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Dopaminergic Inhibition of Adrenergic Neurotransmission as a Model for Studies on Dopamine Receptor Mechanisms

Abstract. *Dopamine and apomorphine produced concentration-dependent inhibition of adrenergic neurotransmission in the isolated, perfused, rabbit ear artery. The inhibitory action of both dopamine and apomorphine was competitively antagonized by haloperidol and several other antipsychotic drugs. The calculated affinities of these drugs for the dopaminergic receptor correlate closely with both the pharmacological potencies of these drugs in vivo and their reported potencies as inhibitors of [^{3}H]haloperidol binding to "dopamine receptors" in brain homogenates.*

The neurotransmitter action of dopamine is mediated by distinct dopamine receptors (*1, 2*). Most antipsychotic drugs are thought to exert their therapeutic actions and to produce extrapyramidal side effects by blocking dopamine receptors (*3*). However, the precise pharmacological characteristics of these receptors have been only poorly defined. Indeed, it has been suggested that there may be more than one type of dopamine receptor in the brain (*4*).

Most of our knowledge of the characteristics of the different types of adrenergic and cholinergic receptors stems from studies on peripheral tissues. Although there are several peripheral dopaminergic receptor systems, none of these have been found suitable for quantitation of the action of dopaminergic agonists and antagonists. Recently, several investigators have demonstrated that some postganglionic sympathetic nerve terminals possess dopaminergic receptors which, when activated, mediate inhibition of nerve-evoked norepinephrine release (*5*). We now report that the inhibitory action of dopaminergic agonists on adrenergic neurotransmission in the isolated, perfused, rabbit ear artery provides an excellent experimental model for quantitative studies on dopaminergic receptor mechanisms.

The preparation of the isolated rabbit ear artery and the perfusion techniques used have been described (*6*). In brief, the artery, mounted in a narrow-bore muscle chamber, was simultaneously perfused intraluminally and superfused extraluminally. Both perfusion and superfusion were at constant flow rates (about 2 ml/min for each), so that the inflow perfusion pressure, recorded with a transducer and Physiograph (E & M Instrument), monitored the degree of vasoconstriction. The perfusion and superfusion fluid was oxygenated Krebs-bicarbonate solution maintained at 35°C. The periarterial adrenergic nerves were excited by field stimulation with square-wave pulses of 0.7-msec duration at supramaximal voltage (60 to 70 volts), applied through two platinum electrodes mounted in the muscle chamber. Except for a few experiments in which norepinephrine was administered intraluminally to produce vasoconstriction, all drugs were administered extraluminally by way of the inflowing superfusion fluid.

Dopamine, at concentrations ranging from 3 to 1000 nM, produced concentration-dependent inhibition of the constrictor response of the rabbit ear artery to brief intermittent periods of nerve stimulation (Fig. 1A). At high concentrations, dopamine also produced vasoconstriction. The vasoconstrictor response to dopamine was blocked by phentolamine and other α-adrenergic antagonists. Both the inhibitory effect on adrenergic neurotransmission and the vasoconstrictor effect of dopamine were markedly potentiated by cocaine, which acts as an inhibitor of neuronal dopamine uptake and thus slows the process of dopamine inactivation. All of the results described in this report were obtained in the presence of 3 μM cocaine.

Propranolol, a β-adrenergic antagonist, had no effect on dopamine-induced inhibition of neurotransmission. Dopamine did not inhibit the vasoconstrictor response to intra- or extraluminally administered norepinephrine. Thus the inhibition of neurotransmission by dopamine appears to result from an interference with the process by which nerve stimulation causes release of norepinephrine.

Apomorphine, which acts as a dopaminergic agonist in behavioral tests, also produced concentration-dependent inhibition of neurotransmission in the ear artery (Fig. 1B). Apomorphine and dopamine were equipotent as inhibitors of neurotransmission; the concentration-effect curves for these two agonists were essentially superimposable over the entire concentration range tested (Fig. 1C). The concentration required to produce 50 percent inhibition of the response to nerve stimulation was 37 ± 6 nM for dopamine and 44 ± 6 nM for apomorphine. Haloperidol antagonized the inhibitory effect of both dopamine and apomorphine. The antagonism was competitive; that is, haloperidol caused a parallel shift to the right of the log concentration-effect curves for the agonists (Fig. 1D).

