydrochloride (E. R. Squibb & Sons, Inc., Princeton, NJ), haloperidol (McNeil Lab., Inc., Fort Washington, PA), and reserpine (Sigma). Drugs were initially dissolved in small volumes of glacial acetic acid and diluted with medium. Radioactive materials used were [2,6-^{3}H]tyrosine (34 Ci/mmole) and [1-^{14}C]dopamine hydrochloride (55 mCi/mmole) obtained from Amersham-Searle Corp. (Arlington Heights, IL) and [1-^{14}C]tyrosine (58 mCi/mmole) obtained from the New England Nuclear Corp. (Boston, MA).

Procedures. The procedures for tissue preparation, incubation and chromatographic separation were precisely the same as those described in the preceding paper [18]. In addition to the three types of experiments described therein, the uptake of [1-^{14}C]DA into striatal slices was examined. Prepared slices (0.5 ml) were added to Corex centrifuge tubes containing 0.5 ml medium, 0.1 μM [1-^{14}C]DA, and drugs. Samples were incubated for 3 min. Uptake was terminated with 1 ml of ice-cold medium.

Quantification and analysis. The dpm in catecholamines was divided by the protein content of the tissue sample to derive specific activities. Blank values, determined in samples that received radioactivity but were incubated at 0°, were subtracted from experimental values. Data from accumulation studies are expressed as moles of CA accumulated per mg protein per min (based on the medium tyrosine specific activity). Data from metabolism studies were expressed as percentage of total radioactivity recovered in fractions containing labeled DA or its metabolites.

One-factor analyses of variance (ANOVA) and Student's *t*-tests were performed on a Hewlett-Packard 9810A calculator. An Amdahl 370, version 6 computer was used for quench and double-label corrections and for two or more factor ANOVAs as programmed in the Statistical Analysis System (SAS) package.

RESULTS

Effects of neuroleptics and apomorphine on ^{3}H-labeled catecholamine accumulation from ^{3}H-tyrosine by slices. Both apomorphine and haloperidol potently inhibited the accumulation of [^{3}H]DA in striatal slices (Fig. 1). Reserpine was later found to be more potent than either apomorphine or haloperidol in this respect. Chlorpromazine (10^{-5} M) caused a slight but significant inhibition (24%), while fluphenazine at the doses tested had no significant effect on [^{3}H]DA accumulation. Haloperidol and apomorphine also inhibited the release of $^{14}CO_2$ from [1-^{14}C]tyrosine by striatal slices (Fig. 2). The effective concentrations of haloperidol and apomorphine were very similar to those that inhibited the accumulation of [^{3}H]DA from [^{3}H]tyrosine.

Apomorphine and haloperidol were also tested at a range of doses in the presence of 26 mM K^+ (Fig. 3), which elevated rates of [^{3}H]DA accumulation by 300%. From this elevated baseline, 26 mM K^+

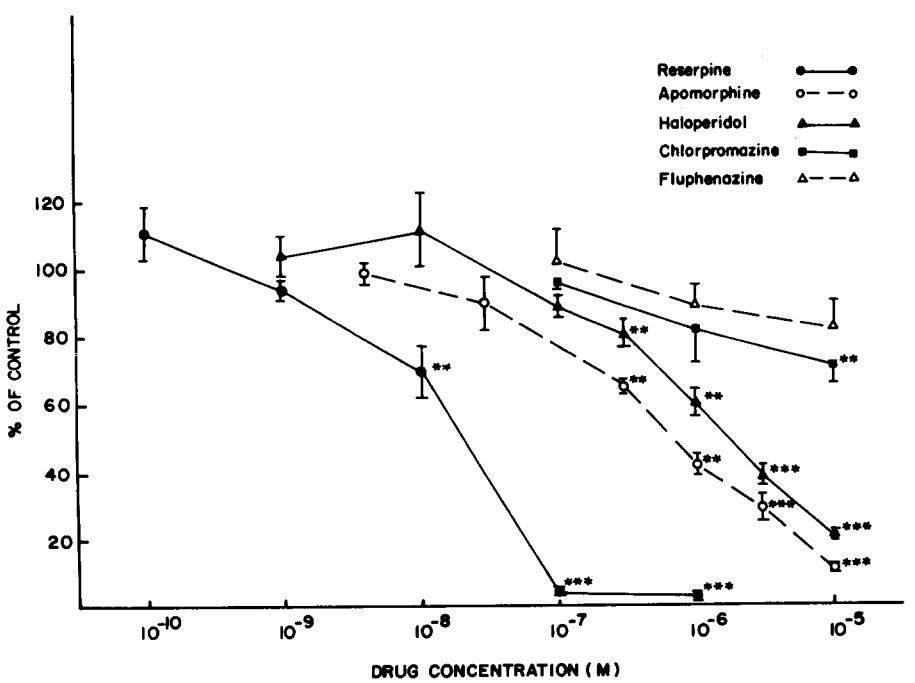

Fig. 1. Inhibition of striatal [^{3}H]DA accumulation by reserpine, apomorphine, haloperidol, chlorpromazine and fluphenazine. Striatal slices were incubated with 4.17 μCi/ml [^{3}H]tyrosine at 8.3 μM tyrosine and 6 mM K^+. Each point is the mean of at least eight determinations. The rate of [^{3}H]DA accumulation for saline (100% value) was 0.89 pmole per mg protein per min. Key: (**) significantly different from saline, $P < 0.01$; and (***) $P < 0.001$ (ANOVA).

