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Neuroendocrine Evidence That Tetrabenazine Is a Dopamine Antagonist (41550)

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Abstract. Tetrabenazine is considered to be a reserpine-like drug because of its ability to block dopamine storage in presynaptic vesicles. We used two methods to determine that tetrabenazine is also a dopamine antagonist. Tetrabenazine displaced the specific [^{3}H]spiperone binding to the dopamine receptors of the anterior pituitary, the corpus striatum, and a transplantable rat pituitary tumor with values for 50% displacement (IC_{50}) of about 15 μM. Under *in vitro* conditions, 0.5 to 10 μM tetrabenazine blocked dopaminergic inhibition of prolactin secretion from rat anterior pituitary glands. One, four, and twenty-four hours after a single tetrabenazine injection (30 mg/kg, ip), the serum prolactin changed from 22 ± 9 ng/ml initially, to 450 ± 52, 254.7 ± 10.4, and 9.3 ± 1.1 ng/ml, respectively. Pituitary glands of the treated rats incubated *in vitro* were refractory to dopaminergic inhibition of prolactin release to an extent that was maximal at one hour but inapparent by 24 hours after injection. *In vivo* and *in vitro*, tetrabenazine induces biological responses characteristic of a dopamine antagonist. These actions are independent of the reserpine-like properties of tetrabenazine. The unusual ability of tetrabenazine both to antagonize dopamine and to block presynaptic dopamine storage may provide a new tool for understanding the physiology of dopaminergic systems.

Tetrabenazine depletes biogenic amines (1–3) to produce an animal model of depression upon which antidepressant drug activity can be evaluated (4, 5). Tetrabenazine is also clinically important in the treatment of involuntary movement disorders (6, 7). Because tetrabenazine blocks vesicular amine storage (8), competitively binds to reserpine receptors (9, 10), and induces reserpine-like behavioral effects (1), tetrabenazine has been thought to act in a manner similar to reserpine (1, 11). Indirect evidence suggests that tetrabenazine may also have properties typical of a dopamine antagonist (2, 12–14). Little attention, however, has been given to the possibility that dopamine antagonism could contribute to the observed effects of the drug.

We found that tetrabenazine was capable of displacing specific [^{3}H]spiperone binding to dopamine receptors in the porcine anterior pituitary (15–18), rat corpus striatum (15), and a rat transplantable pituitary tumor (19). In addition, the ability of tetrabenazine to block dopaminergic inhibition of prolactin secretion from rat anterior pituitary glands incubated under *in vitro* conditions (20) was demonstrated.

Materials and Methods. *A. Radioligand dopamine receptor binding.* Membranes of rat corpus striatum, porcine anterior pituitary, and the 7315a transplantable rat pituitary tumor, which secretes prolactin and ACTH (21), were prepared for dopamine receptor binding studies as previously described (18). In brief, tissue was quickly dissected after sacrifice and placed into the iced buffer used throughout the study (i.e., 15 mM Tris, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, 0.1% ascorbate, 12.5 μM nialamide, 0.1 mM EDTA, pH 7.3). Each tissue was broken with a Polytron (half speed, 15 sec), and the rat striatum and rat tumor were centrifuged at 50,000g for 30 min. The pellet from each tissue was then resuspended in buffer with the Polytron and added to the assay. For the porcine anterior pituitary, after a Teflon–glass homogenization (200 rpm, 15 plunges), an 800g (3 min, three times) centrifugation in 0.32 M sucrose buffer removed nuclei, unbroken cells, and debris. The supernatant was then centrifuged at 110,000g for 60 min and the pellet resuspended for the binding assay in the same way as the striatum and tumor. Because of the

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relatively low density of dopamine receptors in the anterior pituitary (15), this two-step process was adopted to increase the ratio of receptors to protein and thus improve the resolution of the interaction of tetrabenazine with the dopamine receptor.

