Antipsychotic Drugs: Direct Correlation between Clinical Potency and Presynaptic Action on Dopamine Neurons

Abstract. *Neuroleptic (antipsychotic) drugs inhibited the electrically stimulated release of [^{3}H]dopamine from rat striatal slices. The concentrations for 50 percent inhibition (ranging from 11.5 nanomolar for spiroperidol to 800 nanomolar for thioridazine) correlated closely with the average daily dosages of 25 neuroleptic drugs used clinically for schizophrenia. The correlation includes butyrophenones, phenothiazines, reserpine, pimozide, clozapine, and (+)-butaclamol. Clinically inactive isomers* [trans-*thiothixene*, trans-*flupenthixol, and (−)-butaclamol*] *required 20 to 1000 times higher concentrations than the active isomers to inhibit release. Compared to the inhibition of [^{3}H]dopamine release, much higher neuroleptic concentrations were needed to inhibit the electrically stimulated release of other neurotransmitters—[^{3}H]acetylcholine, [^{3}H]GABA (γ-aminobutyric acid). The neuroleptic drugs may block the presynaptic coupling between impulse and neurosecretion.*

There are two types of theories explaining the main tranquilizing actions and side effects of antipsychotic drugs. The receptor-blockade hypothesis states that the neuroleptic drugs specifically attach to dopamine receptors in the nervous system (*1*), thus inhibiting dopamine-sensitive adenylate cyclase (*2*), increasing the firing rate of dopamine neurons (*3*), accelerating dopamine turnover (*4*), and blocking the effects of amphetamine and apomorphine (*5*). There is no simple and direct quantitative relation, however, between any of these neuroleptic actions and their antipsychotic potencies in controlling schizophrenia (*6*); the butyrophenones such as haloperidol and spiroperidol, for example, are 20 to 100 times more potent clinically than chlorpromazine, yet are equal to or weaker than the latter in blocking dopamine-sensitive adenylate cyclase (*2*).

The coupling-blockade hypothesis of neuroleptic action recognizes that the neuroleptics are fat-soluble and surface-active drugs which readily accumulate in cell membranes (*7*), causing membrane expansion (*8*), blocking nerve membrane impulses (*9*), displacing membrane-associated Ca^{2+} (*10*), enhancing the spontaneous release of transmitter—for example, dopamine (*11*)—or modulating the coupling between impulses and neurosecretion (*12*). We now provide the first evidence of a direct correlation between the clinical antipsychotic activity of neuroleptic drugs and their ability to block the impulse-coupled release of dopamine.

The electrically stimulated release of [^{3}H]dopamine was studied on rat neostriatal slices according to the method of Farnebo and Hamberger (*13*) with some variations. Adult male Wistar rats (190 to 230 g) were killed by a blow to the back of the neck, and the brain was removed within 1.5 minutes. Neostriatal slices (1 mm by 1 mm by 0.3 mm) were made with a Smith-Farquhar tissue chopper (Sorvall). The slices were incubated with 10^{-7} *M* [^{3}H]dopamine (8 to 10 c/mmole; New England Nuclear) for 40 minutes at 37°C in a modified Krebs-Henseleit-Ringer medium (118 m*M* NaCl, 4.7 m*M* KCl, 2.5 m*M* $CaCl_2$, 1.25 m*M* $MgSO_4$, 25 m*M* $NaHCO_3$, 1.18 m*M* KH_2PO_4, 11 m*M* dextrose, 0.54 m*M* sodium ethylenediaminetetraacetate, 1.14 m*M* ascorbic acid, and 0.0125 m*M* nialamide); the medium was constantly gassed with a mixture of 95 percent O_2 and 5 percent CO_2. The slices were then quickly rinsed twice with dopamine-free Krebs-Ringer medium and ten slices were placed in each of four circular chambers (5 mm diameter and 13 mm high), the slices being sandwiched between nylon mesh, which separated the slices from the platinum wire electrodes (the interelectrode distance was 8 mm). The four chambers were then simultaneously perfused with dopamine-free Krebs-Ringer solutions, two of which contained the neuroleptic drug being tested, and the other two serving as controls. After being superfused for 30 minutes (flow rate, 0.3 ml/min), the slices were electrically stimulated for 60 seconds (*14*) (biphasic pulses, 25 hz; sawtooth pulse shape of 15 volt maximum and falling to half-maximum at 0.75 msec), and then further superfused for 30 minutes before homogenization. The effluent was collected directly into liquid scintillation vials every 3 minutes, except after the onset of stimulation when three 1-minute samples were taken; the total radioactivity was measured by a Packard spectrometer after the addition of 10 ml of Aquasol (New England Nuclear) to each vial. At least 90 percent of the radioactivity was in the form of [^{3}H]dopamine (*13, 15*).