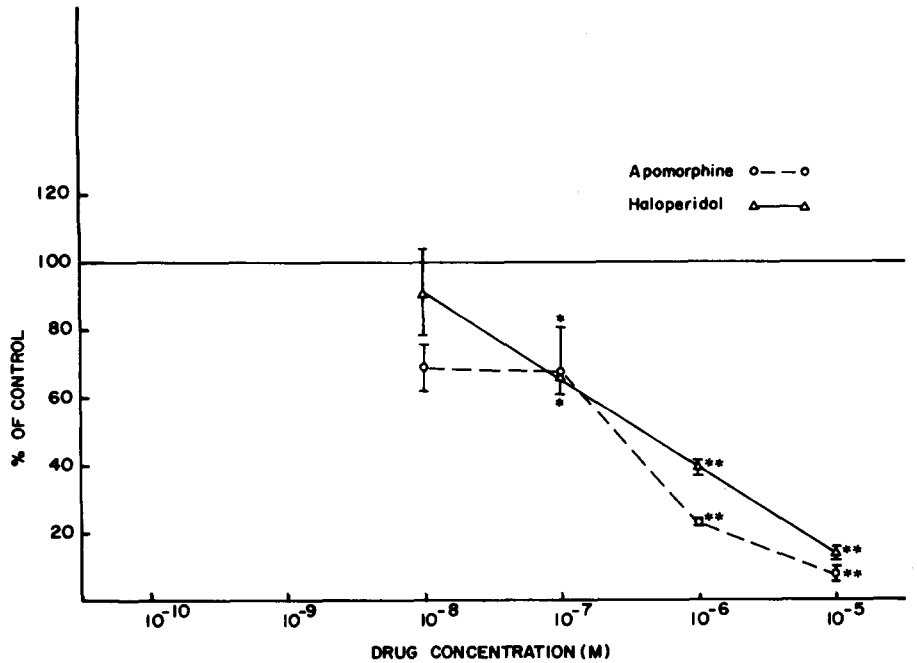

Fig. 2. Inhibition of $^{14}CO_2$ liberation from [^{14}C]tyrosine by apomorphine and haloperidol. Striatal slices were incubated with 83 nCi/ml at 1.5 μM tyrosine and 6 mM K^+ for 20 min. Each point is the mean and standard error of four determinations. The rate of $^{14}CO_2$ accumulation for saline was 1.52 pmoles per mg protein per min. Key: (*) significantly different from saline, $P < 0.05$; and (**) $P < 0.01$ (ANOVA).

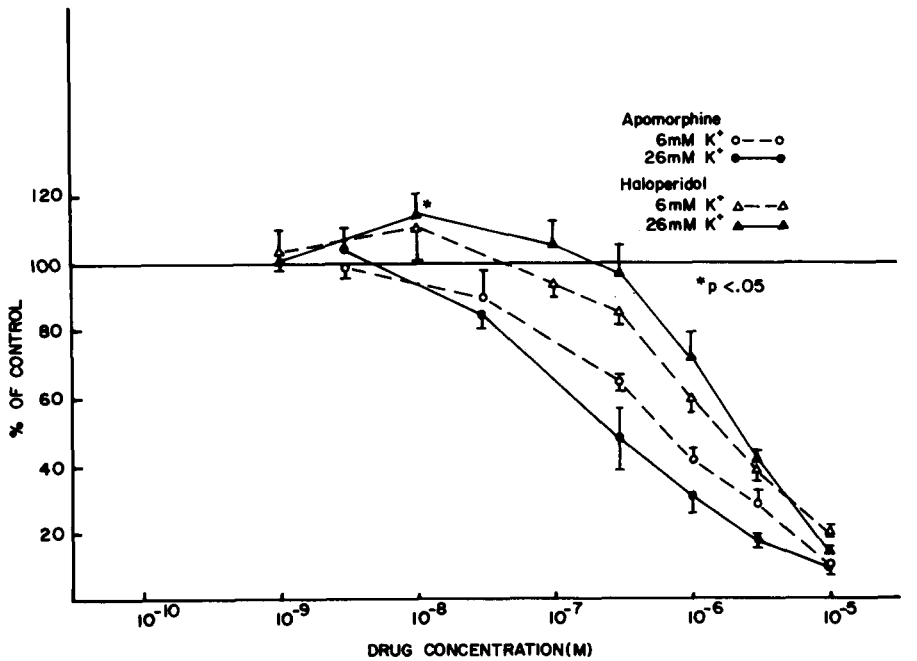

Fig. 3. Interactions of elevated K^+ with haloperidol- and apomorphine-induced inhibition of striatal [^{3}H]DA accumulation from [^{3}H]tyrosine. Curves generated at 6 mM K^+ are the same as presented in Fig. 1. The rate of [^{3}H]DA accumulation for saline at 26 mM K^+ is 3.54 pmoles per mg protein per min. Each point is the mean (± S.E.) of four determinations for apomorphine and at least eight determinations for haloperidol. Key: (*) significantly different from saline, $P < 0.05$ (ANOVA).