[^{3}H]Spiperone (1-phenyl-4[^{3}H]spiperone, 51 Ci/mmole, New England Nuclear), a potent dopamine antagonist (22), was utilized to label the dopamine receptors. Nonsaturable binding was defined as that which was present with 2 μM *d*-butaclamol (gift of Ayerst); thus saturable or specific [^{3}H]spiperone binding was that displaced by the excess of *d*-butaclamol. Increasing concentrations of tetrabenazine were added to the displacement experiments run at equilibrium (1.5 hr at 22–23°). The bound [^{3}H]spiperone was separated from free by filtration (Whatman GF/C filter, 15 sec, 20 ml cold buffer) and the trapped radioactivity was assessed by liquid scintillation spectroscopy (machine counting efficiency, 38–43%). The protein content of the assay tubes was determined by a micro method (23), using bovine serum albumin as the standard.

B. The influence of tetrabenazine on prolactin secretion. Female Sprague–Dawley rats (200–220 g, Flow Laboratories, Dublin, Va.) were housed under conditions of controlled illumination, temperature, and humidity with free access to water and standard rat chow.

The synthesis and release of [^{3}H]prolactin ([^{3}H]Prl) were evaluated as previously described (20). Briefly, following decapitation between 08:00 and 10:30 hr, the anterior pituitary glands were rapidly removed and bisected. Three pituitary halves from three different rats were pooled, weighed, and placed into an incubation flask. Each flask contained 1 ml of Medium 199 (M. A. Bioproducts, Walkersville, Md.), and 10 μCi [^{3}H]leucine (40–50 Ci/mmole, Amersham, Arlington Heights, Ill.). Four flasks comprised each experimental group. The flasks were incubated in a Dubnoff shaker under 95% O_2–5% CO_2 at 37° for 5 hr, after which the medium and glands of each flask were separated. The pituitary glands were homogenized in distilled water (1 ml), mixed with a 10% solution of Triton X-100 (100 μl), and after centrifugation, the supernatant was retained. The medium and homogenate from each flask were then separately analyzed by polyacrylamide gel electrophoresis. The radioactivity in the prolactin band was measured in a liquid scintillation spectrometer (Beckman, LS-233) and the incorporated radioactivity was expressed relative to anterior pituitary wet weight as counts per minute [^{3}H]Prl per milligram. The sum of the [^{3}H]Prl values for medium and homogenate represented the total hormone synthesized *de novo* during the incubation. To evaluate the effects on pituitary prolactin synthesis and release, experimental drugs were added directly to the flasks at the onset of the 5-hr incubation.

Dopamine (Sigma) and tetrabenazine methane sulfonate (generously provided by Dr. W. E. Scott of Hoffman-LaRoche, Inc.) were each dissolved in distilled water.

Animals were also given a single intraperitoneal injection of either tetrabenazine 30 mg/kg or the solvent, and trunk blood was collected 1, 4, 16, and 24 hr later. This tetrabenazine dose and temporal pattern of administration were based on profiles established for the observed behavioral and biochemical effects of the drug (1, 2). Sera were frozen until prolactin radioimmunoassay. Anterior pituitary glands of rats given a single injection of tetrabenazine (30 mg/kg, ip) or solvent 1, 4, or 24 hr earlier were then incubated *in vitro* in the absence or presence of 500 nM dopamine, which reliably inhibits prolactin secretion under these conditions (20).

The serum prolactin concentration (ng/ml) was measured by standard double antibody radioimmunoassay, using materials and protocol supplied by Dr. A. Parlow of the NIAMDD Rat Pituitary Hormone Distribution Program, Bethesda, Maryland. All samples from each experiment were analyzed within a single radioimmunoassay and the results were expressed relative to the rat prolactin standard RP-1.

The mean ± SE was calculated for prolactin in the medium and homogenate or serum of each group. Analysis of variance was applied to the data with $P < 0.05$ indicating that a significant difference existed. The dose-dependent nature of dopamine antagonism by tetrabenazine was further evaluated by linear regression analysis.