The onset of antagonism was slow; at the concentrations used for these experiments, haloperidol had to be administered for approximately 1 hour in order to produce maximal antagonism of the inhibitory action of dopamine. The washing out of the haloperidol required several hours, probably because of the highly lipophilic nature of this agent. Several other antipsychotic drugs produced similar competitive antagonism of dopaminergic inhibition of neurotransmission. Based on experimental data of the type shown in Fig. 1D, we calculated the dissociation constants (K_B) for the receptor-antagonist complex for a series of antipsychotic drugs including the butyrophenones (spiroperidol, droperidol and haloperidol); the diphenylbutylpiperidines (pimozide and penfluridol), and a phenothiazine (perphenazine) (Table 1). (+)-Butaclamol acted as a potent dopaminergic antagonist, whereas (−)-butaclamol did not possess any antagonist activity at con-

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centrations as high as 1 μM. The K_B values for each of the antipsychotic drugs acting as antagonists of apomorphine were not significantly different from the K_B values obtained against dopamine. Thus, according to current concepts of receptor theory, it appears that the effect of both apomorphine and dopamine on adrenergic neurotransmission is mediated by the same type of receptor.

It seems well established that postganglionic sympathetic nerve endings possess α-adrenoceptors which, when activated, mediate inhibition of the release of norepinephrine by nerve impulses (*7*). Evidence obtained in this and other laboratories indicates that the pharmacological characteristics of the prejunctional α-receptors are different from those of α-receptors on vascular smooth muscle (*8*). We have reported that in the isolated rabbit ear artery, clonidine (an agonist) and tolazoline (an antagonist) have greater affinity for the prejunctional α-adrenoceptors than for the postjunctional α-adrenoceptors (*9*).

Table 1. Comparison of the dissociation constants (K_B) of a series of antipsychotic drugs acting as antagonists of dopamine- and apomorphine-induced inhibition of neurotransmission in the ear artery. The inhibition constants (K_i) are shown for the drugs as inhibitors of [^{3}H]haloperidol binding in calf caudate homogenates. The K_B values were calculated from the formula (*15*): K_B = antagonist concentration/(dose ratio − 1). Each value is the mean ± standard error of the mean for four to ten experiments (N is given in parentheses).

Compound	K_B against		K_i for inhibition of [^{3}H]haloperidol binding* (nM)
	Dopamine (nM)	Apomorphine (nM)	
Spiroperidol	0.22 ± 0.09 (7)	0.15 ± 0.05 (6)	0.25 ± 0.02
(+)-Butaclamol	0.36 ± 0.04 (4)	0.25 ± 0.03 (4)	0.54 ± 0.08
Pimozide	0.57 ± 0.10 (5)	0.70 ± 0.10 (5)	0.81 ± 0.09
Perphenazine	1.0 ± 0.30 (7)	0.73 ± 0.20 (7)	
Droperidol	1.2 ± 0.30 (6)	1.1 ± 0.30 (6)	1.0 ± 0.1
Haloperidol	1.4 ± 0.20 (10)	1.0 ± 0.20 (8)	1.4 ± 0.1
Penfluridol	7.4 ± 2.3 (5)	7.3 ± 2.2 (5)	5.6 ± 1.4
(−)-Butaclamol	Inactive†	Inactive†	700 ± 120

*Data from Burt *et al.* (*11*). †Exhibited no dopaminergic antagonist activity at concentrations up to 1 μM.