tended to reduce the inhibition by haloperidol (except at 10^{-8} M) as compared to incubations at 6 mM K^+, while it potentiated that by apomorphine. A concentration of 10^{-8} M haloperidol caused a slight but significant elevation of [^{3}H]DA accumulation in slices exposed to 26 mM K^+. Similar, but nonsignificant increases were observed with 10^{-8} M haloperidol and 10^{-10} M reserpine at standard K^+ (6 mM) (Figs. 3 and 4).

Reserpine, apomorphine and haloperidol all inhibited the accumulation of [^{3}H]DA in substantia nigral slices incubated at 8.3 μM tyrosine (Fig. 4). Surprisingly, apomorphine, haloperidol, and reserpine also inhibited the accumulation of [^{3}H]NE. Similarly, [^{3}H]NE accumulation in cerebellar slices was inhibited by haloperidol and apomorphine to the same extent as in substantia nigral slices (data not shown). Curiously, the effect of apomorphine on substantia nigral slices was diminished by 83 μM tyrosine (Fig. 4B). At a medium concentration of 8.3 μM tyrosine, 10^{-5} M apomorphine inhibited [^{3}H]DA (82%) and [^{3}H]NE (65%) accumulation twice as much as at 83 μM tyrosine (41 and 30% respectively). A concentration of 10^{-5} M haloperidol was equally effective at 8.3 and 83 μM tyrosine.

Accumulation of ^{3}H-protein from [^{3}H]tyrosine. Since haloperidol and apomorphine affected the accumulation of both [^{3}H]DA and [^{3}H]NE, it is possible that they inhibit cellular metabolism nonspecifically. However, the labeling of protein in striatal slices incubated with [^{3}H]tyrosine was not affected by either drug. The mean (± S.E.) protein specific activities for saline, haloperidol (10^{-5} M) and apomorphine (10^{-5} M) were 12.1 + 1.7, 12.1 ± 1.0, and 10.9 ± 1.5 dpm/μg respectively.

Uptake of [^{14}C]DA by slices. Striatal slices were incubated with 0.1 μM [^{14}C]DA for 3 min. Comparisons between 10^{-5} M haloperidol and saline were made both with and without 12.5 μM nialamide (an MAO inhibitor) and 100 μM EGTA, used to diminish release and catabolism of accumulated [^{14}C]DA. Under both conditions, haloperidol decreased the accumulation of [^{14}C]DA in slices and increased the overall formation of [^{14}C]DOPAC (i.e. slice plus