Results. *A. Tetrabenazine interaction with*

dopamine receptor binding. The protein content in this dopamine receptor assay (i.e., 0.2–0.4 mg protein/tube) was within the range (i.e., 0.1–0.8 mg protein/tube) where specific [^{3}H]spiperone binding increased linearly with increasing protein. Specific binding was 60–90% of total binding and the concentration of [^{3}H]spiperone used was 0.2–0.4 n*M* (i.e., 2–4× dissociation constant). In each of the three tissues, specific [^{3}H]spiperone binding to the dopamine receptor was displaced by tetrabenazine with values for 50% displacement (IC_{50}) listed in Table I.

TABLE I. TETRABENAZINE DISPLACEMENT OF SPECIFIC [^{3}H]SPIPERONE BINDING TO THE DOPAMINE RECEPTOR OF THREE DIFFERENT TISSUES

Tissue	IC_{50}[a] (μ*M*)
Corpus striatum	17.4 ± 3.4
Anterior pituitary	14.1 ± 2.3
7315a tumor	16.6 ± 1.7

[a] The IC_{50} was the tetrabenazine concentration that yielded 50% specific [^{3}H]spiperone binding in each experiment. The mean ± SE is given for the three independent experiments performed on each tissue. There were no statistically significant differences between the tissues.

B. The influence of tetrabenazine on prolactin release. We investigated biological manifestations of the tetrabenazine interaction with dopamine receptors. As shown in Fig. 1, anterior pituitary glands of normal female rats synthesized [^{3}H]Prl equivalent to 15,300 ± 1080 cpm/mg pituitary during a 5-hr incubation. Of this total amount, 9500 ± 1150 cpm/mg were secreted into the incubation medium (open bar) and 5800 ± 430 cpm/mg were synthesized but retained within the gland homogenate (shaded bar). Dopamine inhibited [^{3}H]Prl secretion ($P < 0.01$ vs control), led to a small [^{3}H]Prl accumulation within the homogenate and a reduction of total synthesis ($P < 0.05$). Tetrabenazine alone did not influence [^{3}H]Prl synthesis or release, but glands incubated in both dopamine and tetrabenazine together demonstrated that the effects of dopamine were blocked.