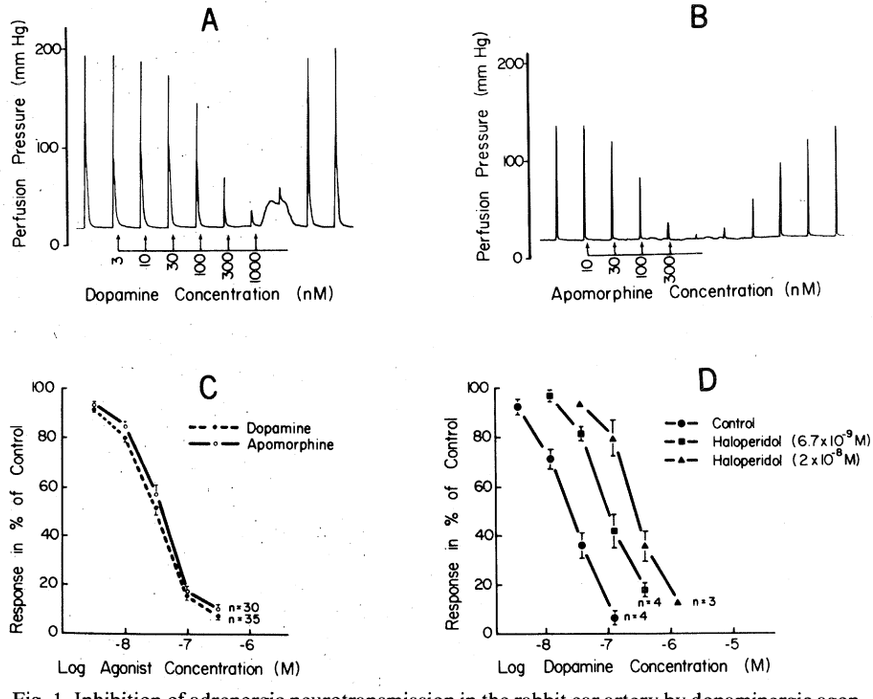

Fig. 1. Inhibition of adrenergic neurotransmission in the rabbit ear artery by dopaminergic agonists and antagonism of this inhibition by haloperidol. (A) Inhibition of the constrictor response to brief intermittent periods of nerve stimulation by increasing concentrations of dopamine in the presence of cocaine (3 μM). Stimulation for 300 msec at 10 hertz every 4 minutes. (B) Inhibition of the constrictor response by increasing concentrations of apomorphine in the presence of cocaine (3 μM). Stimulation parameters as in (A). (C) Concentration-effect curves for dopamine and apomorphine as inhibitors of neurotransmission. The vertical bars show standard errors. (D) Antagonism of dopamine-induced inhibition of neurotransmission by haloperidol. The vertical bars show standard errors.

To examine the possibility that the effect of dopamine might involve the prejunctional α-receptors, we tested haloperidol as an antagonist of the prejunctional action of clonidine and tolazoline as an antagonist of dopamine. The inhibitory effect of clonidine on adrenergic neurotransmission was not affected by haloperidol at a concentration of 0.1 μM (18 experiments). This concentration is approximately 70 times higher than the K_B value for this antipsychotic drug acting as an antagonist of dopamine (Table 1). Tolazoline, which at a concentration of 1 μM causes a one-unit shift of the clonidine log concentration-effect curve, did not affect the dopamine-induced inhibition of neurotransmission (six experiments). These results demonstrate that the effect of dopamine on adrenergic neurotransmission is not mediated by prejunctional α-adrenoceptors.

Two biochemical models have been used extensively for studies on dopaminergic receptor mechanisms. One of these models is based on the ability of dopaminergic agonists and antagonists to bind to and compete for "dopamine receptors" in membranes isolated from dopamine-rich brain tissues. Using this technique, several investigators have demonstrated a very good correlation between the potency of antipsychotic drugs acting as inhibitors of [^{3}H]-haloperidol binding and their clinical potency (*10, 11*). In Table 1, we have included the inhibition constant (K_i) values determined by Burt *et al.* (*11*) for some of the antipsychotic drugs as inhibitors of labeled haloperidol binding in caudate homogenates. The remarkably close agreement of these K_i values with the K_B values obtained in our study shows that the prejunctional dopaminergic receptors on the rabbit ear artery are similar to those on which the antipsychotic drugs act within the central nervous system.

The other biochemical model for dopamine receptor studies is based on the discovery of a dopamine-sensitive adenylate cyclase in brain homogenates (*12*). Antipsychotic drugs act as competitive antagonists of the action of dopamine on this enzyme. However, the relative potencies of the antipsychotic drugs as antagonists of the dopamine-induced adenosine 3′,5′-monophosphate (cyclic AMP) formation do not agree with their relative clinical potencies, their K_i values as inhibitors of haloperidol binding, or K_B values obtained in our studies. Since norepinephrine, like dopamine, produces significant increases in cyclic AMP concentrations in homogenates of brain basal ganglia, and since the effect of both dopamine and norepinephrine is

antagonized by relatively high concentrations of the α-adrenergic antagonist phentolamine (*2, 13*), it seems likely that the dopamine stimulation of cyclase may involve a kind of α-adrenergic receptor or, perhaps, a dopamine receptor different from that of the adrenergic nerve terminal of the rabbit ear artery.