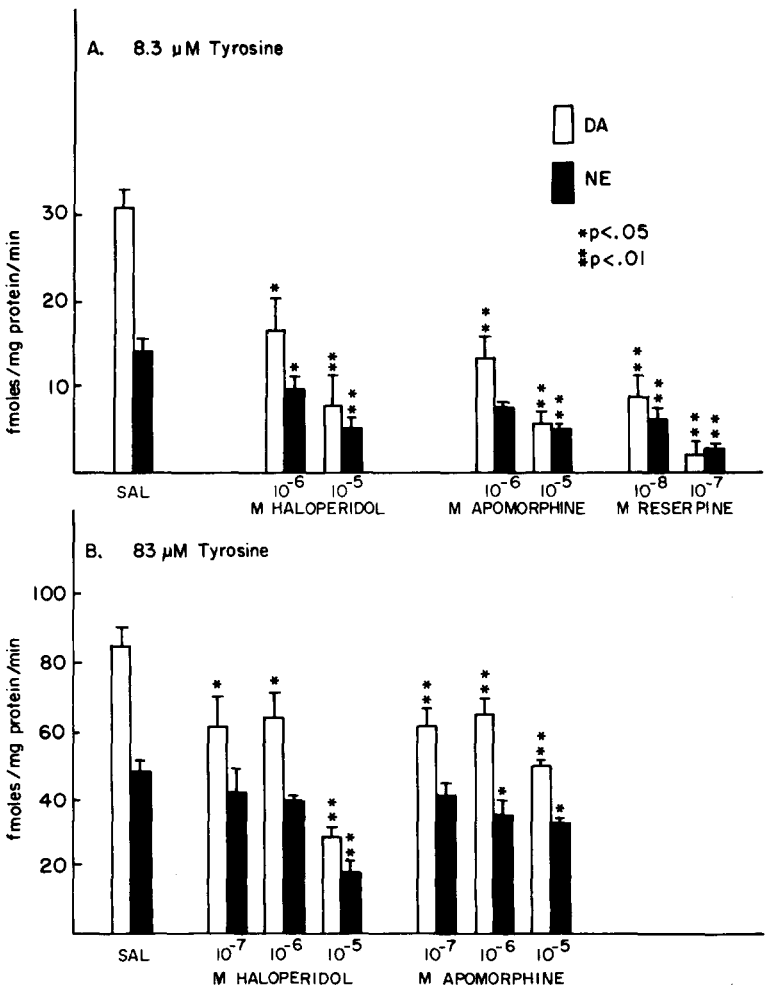

Fig. 4. Inhibition of ^{3}H-labeled catecholamine accumulation from [^{3}H]tyrosine in substantia nigral slices by haloperidol, apomorphine, and reserpine. Open and solid bars represent [^{3}H]DA and [^{3}H]NE respectively. Substantia nigral slices were incubated with (A) 4.17 μCi/ml [^{3}H]tyrosine at 8.3 μM tyrosine and (B) with 16.7 μCi/ml [^{3}H]tyrosine at 83 μM tyrosine. Key: (**) significantly different from saline, $P < 0.01$; and (*) $P < 0.05$ (ANOVA).

Table 1. Inhibition by haloperidol of [^{14}C]DA accumulation in slices, but not of total uptake

Treatment	[^{14}]DA*	[^{14}C]DOPAC†	Total‡
Saline	1.09 ± 0.08	0.69 ± 0.03	1.78 ± 0.08
Haloperidol (10 μM)	0.85 ± 0.06§‖	0.82 ± 0.02¶	1.67 ± 0.07
	−22%		−6%
With 12.5 μM nialamide and 100 μM EGTA:			
Saline	1.69 ± 0.06	0.02 ± 0.02	1.73 ± 0.07
Haloperidol (10 μM)	1.48 ± 0.06‖	0.15 ± 0.09¶	1.63 ± 0.19
	−12%		−6%

* From slices alone, dpm/μg protein.
† Combined from both slices and media, dpm/μg protein.
‡ Sum of [^{14}C]DA and [^{14}C]DOPAC.
§ Significantly different from saline ($P < 0.05$, two-factor ANOVA, Duncan's).
‖ The main effect of haloperidol was significant for DA ($P < 0.025$, ANOVA).
¶ The main effect of haloperidol was significant for DOPAC ($P < 0.05$, ANOVA).

medium; Table 1). The increased [^{14}C]DOPAC found in the medium was probably formed by MAO in dopaminergic terminals and, therefore, reflects additional [^{14}C]DA uptake by the slices. When the total [^{14}C]DOPAC and [^{14}C]DA is considered, there was no significant effect of haloperidol on the accumulation of [^{14}C]DA.

Release and metabolism of labeled DA from preloaded slices. The effects of reserpine, apomorphine, haloperidol, chloropromazine and fluphenazine on the disappearance of labeled DA from slices were measured. Pooled slices were incubated with both [^{3}H]tyrosine and [^{14}C]DA. Following a thorough washing to remove excess radioactivity, 0.5 ml of prepared slices was added to 0.5 ml of unlabeled medium containing various drug and ion concentrations. Radioactive DA in the media (^{3}H and ^{14}C), DA remaining in the slices, total (slice and medium combined) DOPAC, and total residual (tritiated water, homovanillic acid) radioactivity were measured (Fig. 5). Haloperidol (10^{-5} M) and reserpine (10^{-7} M) caused the loss of 30–50% of radioactive DA from the slices (Fig. 5, slice DA). Basal release (i.e. that not induced by elevated K^+) for both [^{3}H]- and [^{14}C]DA was elevated significantly by 10^{-5} M haloperidol (Fig. 5, media DA). However, radioactive DA recovered from the media accounted for only a small percentage of that lost from the slices treated with haloperidol or reserpine. More than 90% of the radioactivity lost from the slices following haloperidol treatment was found in the DOPAC fraction (Fig. 5, DOPAC). More than 80% of the labeled DOPAC was found in the medium. Concentrations of 10^{-7} M reserpine and 10^{-5} M apomorphine both caused small but significant increases in the basal release of [^{14}C]DA but not of [^{3}H]DA. Also, both reserpine and apomorphine caused significant elevations of labeled DOPAC formation; although reserpine was much more powerful than haloperidol, the effect of 10^{-7} M reserpine closely resembled that of 10^{-5} M haloperidol.

Because of the observed similarities between the effects of 10^{-5} M haloperidol and 10^{-7} M reserpine on synthesis, release and metabolism of radioactive DA, comparisons were extended to include tests of Ca^{2+}- and K^+-dependency. Haloperidol and reserpine were tested in separate experiments with 6 or 26 mM K^+, and with 2.6 mM Ca^{2+} or 100 μM EGTA (Fig. 6). Both [^{3}H]- and [^{14}C]DOPAC were greatly increased (more than 400%) by 10^{-5} M haloperidol at both 6 and 26 mM K^+, and with or without Ca^{2+}, suggesting that the effect of haloperidol on DOPAC formation was independent of transmitter release (Fig. 6, DOPAC). Reserpine (10^{-7} M) generated a similar profile, although its effect was smaller than that elicited by 10^{-5} M haloperidol. In addition, 26 mM K^+ caused a small increased of labeled DOPAC accumulation (also observed in Ca^{2+}-depleted media).