In agreement with the results of Farnebo and Hamberger (*13*), the omission of Ca^{2+} or the addition of 10^{-6} *M* tetrodotoxin inhibited the release of [^{3}H]dopamine. The integrated efflux (due to stimulation) was directly proportional to the frequency and voltage of stimulation, and the slices responded to repeated periods of stimulation.

The neuroleptics inhibited the stimulated release of [^{3}H]dopamine (Fig. 1A); the spontaneous efflux (before stimulation)

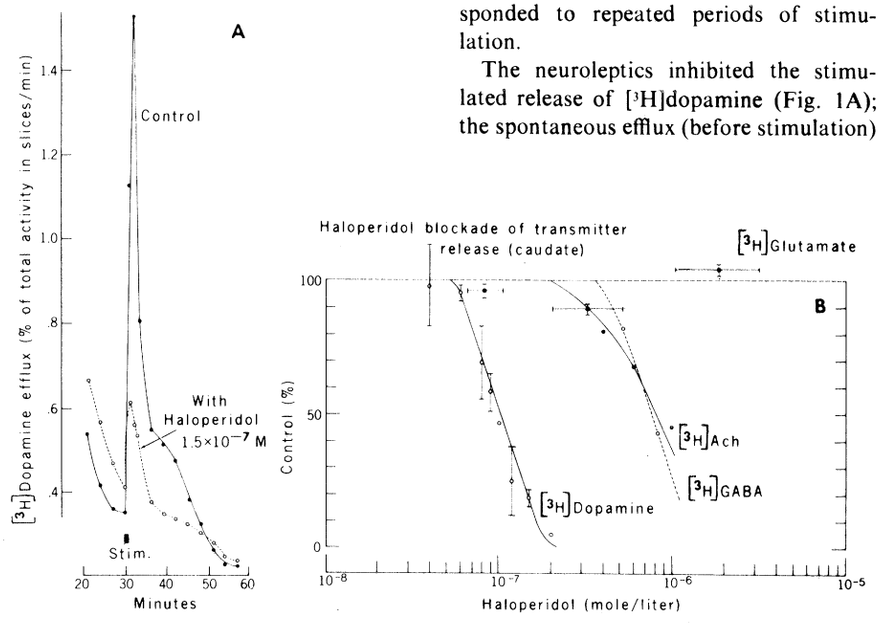

Fig. 1. (A) The efflux of [^{3}H]dopamine from rat striatal slices increased upon electrical field stimulation for 1 minute (at t = 30 minutes). Haloperiodol (1.5×10^{-7} *M*) inhibited the stimulated efflux of [^{3}H]dopamine, as well as increased the spontaneous efflux before stimulation. The method of analyzing the data further is described by Farnebo and Hamberger (*13*). (After 1 hour, more than 90 percent of the released radioactivity was in the form of dopamine.) (B) Showing that the electrically stimulated release of [^{3}H]dopamine was much more susceptible to neuroleptic blockade than the stimulated release of [^{3}H]acetylcholine, [^{3}H]GABA, or [^{3}H]glutamate from striatal slices. Vertical bars are standard deviations for seven experiments; horizontal bars (for glutamate) indicate the range of concentrations tested. Single points represent one experiment consisting of ten slices each in duplicate chambers with the drug, and ten slices each in duplicate control chambers.

was also increased, in agreement with earlier work on synaptosomes (*11*). Compared to the stimulated release of [^{3}H]dopamine, this increased spontaneous efflux is small; in agreement with the procedure of Farnebo and Hamberger (*13*), our results deal only with the stimulated release, the spontaneous level being subtracted. We were able to obtain the drug effects with only 5 minutes of drug exposure before stimulation; washing with drug-free solution returned the release to the level observed without the drug. Although it was assumed that the [^{3}H]dopamine was a valid tracer for endogenous dopamine, this may not necessarily be so since the isotope might have entered different dopamine pools.