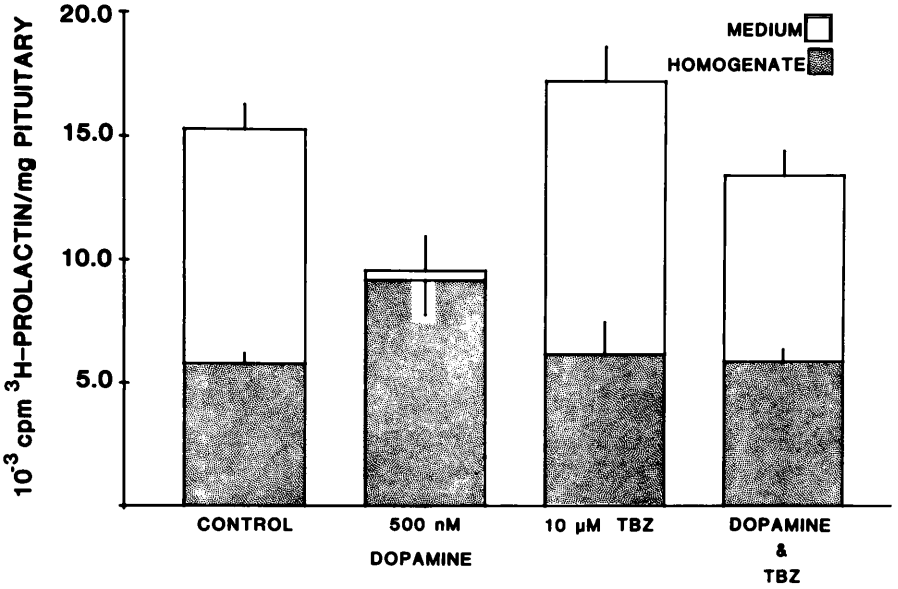

FIG. 1. Tetrabenazine blocks dopaminergic inhibition of [^{3}H]Prl secretion. Three anterior hemipituitary glands from three normal untreated female rats were incubated in a flask in Medium 199 (1 ml) and 10 μCi [^{3}H]leucine for 5 hr. The synthesis and release of [^{3}H]Prl were measured under the influence of dopamine and/or tetrabenazine (TBZ) added to the incubation flasks. The ordinate represents cpm [^{3}H]Prl/mg pituitary as the mean ± SE of the four flasks in each experimental group. The open bars represent [^{3}H]Prl released into the incubation medium and the shaded bars, [^{3}H]Prl synthesized but retained within the homogenate. The sum of the values of medium and homogenate is the total hormone synthesized during the incubation.

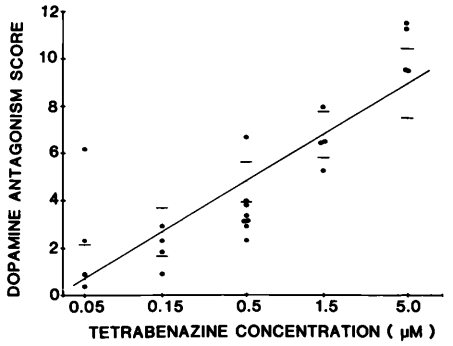

FIG. 2. The dose-related ability of tetrabenazine to reverse dopaminergic inhibition of [^{3}H]Prl secretion. The incubation conditions are described in the Fig. 1 legend. Increasing concentrations of tetrabenazine (shown logarithmically on the abscissa) were added to incubation flasks containing 500 n*M* dopamine. A dopamine antagonism score was derived from the ratio of [^{3}H]Prl released in the presence of tetrabenazine and dopamine compared to the mean value for [^{3}H]Prl released in the presence of dopamine alone, where greater dopamine antagonism by tetrabenazine yielded a greater score. Each point on the graph was the score for an individual incubation flask. The regression line and 95% confidence limits (horizontal bars at each concentration) for the data demonstrated a high correlation of tetrabenazine concentration with dopamine antagonism ($P < 0.0001$; $r^2 = 0.689125$). Dopamine alone inhibited [^{3}H]Prl secretion about 90%.

The effect of 500 n*M* dopamine alone on [^{3}H]Prl secretion was compared with the same dopamine concentration and increasing amounts of tetrabenazine. The data in Fig. 2 demonstrate a highly significant dose-related ability of tetrabenazine to antagonize dopaminergic inhibition of [^{3}H]Prl secretion. Tetrabenazine concentrations greater than 0.15 μM significantly blocked the dopamine effect ($P < 0.05$). Tetrabenazine had no effect on synthesis or release of ^{3}H-growth hormone (data not shown).

The serum prolactin values after injection of either tetrabenazine or solvent are given in Table II. As seen in Fig. 3, one hour after solvent injection, pituitary [^{3}H]Prl secretion could be inhibited by dopamine *in vitro* ($P < 0.01$ compared to control). One hour after injection of tetrabenazine, however, [^{3}H]-prolactin secretion was refractory to dopaminergic inhibition. Prolactin synthesis and release by glands of tetrabenazine injected animals was unchanged from solvent-injected controls. As seen in Fig. 4, tetrabenazine treatment 4 hr earlier blunted the action of dopamine compared to the dopaminergic effect on glands of solvent-injected rats ($P < 0.05$). The anti-dopaminergic effect of tetrabenazine was less prominent at 4 hr than at 1 hr (Fig. 3). Twenty-four hours after a single dose of tetrabenazine, the full inhibitory effect of 500 n*M* dopamine *in vitro* was again apparent. Compared to solvent-injected controls, [^{3}H]Prl secretion was increased in glands of rats given tetrabenazine (30 mg/kg) both 4 and 24 hr earlier ($P < 0.05$).

Discussion. Kuczenski (13) observed two effects after tetrabenazine injection: striatal dopamine synthesis was increased 100% within 15 min, and striatal dopamine content fell 90% by 30 min. In contrast, reserpine never stimulated dopamine synthesis at any dose or time after injection, despite near total dopamine depletion. Increased dopamine synthesis or turnover has been taken as biochemical evidence of pre- and/or postsynaptic dopamine receptor blockade (24, 25). A tetrabenazine analog, Ro 1-9564, also increased striatal dopamine turnover but without causing dopamine depletion, suggesting characteris-

TABLE II. THE SERUM PROLACTIN CONCENTRATION IN FEMALE RATS AFTER TETRABENAZINE ADMINISTRATION

	Interval after injection (hr)[a]			
	1	4	16	24
Control	22 ± 9 (6)	24.5 ± 4.1 (11)	7.9 ± 2.5 (6)	11.9 ± 1.5 (12)
Tetrabenazine	450 ± 52 (12)[b]	254.7 ± 10.4 (10)[b]	30.8 ± 7.1 (5)[b]	9.3 ± 1.1 (11)[c]

[a] Animals were given a single intraperitoneal injection of tetrabenazine (30 mg/kg) or solvent and sacrificed after these four subsequent periods. The values are the serum prolactin concentrations, ng/ml, mean ± SE. The number of animals in the group is in parentheses.

[b] $P < 0.01$ compared with the respective control.

[c] Not significantly different from control.

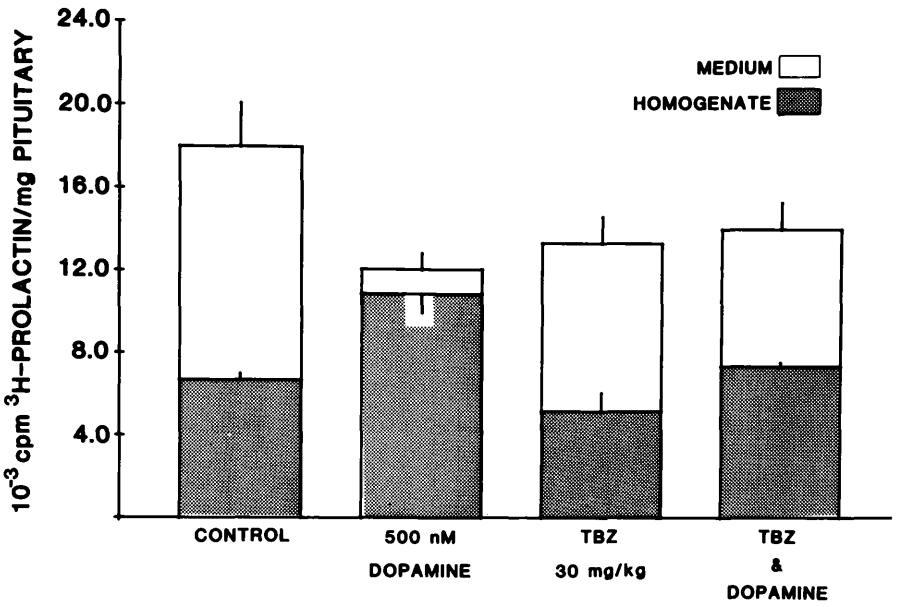

FIG. 3. The influence of tetrabenazine (TBZ) *in vivo* on dopaminergic inhibition of [^{3}H]Prl synthesis and release *in vitro*. Normal female rats were injected with TBZ or solvent and sacrificed one hour later. Their anterior pituitary glands were incubated in [^{3}H]leucine in the presence or absence of dopamine and the values for [^{3}H]Prl synthesis and release were obtained as described in Fig. 1.

tics of a pure dopamine antagonist (14). Increased dopamine turnover by tetrabenazine was also defined clinically by McLellan *et al.* (12), who gave tetrabenazine to patients with Huntington's Chorea and found that the cerebrospinal fluid concentration of homovan-