K^+ induced the release of both [^{3}H]- and [^{14}C]DA from the slices (Fig. 6, media DA). K^+-induced release was still observed for both [^{3}H]- and [^{14}C]DA in Ca^{2+}-depleted media, although the amount released was considerably less than that observed in the presence of 2.6 mM Ca^{2+}. Haloperidol not only elevated basal release, but also potentiated K^+-induced release in both 2.6 mM Ca^{2+} and Ca^{2+}-depleted media. Reserpine significantly elevated basal release of [^{14}]DA, and K^+-induced release of both [^{3}H]- and [^{14}C]DA at 2.6 mM Ca^{2+}, but did not significantly elevate K^+-induced release in Ca^{2+}-depleted conditions, although the pattern of results was similar to that observed with haloperidol. No treatment combination had any consistent effect on the residual ^{3}H and ^{14}C. Changes in slice DA radioactivity inversely reflected the combined changes in media DA and DOPAC radioactivities.

DISCUSSION

Effects of apomorphine on DA metabolism. Presynaptic autoreceptors apparently regulate depolarization-induced release of DA in the striatum [11, 12]. However, since apomorphine also potently inhibits DA synthesis *in vivo* [19] and *in vitro* (Fig. 4; Refs. 13 and 20), several groups have proposed

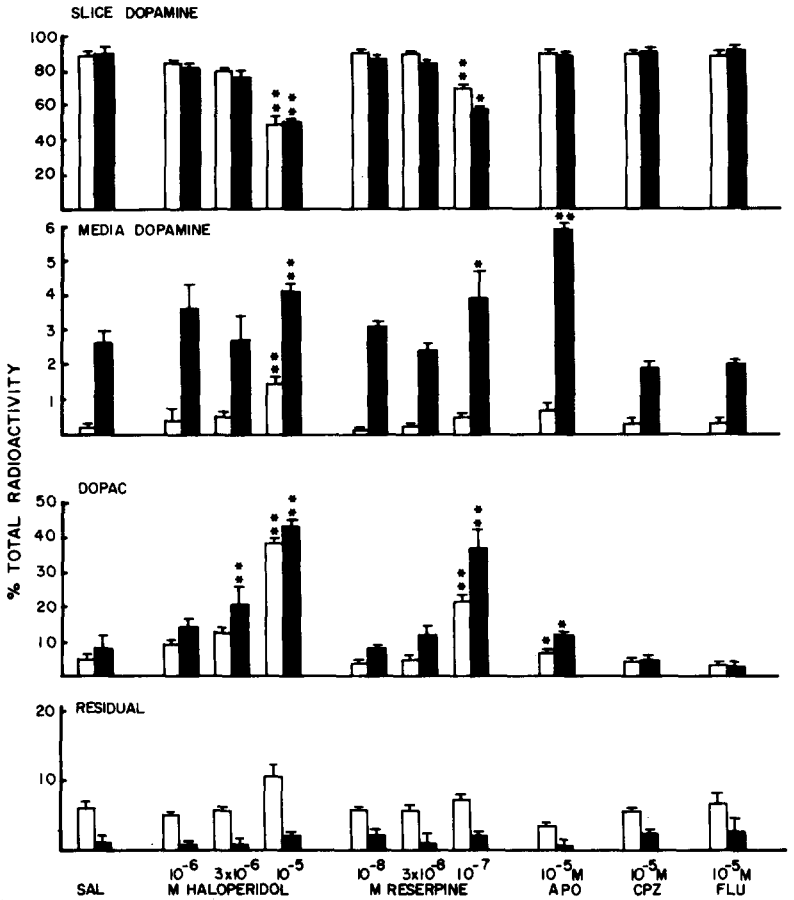

Fig. 5. Effects of haloperidol, reserpine, apomorphine, chlorpromazine and fluphenazine on the release and metabolism of preloaded labeled DA in striatal slices. Pooled striatal slices were exposed to [^{3}H]tyrosine (250 μCi/10 ml; 740 nM) for 10 min. Then 10 μCi/10 ml [^{14}C]DA (18 μM) was added, and the slices were equilibrated for an additional 10 min. Slices were then washed four times. Aliquots of 0.5 ml of slices were added to 0.5 ml of media containing ionic and drug treatments and incubated for 10 min. Open and solid bars represent the distribution of ^{3}H and ^{14}C respectively. DOPAC and residual fractions were pooled from the slices and the media. The sum of slice DA, medium DA, DOPAC, and residual equals 100% for each label. Key: (*) significant difference from saline for the same label ($P < 0.05$), (**) $P < 0.01$ [ANOVA for haloperidol and reserpine; Student's t-test for apomorphine (APO), chlorpromazine (CPZ), and fluphenazine (FLU)].

that presynaptic autoreceptors may modulate DA synthesis as well [6, 19, 21]. DA inhibits its own synthesis in striatal synaptosomes [22], and both apomorphine- and DA-induced inhibitions of DA synthesis have been reported to be reversed by haloperidol or fluphenazine [13, 14].

An alternative site of action of apomorphine is tyrosine hydroxylase (TH). TH undergoes a presumed conformational change following electrical or K^+ stimulation, resulting in an increased affinity of the enzyme for pterin cofactor and reduced sensitivity to end-product inhibition [23, 24]. Thus, if apomorphine inhibits TH by end-product inhibition, K^+ activation should reduce the relative potency of apomorphine. Instead, we found that 26 mM K^+ potentiated the inhibition of [^{3}H]DA accumulation by apomorphine. This result was probably not due to some change in release or metabolism, since 10^{-5} M apomorphine did not cause a detectable depletion of [^{3}H]DA from preloaded slices (Fig. 5, slice DA). Therefore, a mechanism other than end-product inhibition of TH is likely to be responsible for the apomorphine-induced inhibition of DA synthesis.

The inhibition of CA synthesis caused by apomorphine was not limited to DA terminals but was also apparent in the accumulation of [^{3}H]NE from [^{3}H]tyrosine in substantia nigral and cerebellar slices. Starke *et al.* [2] have reviewed the repeated observation that DA inhibits release from NE terminals in some peripheral tissues, apparently by means of a presynaptic receptor similar to DA receptors found in the CNS [25]. Therefore, the ability of apomorphine to inhibit both NE and DA syntheses *in vitro* does not necessarily argue against the hypothesis that inhibition is mediated by an autoreceptor; the same receptor might be present on both terminal populations.

Effects of haloperidol on catecholamine synthesis and release. In vitro inhibition of DA synthesis by haloperidol has only been briefly reported before. Christiansen and Squires [13] commented that halo-

