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Mitochondrial toxin inhibition of [^{3}H]dopamine uptake into rat striatal synaptosomes

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Abstract

Administration of the mitochondrial inhibitors malonate and 3-nitropropionic acid (3-NP) to rats provides useful models of Huntington's disease. Exposure to these inhibitors has been shown to result in increased extracellular concentrations of striatal dopamine (DA), which is neurotoxic at high concentrations. The cause of this increase is unknown. The purpose of this study was to determine whether mitochondrial inhibition alters dopamine transporter (DAT) function. Striatal synaptosomes were incubated in the presence of several structurally unrelated inhibitors of mitochondrial Complexes I, II, and IV, and [^{3}H]DA uptake was measured. Although all of the toxins inhibited [^{3}H]DA uptake, there was a large variation in their inhibitory potencies, the rank order being rotenone $\gg$ cyanide $>$ azide $>$ 3-NP $\gg$ malonate. Examination of the kinetic parameters of [^{3}H]DA uptake revealed that inhibition was due to a reduction in maximum velocity (V_{max}), with no change in affinity (K_m). The addition of either ATP or of ADP plus P_i to synaptosomes treated with 3-NP, or of the reactive oxygen species spin trap α-phenyl-*N*-*tert*-butyl nitrone to synaptosomes exposed to either malonate or cyanide failed to prevent mitochondrial toxin-induced inhibition of DAT function. The lack of effect of high energy substrates or of a free radical scavenger suggests that the mechanism by which extracellular DA is increased by several mitochondrial toxins involves factors other than mitochondrial ATP production or oxidative stress. Taken together, the results suggest that one mechanism whereby mitochondrial toxins increase extracellular concentrations of DA is *via* interaction with the DAT at a site other than the substrate site, i.e. noncompetitive inhibition of the DAT.

Keywords: Malonate; 3-Nitropropionic acid; Rotenone; Dopamine transporter; Striatum; Huntington's disease

1. Introduction

The systemic administration of the mitochondrial toxin 3-NP to adult rats generates relatively selective striatal injury similar to that observed in Huntington's disease [1,2]. The mechanism of 3-NP-induced damage involves irreversible inhibition of the mitochondrial enzyme succinate dehydrogenase (Complex II) and subsequent depletion of cellular ATP. In the central nervous system, inadequate energy stores lead to a reduction in the neuronal membrane potential, which can indirectly activate the NMDA subtype of EAA receptors [3,4]. Overactivation of NMDA receptors results in excessive Ca^{2+} influx and the subsequent stimulation of numerous "cell death" pathways including an increase in phosphatase and lipase activity and the production of oxygen- and nitrogen-based free radicals [5].

Several recent reports indicate that the neurotransmitter DA contributes to striatal damage induced by impaired energy metabolism. For instance, removal of DA afferents significantly reduces the severity of striatal lesions caused by either systemic administration of 3-NP [6,7] or direct intraparenchymal injection of malonate, another Complex II inhibitor [6,7]. Addition of DA to cultured striatal neurons potentiated apoptotic cell death caused by methyl malonate [8]. Also, 3-NP toxicity was enhanced in animals administered amphetamine, an agent that stimulates DA release from neurons [9].

Although DA is known to be neurotoxic at high concentrations, the primary mechanism by which DA contributes to mitochondrial toxin-induced brain damage in

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Abbreviations: DA, dopamine; DAT, dopamine transporter; DOPAC, dihydroxyphenylacetic acid; EAA, excitatory amino acid; NDMA, *N*-methyl-D-aspartate; 3-NP, 3-nitropropionic acid; PBN, α-phenyl-*N*-*tert*-butyl nitrone; P_i, inorganic phosphate; ROS, reactive oxygen species.

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the intact animal has not been elucidated. Several recent reports, however, have demonstrated a 100- to 300-fold increase in extracellular DA concentrations in the striatum during intraparenchymal infusion of malonate [10,11], suggesting that altered dopamine function might be involved. In addition, increased extracellular concentrations of the DA metabolite DOPAC have been measured in the striatum of animals chronically treated with 3-NP [1], likely resulting from enhanced DA oxidation by monoamine oxidase.

One mechanism that could account for the observed increases in synaptic DA following mitochondrial poisoning is inhibition of the DAT, which is responsible for the removal of endogenous DA after release into the synapse. The DAT is localized predominantly on DA terminals and is in high concentration in the densely innervated striatum [12,13]. The present study tested the hypothesis that impaired mitochondrial function would result in diminished DA uptake *via* reduced activity of the DAT. Thus, we examined the effects of five structurally unrelated mitochondrial toxins on DAT function: rotenone which inhibits NADH dehydrogenase (Complex I), 3-NP and malonate which inhibit succinate dehydrogenase (Complex II), and cyanide and azide which inhibit cytochrome oxidase (Complex IV) [14].

2. Materials and methods

2.1. Animals

The striata from a single rat (male Sprague–Dawley, 250–300 g; Harlan Laboratories) were dissected on ice, pooled, and used for each experiment. All animal use procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2. Materials

[^{3}H]DA (3,4-ethyl-2[*N*-^{3}H]dihydroxyphenylalanine; specific activity 27.5 Ci/mmol) was obtained from New England Nuclear, and nomifensine from RBI. DA, pargyline, ATP, and ADP were purchased from the Sigma Chemical Co. All other chemicals, including P_i, were reagent grade and obtained from Fisher Scientific.

2.3. Synaptosomal preparation

[^{3}H]DA uptake was determined using a previously described method [15] with minor modifications. Striata were homogenized in 20 mL of ice-cold 0.32 M sucrose containing 5 mM $NaHCO_3$ (pH 7.4) with 16 up-and-down strokes using a Teflon pestle homogenizer (clearance approximately 0.003 inch). The resulting crude synaptosomal preparation was centrifuged at 2000 *g* for 10 min at 4°, and the supernatant was centrifuged at 20,000 *g* for 15 min at 4°. The pellet was resuspended in 2.4 mL of assay buffer containing 125 mM NaCl, 5 mM KCl, 1.5 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.5 mM KH_2PO_4, 10 mM D-glucose, 25 mM HEPES, 0.1 mM EDTA, 0.1 mM pargyline, and 0.1 mM ascorbic acid and saturated with 95% O_2/5% CO_2, pH 7.4. Protein concentrations were determined using the Bradford protein assay (Pierce).

2.4. [^{3}H]DA uptake assay

The effects of mitochondrial inhibitors of Complex I (rotenone, 0.1 nM to 10 μM), Complex II (3-NP, 10 μM to 10 mM; and malonate, 0.1 to 300 mM), and Complex IV (azide, 0.1 to 10 mM; and cyanide, 1 μM to 10 mM) on [^{3}H]DA uptake were determined. Since high concentrations of 3-NP, malonate, azide, and cyanide were employed to generate the complete concentration–response relationship, the potential effect of a similarly high osmolality on [^{3}H]DA uptake was assessed by determining the effect of inulin (100 μM to 125 mM) included in the assay buffer. Each of the mitochondrial toxins was dissolved in assay buffer, except for rotenone. In the experiments determining inhibitory affinity (K_i), rotenone was dissolved initially in 100% DMSO, and this solution was then serially diluted with assay buffer, such that the highest concentration of rotenone tested included 1% DMSO. In each experiment, a contemporaneous control condition was included in which specific [^{3}H]DA uptake was determine with 1% DMSO in the assay buffer.

Assays were performed in triplicate in a final volume of 500 μL. Aliquots of striatal synaptosomes containing 20 μg protein (40–60 μL) were added to test tubes containing 50 μL of various concentrations of mitochondrial toxin and sufficient assay buffer to yield a volume of 450 μL. The tubes were then placed in an oxygenated metabolic shaker at 34° for 5 min. Synaptosomes were incubated for 10 min with [^{3}H]DA (0.1 μM final concentration, isotopically diluted with non-radioactive DA) in a final volume of 500 μL. Tubes were incubated for 10 min at 34° in the metabolic shaker. The reaction was stopped by the addition of 3 mL of ice-cold assay buffer. