Concentration–Effect Relationships of Tetrabenazine and Dihydrotetrabenazine in the Rat

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Abstract □ To investigate the pharmacodynamics of tetrabenazine [1,3,4,6,7,11*b*-hexahydro-3-isobutyl-9,10-dimethoxy-2-*H*-benzo(*a*) quinolizine-2-one; **1**], the drug of choice in the control of Huntington's chorea and tardive dyskinesia, and its major metabolite, dihydrotetrabenazine (**2**), ip doses of 3 mg/kg of the drug and the metabolite were administered to rats. Animals were decapitated at 0.0, 0.5, 2, 5, and 12 h. Brain and serum concentrations of **1** and **2**, and brain concentrations of dopamine, norepinephrine, and serotonin were measured. Time courses of **1** and **2** in the brain (site of action) were parallel to those in serum, indicating that the brain is a part of the central compartment for both compounds. Despite its greater polarity, **2** was able to readily cross the blood–brain barrier. The monoamine depletions at any time following the administration of **2** were at least equal to or greater than those observed following the administration of the same dose of **1**. After both compounds, the maximum serum and brain concentrations and maximum depletions were observed with the first sample at 0.5 h, with dopamine and serotonin being the most and least affected, respectively. The brain levels of the amines returned to the control values a maximum of 12 h after ip injections of either **1** or **2**. Sigmoidal relationships were found between the monoamine levels and the corresponding log brain or serum concentrations of **1** or **2**. The concentration–response curves of **2** following the administration of **1** and **2** were superimposable, suggesting that the observed activity after tetrabenazine injection is mainly, if not entirely, due to the formation of the active metabolite. These results might be of great clinical significance, as area under the plasma concentration–time curves of **2** in patients have been reported to be ~150-fold greater than those of **1** following po doses of **1**.

The effectiveness of tetrabenazine [1,3,4,6,7,11*b*-hexahydro-3-isobutyl-9,10-dimethoxy-2-*H*-benzo(*a*)quinolizine-2-one; **1**] in the control of hyperkinetic disorders such as tardive dyskinesia and Huntington's chorea is now well established.[1-6] The clinical effects of **1** have been attributed to its ability to deplete brain catecholamines and serotonin (5-HT),[7] as well as blockade of both pre- and postsynaptic dopamine (DA) receptors.[8-10] Limited available pharmacokinetic data indicate that **1** undergoes very extensive metabolism resulting in the formation of nine metabolites,[11] and has a low oral bioavailability of 6% in humans[12,13] and 17% in rats.[12] Following po administration of therapeutic doses of **1** to patients with tardive dyskinesia, the steady-state area under the plasma concentration–time curve of the major metabolite of tetrabenazine, dihydrotetrabenazine (**2**), was shown to be 82.6–199-fold higher than that of the parent drug.[12] In vitro studies indicate that **2**, like **1**, is capable of depleting catecholamines from their storage sites.[14,15] However, the extent of its in vivo effects has been questioned as, utilizing chromaffin granule membranes, the activity of **2** was shown to be only half of that of **1**.[14] We have previously posed the question of whether the efficacy of the administration of **1** is due to the very low concentrations of **1** and/or the relatively high concentrations of **2**.[12] Roberts et al.[13] have also suggested the possibility of **2** being "more therapeutically important than" **1**. In this study, attempts were made to correlate the serum concentrations of **1** and **2** with their corresponding brain concentrations following ip injections of **1** and **2**, and to compare the pharmacodynamics of **1** and **2** in the rat.

Experimental Section

Materials—Dopamine (DA), norepinephrine (NE), serotonin (5-HT), and 3,4-dihydroxybenzylamine were purchased as either the free or salt forms from Sigma (St. Louis, MO). Tetrabenazine (**1**; lot #61-84) was a gift from Hoffmann-LaRoche of Canada (Etobicoke, Ontario), while dihydrotetrabenazine (**2**) was prepared by reduction of **1** by sodium borohydride in methanol.[15] The melting point and mass spectrum of the synthesized powder were identical to those of an authentic sample supplied by Hoffmann-LaRoche.[12] 5,6-Benzoquinoline (B.D.H. Laboratory, UK) was used as internal standard (IS) in the assay of **1** and **2** in the serum or brain samples. All the other reagents were analytical grade, except acetonitrile which was HPLC grade.