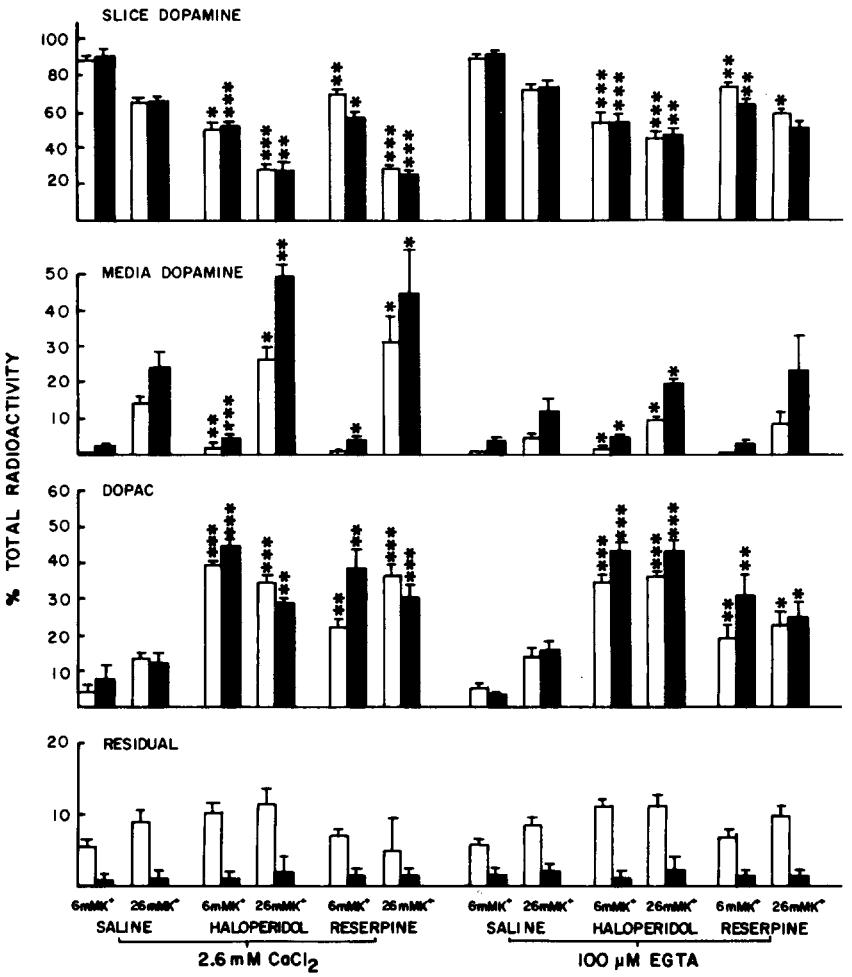

Fig. 6. Comparisons of the effects of haloperidol and reserpine on the release and metabolism of labeled DA in striatal slices. Slices were prepared as described in Fig. 5. Open and solid bars represent ^{3}H and ^{14}C respectively. Orthogonal comparisons were performed between treatment groups differing in only one variable using Student's *t*-test. This was justified since the effects of K^+ and Ca^{2+} were so large that variance was not homogenous, invalidating the ANOVA. Significance levels indicate the effect of 10^{-5} M haloperidol or 10^{-7} M reserpine as compared with the saline value with the same label and at the same K^+ and Ca^{2+} concentrations (i.e. [^{14}C]DOPAC for 26 mM K^+, 2.6 mM Ca^{2+} and haloperidol was compared to [^{14}C]DOPAC for 26 mM K^+, 2.6 mM Ca^{2+} and saline. Key: (*) $P < 0.05$; (**) $P < 0.01$; and (***) $P < 0.001$.

peridol at concentrations greater than 10^{-6} M inhibited [^{3}H]DA synthesis from [^{3}H]tyrosine in striatal synaptosomes. Such an inhibition of DA synthesis contradicts a model of autoreceptor regulation of DA synthesis, which would predict that DA-receptor blockers would increase DA synthesis or have no effect at all. Also, while apomorphine clearly inhibits synthesis *in vivo* [19] and *in vitro* [13, 20], haloperidol *in vivo* dramatically increases DA synthesis [15–17], but not *in vitro*.

The mechanisms by which apomorphine and haloperidol inhibit the accumulation of [^{3}H]DA from [^{3}H]tyrosine appear to differ. First, 26 mM K^+ potentiated apomorphine-induced, while it lessened haloperidol-induced, inhibition of synthesis. Also, apomorphine-induced inhibition of the accumulation of ^{3}H-labeled catecholamines in substantia nigral slices was halved by increasing the concentration of medium tyrosine, but no such effect was observed with haloperidol.

An attempt was made to determine whether the decreased accumulation of [^{3}H]DA observed in the presence of haloperidol was due to decreased synthesis, increased spontaneous release, or decreased reuptake. When DA synthesis was estimated by the liberation of $^{14}CO_2$ from [1-^{14}C]tyrosine, haloperidol again was inhibitory, suggesting that the haloperidol-induced decrease in ^{2}H-labeled CA accumulation reflected a real decrease in CA synthesis.

Inhibition of DA reuptake by haloperidol has been reported previously [26, 27]. Thus, part of the haloperidol-induced decrease in [^{3}H]DA accumulation could be due to decreased reuptake of release, newly synthesized [^{3}H]DA. However, the decrement in the striatal accumulation of [^{14}C]DA from the

media was much smaller than would be necessary to account for the decreased accumulation of [^{3}H]DA from [^{3}H]tyrosine. Also, it has been suggested that apparent decreases in uptake might actually reflect enhanced release [28, 29]. We found that [^{14}C]DOPAC in the medium (presumably formed in DA terminals) could account for the haloperidol-induced decrease of [^{14}C]DA accumulation in slices, indicating that haloperidol did not block reuptake but caused increased oxidation of DA subsequent release of labeled DOPAC.

In subsequent studies of amine release and catabolism, incubation with 10^{-5} M haloperidol caused the loss of both [^{3}H]DA synthesized from [^{3}H]tyrosine and [^{14}C]DA preloaded in striatal slices. The amount of labeled DA found in the medium was elevated, confirming several reports that haloperidol elevated the basal efflux of DA [9, 10, 27]. However, the increased efflux of labeled DA accounted for less than 15% of the label lost from the slices; more than 80% of the missing label was located in a fraction containing DOPAC. Both our results and those of previous studies using MAO inhibitors to prevent the formation of DOPAC [27] indicate that haloperidol does increase the basal efflux of DA to a small extent. However, the assumption of other earlier studies (e.g. Refs. 9 and 10) that the total efflux of label induced by haloperidol was associated with [^{3}H]DA is clearly incorrect.

The increased formation of labeled DOPAC might have been due to efficient reuptake and degradation of released DA. However, this does not seem likely since the bulk of the radioactivity released by elevated K^+ concentrations from striatal slices preloaded with labeled DA remains as DA (Fig. 6; Ref. 30). Also, the elevation of labeled DOPAC by 10^{-5} M haloperidol was independent of both K^+ and Ca^{2+} concentrations and, therefore, probably independent of vesicular release. Nevertheless, the increased formation of labeled DOPAC and increased spontaneous release of labeled DA suggest an increase in the cytoplasmic concentration of DA. These results resemble those obtained with reserpine [30] and papaverine [31].