The inhibitory effect of dopaminergic agonists on neurotransmission in the rabbit ear artery represents a pharmacological model for quantitative studies on dopaminergic receptor mechanisms. Since both antagonist and agonist activity can be assayed, this model may prove useful for the development of new dopaminergic antagonists for the treatment of psychotic disorders, as well as dopaminergic agonists for the treatment of parkinsonism, a disease associated with depletion of striatal dopamine levels (*14*).

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Maternal Behavior as a Regulator of Polyamine Biosynthesis in Brain and Heart of the Developing Rat Pup

Abstract. *Rat pups removed from the mother and placed in a warm incubator for 1 hour or more show a 50 percent reduction in ornithine decarboxylase activity in the brain and heart. The decline is not caused by lack of nutrition. Instead, these studies suggest that active maternal behavior is necessary to maintain normal polyamine metabolism in brain and heart of the pup during development.*

Data from various mammalian species suggest that early interruption of mother-infant interaction may have biochemical, physiological, and behavioral consequences (*1*). Ornithine decarboxylase (ODC) (E.C. 4.1.1.17) is the first and probably rate-limiting step in polyamine biosynthesis, and it is elevated in tissues undergoing rapid growth and differentiation (*2*). In the brains and hearts of preweanling rats, a developmental pattern of ODC activity exists, with enzyme activity reaching a peak during periods of maximum DNA and RNA synthesis and returning to low levels as the period of rapid brain growth ends (*3*). Thyroxine and cortisol alter this developmental brain ODC activity pattern in a direction similar to the effects of these hormones on brain morphology, and protein synthesis, and behavioral development; it has been suggested that ODC activity is a sensitive index of maturation of the central nervous system (CNS) (*4*). We have reported that removal of rat pups from the mother prior to weaning for periods as short as 1 hour leads to a reduction of as much as 50 percent in brain and heart ornithine decarboxylase activity, as well as its immediate polyamine product, putrescine. The decline in ODC activity is not caused by alterations in the body temperature of the pups, since temperatures of deprived and nondeprived pups are the same, and the ODC activity is reversed rapidly by return to the biological mother or an accepting (lactating) foster mother (*5*).

Hofer (*6, 7*) has reported that a fast and readily reversible decrease in heart rate occurs in 14-day-old rat pups after they are removed from the mother. These alterations in heart rate have been shown to be related to nutrition (*6, 7*). The purpose of our experiments was to examine the role of nutrition and other factors in the maternal deprivation-induced decline in ODC activity in brain and heart tissue.

Gravid Sprague-Dawley rats (Zivic-Miller) were obtained 1 week before delivery, housed individually, and given free access to food and water. These rats delivered normally on day 21 of gestation. On the day of an experiment, pups were transferred from the vivarium to our laboratory where all subsequent studies were performed. For the maternal deprivation experiments, all pups were removed from the mother and either placed individually in a warm incubator or returned to the mother in the nest. At the end of an experiment, pups were decapitated, and ODC activity was determined by measuring the release of CO_2 from DL-[1-^{14}C]ornithine (43 mc/mole) (*8*), a reaction stoichiometric with the formation of putrescine (*3*). Enzyme activity was proportional to the tissue added and was linear for at least 1.5 hours. The cofactor, pyridoxal phosphate, at a concentration of 50 μM was used in all assays.

In the first set of experiments, lactating rats were anesthetized, and their nipples were ligated; they were then allowed to recover for 2 hours. Ten-day-old pups were then either placed with these mothers for 2 hours—the time twice that normally necessary to effect a decline in ODC activity induced by maternal deprivation—or allowed to remain with the biological mother. Pups placed with a lactating female whose nipples were ligated did not show a decrease in brain ODC activity (Table 1). In fact, a small but significant increase in enzyme activity occurred in these pups. Hofer and Weiner (*9*) have shown that pups placed with nipple-ligated lactating mothers spend more time with the mothers, presumably because the pups are continuously hungry. This may account for a slight increase in their brain ODC activity. These data suggest that

Table 1. Brain ODC activity of 10-day-old pups removed from the biological mother and placed for 2 hours either in a warm incubator or with a lactating foster mother whose nipples had been ligated. ODC activity is expressed as a percent of that of the nondeprived littermate controls.

Experimental condition of pups	*N*	ODC activity (% control)
With biological mother	44	100 ± 10
In incubator	28	24 ± 2*
With nipple ligated lactating female	45	140 ± 10*

*Significant difference from controls ($P < .05$, by Student's *t*-test).

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