Doses and Sample Collection—Male, Sprague-Dawley rats weighing 200–250 g were kept in the department at least 3 d before dosing. At 0.5, 2, 5, and 12 h following ip injections of 3 mg/kg of either **1** or **2**, rats (n = 4) were decapitated. The brains were rapidly removed and dissected into two identical halves, transferred to tubes kept on dry ice and hexanes, weighed, and then stored at −70 °C until the time of analysis.

Serum samples were obtained from blood by centrifugation and stored at −20 °C until the time of analysis of **1** and **2**.

Assay Procedure—Serum concentrations of **1** and **2** were analyzed utilizing a previously reported HPLC method.[12] The brain concentrations of **1** and **2** were measured by a method which is described here. One half of each brain sample was homogenized in glass tubes with a motorized tissue homogenizer (Bodine Electric, Chicago, IL, USA) after addition of 4 mL of an ice cold 0.5 M solution of perchloric acid containing 0.5% mercuric acetate as a protein precipitant and 0.2 mL of IS (50 ng/mL). One milliliter of the homogenate was transferred to a microcentrifuge tube and centrifuged (microcentrifuge model 235A, Fisher, Edmonton, Alberta, Canada) for 10 min. The supernatant was then transferred to a glass tube and excess sodium carbonate was added. The drug and its metabolite were then extracted with 3 mL of ethyl acetate. The organic layer was evaporated to dryness in a Savant Speed Vac Concentrator/Evaporator (Emerston Instrument, Scarborough, Canada). The dehydrogenation of **1** and **2**, and the chromatography conditions were the same as described previously.[12,16]

The concentrations of **1** and **2** were calculated from standard curves obtained by spiking drug-free rat brains with known amounts of **1** and **2** to final concentrations of 5 to 500 ng/g for **1**, and 10 to 1000 ng/g for **2**. Excellent linearity (r = 0.990–0.999) was observed between ratios (1:IS and 2:IS) and their corresponding brain concentrations (n = 4). The inter- and intraday CV values were <10%. The recovery from brain (n = 4) of **1** and **2** was estimated by measuring the response in the presence and absence of brain samples and was found to be 71.6 ± 1.7 and 68.3 ± 1.9% for **1**, and 64.6 ± 2.1 and 68.2 ± 2.7% for **2**, at concentrations of 50 and 250 ng/g, respectively.

Concentrations of DA, NE, and 5-HT were determined in the other half of the brain samples using a direct-injection HPLC method[17] with a modified mobile phase (55 mM sodium dihydrogen phosphate, 0.85 mM sodium octyl sulfate, 0.37 mM EDTA, and 9% acetonitrile, adjusted to pH 3.0 with phosphoric acid). Brain samples were homogenized in a 0.1 M solution of perchloric acid containing 0.01% EDTA and 50 ng/mL of 3,4-dihydroxybenzylamine as the IS. The concentrations of the amines were determined from a standard curve. Average brain concentrations of the monoamines in eight

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untreated rats served as baseline values.

Data Analysis—The pharmacologic effects of 1 and 2 were determined by their ability to decrease brain concentrations of DA, NE, and 5-HT. Brain concentrations of the monoamines were plotted against the corresponding log serum concentrations of 1 and 2.

Utilizing the baseline values in the control brains, the percent changes in the concentrations of the amines following treatment with 1 or 2 (E) was calculated by the following equation:

$$E = [1 - (N/N_b)] \times 100 \quad (1)$$

where N is the brain concentration of the amine and N_b is the corresponding average baseline value of the amine. Plots of C/E versus C (brain or serum concentrations of 1 or 2) were constructed utilizing the following equation, based on the maximum effect (E_{max}) model[18]:

$$C/E = C/E_{max} + K_d/E_{max} \quad (2)$$

where K_d is the affinity constant which equals the concentration of 1 or 2 eliciting half of the maximum effect (E_{max}). The E_{max} value in each case was determined from the inverse of the slope, and K_d values were calculated by multiplying the y-intercepts by E_{max}.

Utilizing plots of E versus time, the predicted maximum effect after ip administration of 3 mg/kg of 1 or 2 was calculated.[19] The slopes of these lines were also calculated and compared. The data points obtained 12 h after injection were not incorporated in the calculation as, in all cases, the monoamine concentrations returned to the baseline values before this time.

Linear regression was used to examine the relationships between serum and brain concentrations of 1 or 2. Homogeneity of the slopes of the plots of E versus time was tested by analysis of covariance.[20] The paired t test at $\alpha = 0.05$ was used to examine the significance of the observed differences. The data are reported as mean ± SD.

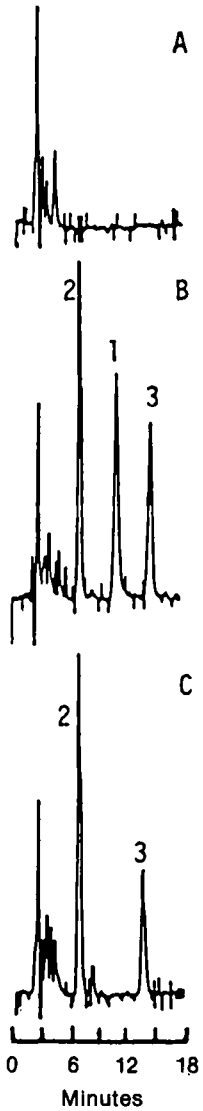

Figure 1—*Chromatograms of brain samples before* (A), *and 2 h after ip injection of 3 mg/kg of either tetrabenazine* (B) *or dihydrotetrabenazine* (C). *Peaks:* 1, *tetrabenazine;* 2, *dihydrotetrabenazine;* 3, *internal standard.*

Results and Discussion

Figure 1 depicts chromatograms of brain samples before and 2 h after ip injections of 1 and 2. The average time courses of 1 and 2 in the brain and serum following ip administrations of 1 and 2 are shown in Figure 2. Similar to serum, the maximum brain concentrations of 1 and 2 occur within 0.5 h, and the decline in the brain concentrations is parallel to that in serum, which suggests that the brain is a part of the central compartment. Following the administration of both compounds, strong correlations were found between serum and brain concentrations of the drug and the metabolite ($r > 0.958$). No tetrabenazine (1) was found in serum or brain following administration of 2.

The site of action of 1 is in the central nervous system. Consequently, 2 can elicit in vivo activity only if it crosses the blood–brain barrier (BBB) and/or is formed from 1 within the brain. Presence of 2 in the brain after its administration (Figures 1 and 2) indicates that 2, despite its greater polarity

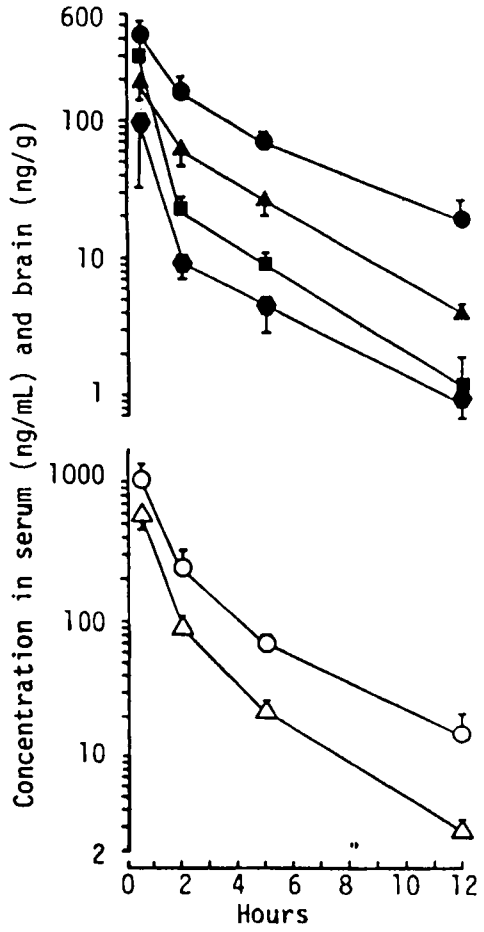

Figure 2—*Mean time courses of tetrabenazine and dihydrotetrabenazine after 3-mg/kg single ip doses of the drug (top) and the metabolite (bottom). Key:* (○, ●) *dihydrotetrabenazine in brain;* (△, ▲) *dihydrotetrabenazine in serum;* (■) *tetrabenazine in brain;* (●) *tetrabenazine in serum. Vertical bars represent SD (n = 4).*

than 1, is capable of crossing the BBB. The possibility of the formation of the metabolite from tetrabenazine inside the brain, however, remains to be proven.

The observed brain:serum concentration ratios of 2 were 3.16 ± 1.35 and 3.26 ± 1.72 after the administration of 1 and 2, respectively. The difference between these two ratios was insignificant, indicating that the presence of 1 in the brain had no effect on the brain:serum concentration ratios of 2. A brain:serum concentration ratio of 2.15 ± 0.85 was observed for 1. The greater brain:serum concentration ratio for 2 as compared with that of 1 may be attributed to a difference in binding of these compounds to the plasma or brain proteins. In humans, the plasma protein binding of the metabolite has been shown to be less extensive than that of the parent drug.[13] This, however, does not exclude the possibility of a more extensive binding of 2 to the brain proteins.

In plasma of patients taking oral doses of 1, Mehvar et al.[16] observed at least three unknown compounds, presumably representing other metabolites of the drug. The very high fluorescence intensities of these peaks were attributed to their high plasma concentrations. In the brain, however, only the fluorescence intensities of the peaks representing 1 and 2 were significant (Figure 1).

The effects of 1 and 2 on the concentration of monoamines are depicted in Figure 3. Both 1 and 2 were capable of decreasing brain levels of the amines. Their effects, however, were more intensive on DA than NE and 5-HT; the latter was least affected by 1 and 2. Consistent with their maximum serum and brain concentrations, the maximum pharmacologic effects of both 1 and 2 occurred within 0.5 h and, except for DA, the brain levels of NE and 5-HT returned to their normal values within 5 h. The brain levels of the DA 12 h after injection of either 1 or 2, although insignificant, tended to be greater than the baseline values (Figure 3). This increase might be attributed to compensatory mechanisms or to the blockade of presynaptic DA receptors.[9]

The percent reductions in the monoamine levels at any time following metabolite injection were greater than the corresponding value after injection of the same dose of the parent drug (Figure 3). The difference, although in some instances insignificant, may be attributed to the greater concentrations of 2 achieved after injection of 2, compared with those observed as a result of injection of the parent drug (Figure 2). These observations suggest that the catecholamine- and 5-HT-depleting effects observed following the administration of 1 may be due to formation of its dihydrome-

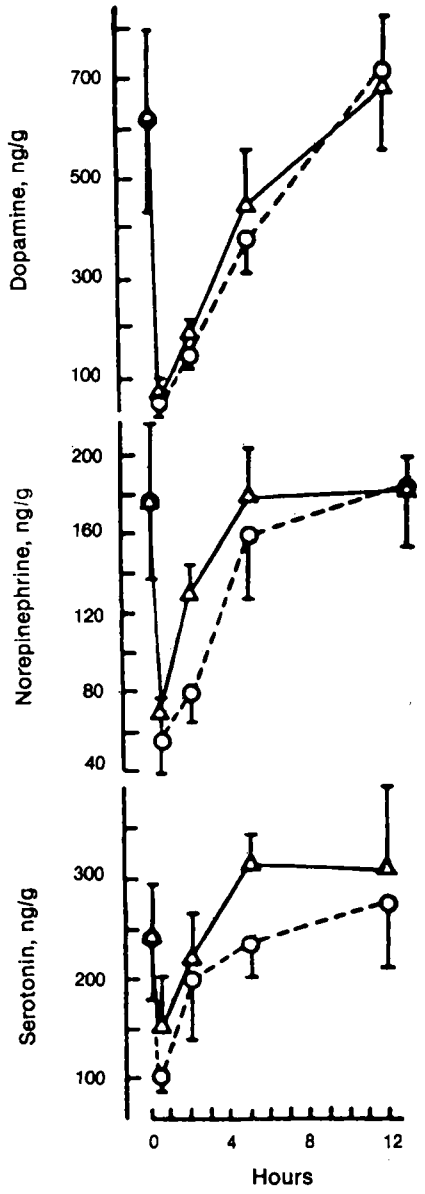

Figure 3—*Mean time courses of monoamines in brain after ip injections of 3 mg/kg of tetrabenazine (△) and dihydrotetrabenazine (○). Vertical bars represent SD (n = 4).*

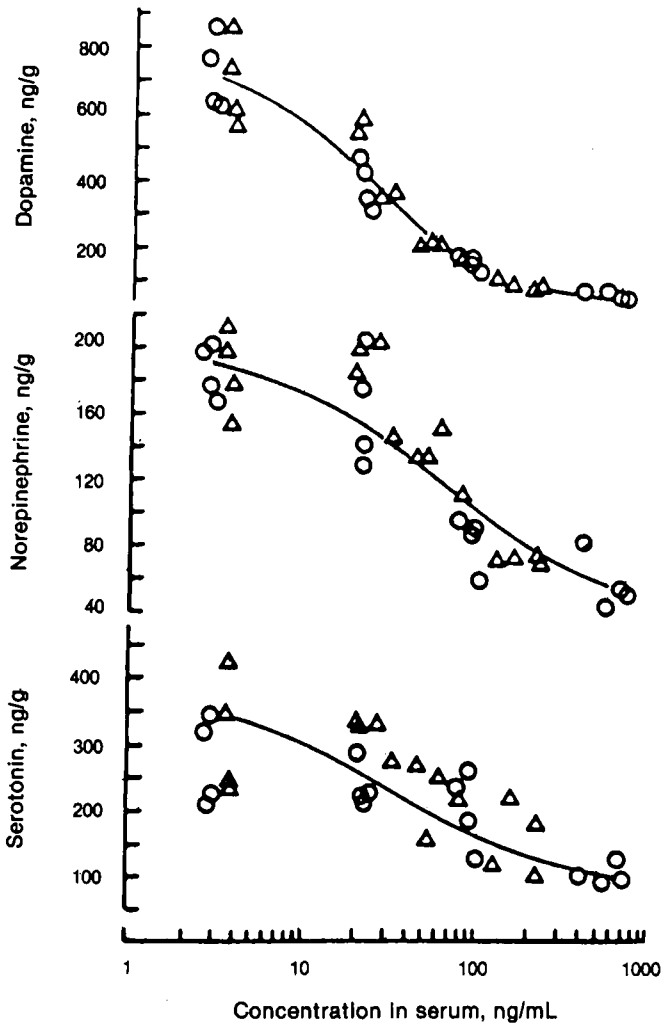

Figure 4—*Response versus log serum concentrations of dihydrotetrabenazine after ip injections of tetrabenazine (△) or dihydrotetrabenazine (○).*

tabolite, and that contribution of the parent drug to these effects is, at most, minimal. Similar to our observations, rapid reductions in the brain levels of DA, NE, and 5-HT were previously reported in rats receiving sc doses of 0.5–10 mg/kg of 1; the maximum effect (70–75% reduction in DA with 2 mg/kg dose) was observed 15 min following drug administration.[21] After iv injections of 30 mg/kg of 2 to rabbits, Pletcher et al.[7] observed a decrease in the brain levels of NE and 5-HT. To our knowledge, in vivo depletion of DA by 2, however, has not yet been addressed in the literature.

The plots of monoamine levels in the brain versus log serum concentrations of 2 after ip injections of 1 or 2 showed characteristics of sigmoidal log concentration–response relationships[19] (Figure 4). The same relationships were found when the levels of the amines were plotted versus log brain concentrations of 2. Examination of Figure 4 reveals that the sigmoidal curves representing monoamine levels and serum 2 concentrations after the administration of 1 are superimposable with those obtained after the administration of 2. This indicates that the monoamine depletion observed after the administration of 1 is mainly, if not entirely, due to the formation of 2.

The regression parameters obtained from the plots of E versus time are listed in Table I. The y-intercept of such a plot is an indication of the maximal effect attainable by that particular dose if administered intravenously.[19] In the present study, both the drug and the metabolite were injected via the ip route. Consequently, the calculated y-intercepts (Table I) are not precise estimates of the maximum attainable effects after 3-mg/kg doses of 1 or 2. This parameter, however, may be utilized for comparison of the potency of 1 and 2. As in all cases, the y-intercepts were greater following the administration of 2; one may suggest a potency greater than the unchanged drug for the metabolite. The slopes of the lines, however, were not statistically different (Table I). This might indicate that, in both cases, the same moiety was responsible for the amine-depleting activity observed after ip injections of 1 or 2.

In plotting C/E versus serum and brain concentrations of 2 for DA, only data points obtained at 0.5 and 2 h after injection of 1 and 2 were used (Figure 5). Some of the 5-h and all of the 12-h samples had DA concentrations equal to the baseline values. This made the estimation of the exact time at which the levels reached the baseline values impossible. Incorporation of 5- and 12-h data points, therefore, could have distorted the pattern of the dependency of C/E upon C. The E_{max} and K_d values calculated from these plots are presented in Table II. Closeness of the values following the administration of 1 and 2 again suggests that the active moiety in both cases is 2, not 1, and that the presence of 1 does not affect the affinity (K_d) or maximum effect (E_{max}) produced by 2.

It has been shown in vitro that 1 is capable of inhibiting the active ATP-dependent transport mechanism of catecholamines[22,23] in the chromaffin granules, the catecholamine storage organelles. The drug also appears to deplete DA and NE from crude preparations of hypothalamic and striatal synaptosomes.[24] The ability of 2 to bind to the membrane of purified bovine chromaffin granules and to inhibit NE uptake has also been demonstrated.[15,22,23] Scherman and Henry[14] investigated the effect of pH on the in vitro activity of 1 and 2 and suggested that at pH 7.5, 1 is twice as active as 2 in binding to chromaffin granules. They attributed their finding to the difference between the pK_a of 1 and 2, as only the neutral form was assumed to be able to bind to the membrane.[14] These in vitro results suggest that the monoamine-depleting activity observed following the administration of 1 is, at least, mainly due to the unchanged drug, a postulate that is contrary to our in vivo findings. This discrepancy may be due to a more complex in vivo distribution process compared with an in vitro situation. Pletcher[25] also noticed such a discrepancy between in vivo and in vitro results and suggested that in situ, the degree of transportation of the various drugs across the neuronal membrane is different; this results in different intraneuronal drug concentrations.

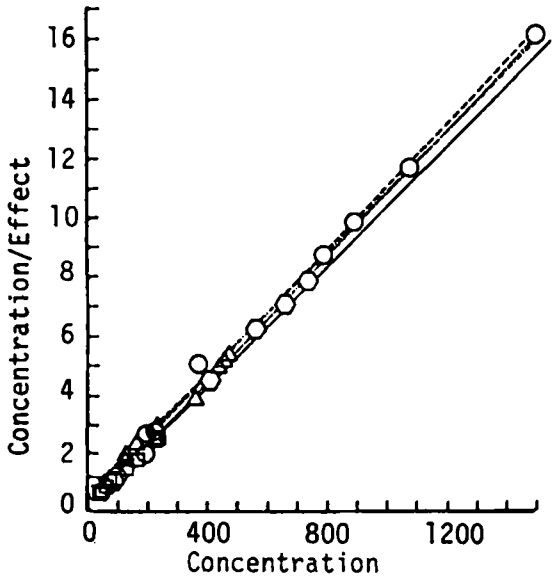

Figure 5—*Serum or brain dihydrotetrabenazine concentration/effect (% reduction in brain dopamine level) versus serum (—□—, ···○···; ng/mL) and brain (– –○– –, —△—; ng/g) concentration of dihydrotetrabenazine following ip administration of 3 mg/kg of tetrabenazine (–·–△–·–, —□—) or dihydrotetrabenazine (—○—, ···○···).*

Table II—Maximum effect (E_{max}) and Affinity Constant (K_d) Values after Single Doses of Tetrabenazine (1) or Dihydrotetrabenazine (2)[a]

Drug	K_d		E_{max}, %	
	Plasma, ng/mL	Brain, ng/g	Plasma, ng/mL	Brain, ng/g
Tetrabenazine (1)	23.8	71.0	100	103
Dihydrotetrabenazine (2)	22.7	50.8	95.7	96.2

[a] Obtained from plots of brain or plasma concentrations of dihydrotetrabenazine (2): effect (percent reduction in the brain levels of dopamine) versus concentration after ip doses of 3 mg/kg of 1 or 2.

Table I—Regression Parameters[a]

	r		Slope		y-intercept, %	
	1	2	1	2	1	2
Dopamine (DA)	0.999	0.998	−13.4	−11.7	96.6	98.7
Norepinephrine (NE)	0.970	0.992	−13.3	−13.5	61.4	78.1
Serotonin (5-HT)	0.996	0.905	−14.7	−11.2	43.2	54.3

[a] Obtained from the plots of percent effect versus time following single ip injections of 3 mg/kg of tetrabenazine (1) or dihydrotetrabenazine (2).

Such differences in the intraneuronal concentrations of 1 and 2, therefore, might explain the discrepancy between our results and those obtained in vitro.

In addition to depletion of brain monoamines, 1 is reported to block both pre- and postsynaptic DA receptors.[8-10] This conclusion has been based both on in vivo and in vitro observations following the administration of 1. In vitro reports indicate that in rats, 1 displaces [^{3}H]spirperone from binding to the striatal DA receptors with reported apparent inhibitory constants (K_i) of 5.2 μM[8] and 2.1 μM.[9] Reches et al.[9] were able to observe DA-receptor blockade following ip administration of 5 mg/kg of 1. Considering the relatively high K_i values mentioned above, an ip dose of 1 > 5 mg/kg is expected to be required in vivo to block the DA receptors. Consequently, formation of a more potent metabolite following in vivo administration of 1 was postulated as one possibility.[9] Nevertheless, because in this study we compared only the monoamine-depleting activities of 1 and 2, the blockade of DA receptor by 2 remains to be proven.

In rats, the reduction in spontaneous exploratory behavior as a result of tetrabenazine (1) therapy has been correlated with DA and NE depletion.[26] In patients with tardive dyskinesia, the clinical effects of 1 seem to be short lasting,[27] as is its depleting effect in the rat. Our study suggests that in the rat, the monoamine-depleting activity observed after the administration of 1 is due to the formation of the active metabolite, dihydrotetrabenazine (2). If these results are extrapolated to humans, then a relatively high plasma concentration of the dihydrometabolite, rather than a very low concentration of the parent drug, observed following po administration of 1,[12,13] may be held responsible for clinical effects.

Our present investigation in the rat shows that the monoamine-depleting activity observed following the administration of either the drug or the preformed metabolite is well related to the brain or serum concentrations of the metabolite. Further investigations on patients treated with 1 for different abnormal movement disorders may also reveal the existence of a therapeutic plasma concentration range for the metabolite, dihydrotetrabenazine (2). The appropriateness of substituting 2 for 1, as suggested earlier,[13] may not, however, be justified before conducting further pharmacokinetic studies on the metabolite, given as such to humans.

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