The effect of 10^{-7} M reserpine and 10^{-5} M haloperidol on release were complex but, like their effects on DOPAC formation, essentially identical. Both agents increased basal efflux of labeled DA and facilitated the release of DA induced by 26 mM K^+. Haloperidol also elevated the Ca^{2+}-independent, K^+-induced release of labeled DA. Reserpine did not significantly elevate the release of labeled DA in Ca^{2+}-depleted media, although the pattern of results was similar to that observed with haloperidol. Facilitation of basal release by haloperidol has been reported before [8, 9]. However, most studies of field-stimulated [8, 9], K^+-induced [12] and veratridine-induced [10] release of preloaded, labeled DA found that haloperidol reduced the amount of label released. Two exceptions have been reported. One [32] indicated that 10^{-5} M haloperidol, the dose used in this study, facilitated K^+-stimulated release, while lower doses inhibited it. The second reported that haloperidol at concentrations of 10^{-9} to 10^{-7} M enhanced electrically-stimulated, but not K^+-stimulated [^{3}H]DA release [11].

Interestingly, apomorphine also elicited an increase in labeled DOPAC formation and passive release of labeled DA and, thus, may also exhibit reserpine-like characteristics to a lesser degree. However, apomorphine-induced inhibition of DA synthesis can be blocked by neuroleptics [13, 14], and activation of TH to a form resistant to end-product inhibition did not alter the observed inhibition of synthesis. For these reasons apomorphine-induced inhibition of synthesis probably works for the most part by a different mechanism.

The neuroleptics and reserpine have a number of similar effects. Reserpine has antipsychotic activity [33] and, like the neuroleptics used in this study, causes Parkinsonian symptoms [33, 34]. All these agents induce catalepsy at high doses [35]. They also have anesthetic properties indicated by their ability to block impulse conduction along peripheral nerves [36]. Reserpine and haloperidol both inhibit catecholamine synthesis *in vitro* and activate TH *in vivo* [17, 37]. Apomorphine reverses the inhibition caused by 10 mg/kg reserpine [38] and by 0.1 mg/kg haloperidol [39]. By contrast, however, reserpine causes a profound depletion of monoamines. While haloperidol has been reported to cause a modest depletion of striatal DA [40], most investigators have not found such an effect [16, 17].

The effects of haloperidol and reserpine observed in this study do not appear to be receptor-mediated. The ranked efficacies of reserpine, haloperidol, chlorpromazine and fluphenazine do not correlate with any of the measures of DA-receptor binding or antagonism [41]. Fluphenazine is typically as effective as haloperidol, while chlorpromazine is less potent by an order of magnitude, in reversing apomorphine-induced stereotypy, in competing with labeled DA for binding [41], and in causing extrapyramidal side-effects [34]. In contrast, fluphenazine had little potency in our preparations. Also, reserpine does not reverse apomorphine-induced stereotypy [35], but it potently inhibited the accumulation of ^{3}H-labeled catecholamines from [^{3}H]tyrosine (Fig. 3).

Inhibition of synthesis, coupled with increased spontaneous release, and increased degradation can most easily be explained by a disturbance of vesicular storage. Vesicular disruption might elevate the cytoplasmic concentration of DA sufficiently to inhibit TH. The inhibitory potency of haloperidol on the accumulation of [^{3}H]DA from [^{3}H]tyrosine was ameliorated by the activation of synthesis (and presumably of TH) induced at 26 mM K^+. Such a result correlates with the reduced susceptibility of TH to end-product inhibition following allosteric activation under depolarizing conditions. The increased cytoplasmic concentrations of DA would permit greater basal or passive release because of an increased concentration gradient across the cell membrane. An increased cytoplasmic concentration of DA would also provide more substrate for degradation by MAO.

DA-receptor antagonism, anesthetic and reserpine-like properties, all decrease synaptic efficacy, consequently decreasing feedback inhibition and increasing DA cell firing rates. The increase in

DOPAC concentrations detected *in vivo* following haloperidol treatments might be due either to increased release [42] or to altered vesicular storage. Similarly, the increases in DA synthesis and TH activation might be due to increased firing of DA cells or to a depletion of terminal DA. Kainic acid lesions of the striatum have been reported to suppress the activation of TH by haloperidol treatments, but not the activation of DA synthesis [43, 44]. This finding implicates a postsynaptic receptor and long-loop feedback as the means of regulating enzyme activation. However, the sustained increase of DA synthesis in the kainic-acid-lesioned striatum suggests that haloperidol also affects the terminals directly. By transiently inhibiting synthesis or depleting amine concentrations in particular compartments, haloperidol might cause an activation synthesis *in vivo* independent of its activation of TH.

Whatever the mechanism, *in vitro* biochemical studies examining the effects of micromolar concentrations of haloperidol on DA metabolism are probably not due to receptor antagonism. Similarly, *in vivo* studies using 1–10 mg/kg haloperidol may also be suspect. If one assumes even, whole animal distribution of injected haloperidol and that mammals are 80% water, 2.5 mg/kg results in a concentration of 10^{-5} M. Since nervous tissue is lipid rich, these figures may be conservative. Thus, our data would suggest that doses above 0.5 mg/kg have effects on DA metabolism unrelated to receptor antagonism. Perhaps relevant to this is a report that 0.4 mg/kg haloperidol has neurochemical and behavioral effects not related to DA-receptor antagonism [45].

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