The stimulated release of [^{3}H]dopamine was more readily blocked than that of other neurotransmitters. Striatal slices, incubated for 40 minutes with either [^{3}H]choline chloride (2.34 c/mmole; $3.2 \times 10^{-7}M$), γ-[^{3}H]aminobutyric acid (GABA) (10 c/mmole; $1.04 \times 10^{-7}M$ with 2 mM aminooxyacetic acid), or with L-[^{3}H]glutamic acid (16.2 c/mmole; $1.03 \times 10^{-7}M$), responded to electrical stimulation by releasing ^{3}H-labeled transmitters or their metabolites (50 percent of the ^{3}H released in the choline experiments was in the form of [^{3}H]acetylcholine, while more than 80 percent of the other two transmitters was unmetabolized). The concentrations of neuroleptic drugs required to block these effluxes, however, were much higher than those needed to block the [^{3}H]dopamine efflux (Fig. 1B), suggesting that the dopaminergic neurons are particularly vulnerable to neuroleptics.

In addition to the IC_{50} values (that is, the concentrations that inhibited the stimulated release of dopamine by 50 percent) shown in Fig. 2, values for other drugs are as follows (in nanomoles per liter): β-flupenthixol dihydrochloride, 13,000; (+)-butaclamol hydrochloride (*16*), 150; (−)-butaclamol hydrochloride (*16*), > 100,000; the values are reproducible to within 15 to 20 percent.

The IC_{50} values correlate directly with the clinical dosages used to control schizophrenia (Fig. 2) (*17*). Reserpine, as well as trazodone, both employed in high dosage for schizophrenia (*17*), also fit in Fig. 2. Farnebo and Hamberger (*13*) found that $10^{-6}M$ and $10^{-5}M$ pimozide enhanced the stimulation-induced overflow of [^{3}H]dopamine; they also found that $10^{-7}M$ and $10^{-6}M$ chlorpromazine increased the overflow. We have tried to replicate their methods as closely as feasible; our present results are the same whether we use different frequencies of stimulation, whether we stimulate the slice chambers in series or parallel, or whether we use square-wave pulses. We have no explanation for the contradiction between the results of Farnebo and Hamberger and our own. The following drugs had very weak inhibitory actions on the release of dopamine and would be predicted to be weak antipsychotic agents clinically:

Promazine hydrochloride (6.1 μM)
Promethazine hydrochloride (16 μM)
l-Propranolol hydrochloride (16 μM)
d-Propranolol hydrochloride (190 μM)
Imipramine hydrochloride (1.6 μM)
Desipramine hydrochloride (3.4 μM)
l-Methadone hydrochloride (2.8 μM)
d-Methadone hydrochloride (9.8 μM)
Ethanol (0.12M)

The possible mechanisms underlying inhibition by neuroleptic drugs of impulse-triggered release of [^{3}H]dopamine include the following. (i) The neuroleptic drugs might be inhibiting nerve impulse conduction (*9*). Small diameter fibers are particularly vulnerable to the blocking actions of neuroleptic drugs, and dopaminergic neurons are small (0.2 μM) (*18*). The lowest concentrations at which chlorpromazine, haloperidol, and clozapine inhibited the release of [^{3}H]dopamine were $3.8 \times 10^{-7}M$, $5.2 \times 10^{-8}M$, and $2.5 \times 10^{-7}M$, respectively; these concentrations are about the same as those in the extracellular water of patients on maintenance doses of these drugs (namely, $3 \times 10^{-7}M$, $6 \times 10^{-8}M$, and $5 \times 10^{-7}M$, respectively). Since anesthetics (for example, chloral hydrate administered intravenously) actually increase the firing rate of nigral dopamine neurons (*3*), impulse inhibition by neuroleptic drugs, if it exists in striatal tissue, could occur in the small presynaptic dopaminergic neuron terminals; the increase induced by neuroleptic and anesthetic drugs on the nigral neuron firing rate (*3*) might arise by neuronal feedback as a consequence of presynaptic depression by neuroleptics. (ii) The neuroleptics might act directly on presynaptic dopamine receptors to block the local negative feedback which extracellular dopamine appears to have on presynaptic terminals (*19*). Such neuroleptic action, however, would increase the release of dopamine (*13*). (iii) The neuroleptic drugs might inhibit the coupling between the impulse and the neurosecretion of dopamine, by interfering with the Ca^{2+} influx in presynaptic nerve terminals (*10, 12*); our current work shows that high Ca^{2+} concentration can antagonize the haloperidol action described above. Whatever the mechanism

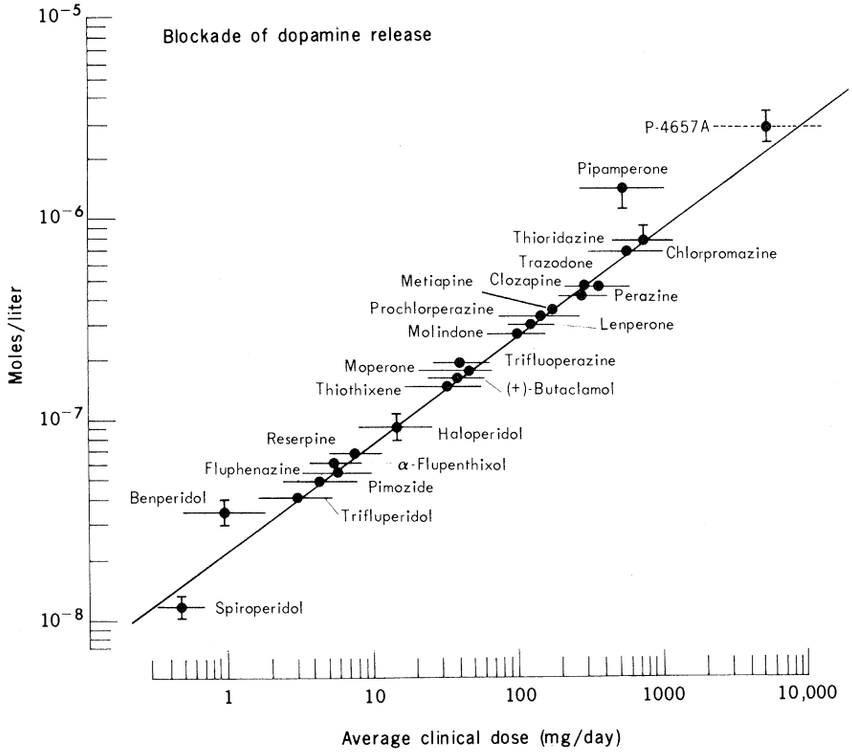

Fig. 2. The neuroleptic IC_{50} values (the neuroleptic concentrations which inhibited the stimulated release of dopamine by 50 percent) correlate with the average clinical doses (in milligrams per day) for controlling schizophrenia. The horizontal bars indicate the range of clinical doses (*17*). The vertical bars show the 20 percent variation in the IC_{50} values. The correlation includes such diverse compounds as phenothiazines, butyrophenones, reserpine, pimozide, trazodone, clozapine, and (+)-butaclamol. *Trans*-thiothixene (P-4657A) is of the order of one-hundreth the potency of its *cis*-isomer (*17*).

for neuroleptic inhibition of stimulated [^{3}H]dopamine release, a presynaptic site of neuroleptic action might explain many of the neuroleptic effects in brain. The neuroleptic inhibition of impulse-secretion coupling in the dopamine preterminals may possibly elicit the neuronal feedback activation of nigral cell firing (*3*) as a compensatory mechanism; the increased firing might activate tyrosine hydroxylase (*20*) and thus possibly lead to the observed increase in brain dopamine turnover (*21*) as an overcompensation.

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Golgi Complex—Endoplasmic Reticulum Transition Region Has Rings of Beads

Abstract. *The smooth surface of the rough endoplasmic reticulum that makes the forming face of the Golgi complex has beadlike structures arranged in rings at the base of transition vesicles. The beads can only be seen easily after staining in bismuth salts. They are 10 to 12 nanometers in diameter and occur in a variety of cell types and organisms.*

Gomori-type reactions involving the precipitation of lead phosphate after the enzymatic formation of free phosphate give a questionable localization of energy-releasing phosphatases (*1*). However, if phosphates are part of a cell structure, either naturally or through fixation, it may be possible to show their presence by an increased electron opacity after reaction with bismuth salts. Bismuth oxynitrate has been successfully used to stain DNA because of its reaction with phosphate (*2*).

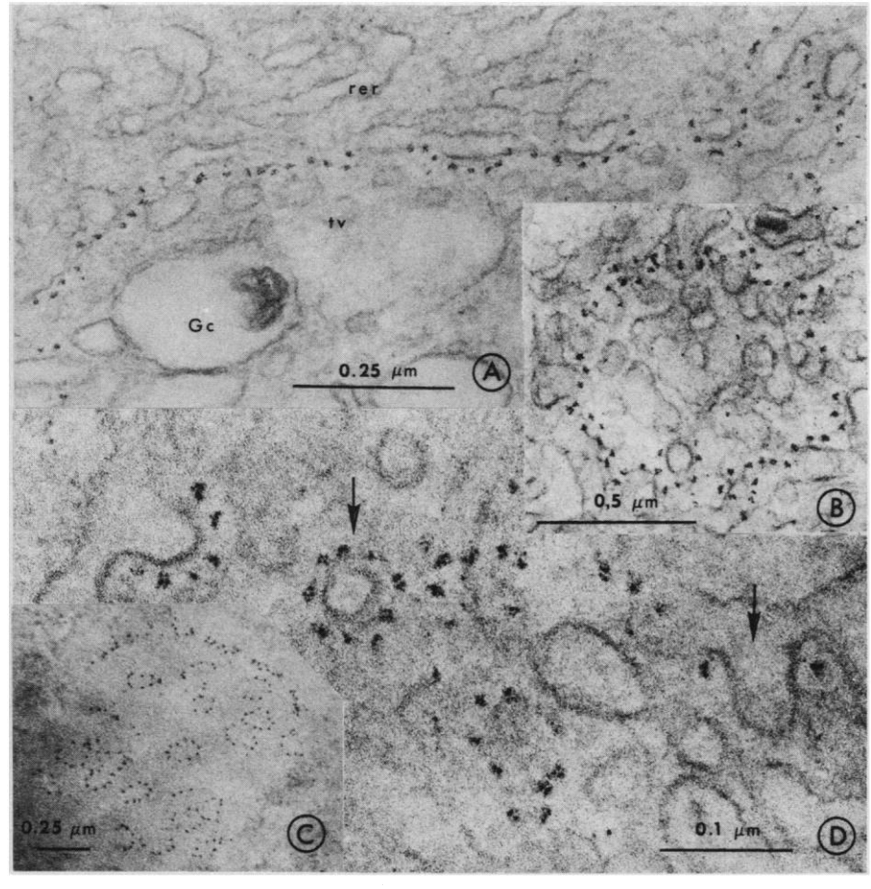

Fig. 1. (A) Beads at the smooth surface of the cisterna of rough endoplasmic reticulum on the forming face of the Golgi complex from the silk gland of *Calpodes*. Abbreviations: *rer*, rough endoplasmic reticulum; *tv*, transition vesicles; *Gc*, saccular region of the Golgi complex. (B) Beads on the smooth tubular endoplasmic reticulum where transition vesicles arise in the small Golgi complexes of oenocytes. (C) Pattern of rings formed by beads in a 0.25-μm section of the Golgi complex from an epidermal cell viewed at 100 kv; this section was not fixed with osmium tetroxide. (D) Beads form rings at the base of transition vesicles. Arrows mark the two views interpreted in Fig. 2.