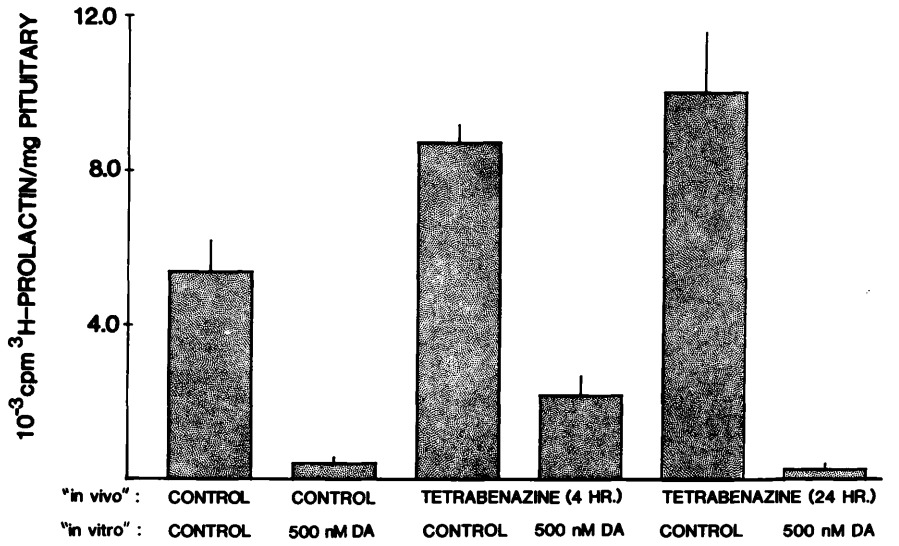

FIG. 4. The temporal profile of blockade of dopaminergic inhibition of [^{3}H]Prl release *in vitro* by tetrabenazine *in vivo*. Four and twenty-four hours after a single dose of tetrabenazine (30 mg/kg, ip) or solvent, rat anterior pituitary glands were incubated in [^{3}H]leucine in the presence or absence of dopamine. Each bar represents the value for [^{3}H]Prl released into the incubation medium.

illic acid, a dopamine metabolite, was increased. Finally, Pletscher *et al.* (2) reported that tetrabenazine blocked physiological effects of the dopamine agonist, apomorphine.

Our biochemical and neuroendocrine data confirm and extend the impressions that tetrabenazine can antagonize dopamine. Tetrabenazine displaced the specific binding of [^{3}H]spiperone to dopamine receptors in subcellular particulates of three different tissues with an IC_{50} in each of about 15 μM (Table I). Reches *et al.* (25) have reported similar data. Tetrabenazine has a relatively low affinity for the dopamine receptor compared to other dopamine antagonists. We have not yet distinguished whether tetrabenazine competes for the dopamine receptor site directly or modifies receptor function indirectly, such as by altering contiguous membrane properties. Under *in vitro* conditions tetrabenazine blocked dopaminergic inhibition of prolactin release (Fig. 1) in a dose-related manner (Fig. 2) at concentrations that also decreased dopamine receptor binding. One hour after tetrabenazine injection, animals were hyperprolactinemic (Table II), and prolactin secretion was refractory to dopaminergic inhibition *in vitro* (Fig. 3). Thus, *in vivo* dopamine receptor blockade appears to have been at least partially responsible for the hyperprolactinemia. This impression is supported by the observation that 30 min after tetrabenazine (5 mg/kg) prolactin levels were increased 40-fold and prolactin secretion could not be reduced by apomorphine (26). We observed that dopamine antagonism was still apparent 4 hr after one dose of tetrabenazine (Fig. 4) but was of smaller magnitude than at 1 hr. It is important to recognize that metabolites formed after *in vivo* administration of tetrabenazine (1) might also possess anti-dopaminergic properties in the pituitary gland. Tetrabenazine had no direct effect on the basal secretion of [^{3}H]Prl or ^{3}H-growth hormone, suggesting a specificity to its action. Tetrabenazine has also antagonized dopaminergic actions as measured by anterior pituitary secretion of radioimmunoassayable prolactin (27).

Tetrabenazine is thus apparently able to interrupt dopaminergic transmission by two independent mechanisms: antagonism at the dopamine receptor and blockade of dopamine storage in presynaptic granules. This unique situation may offer future insights into the physiology of dopaminergic systems.

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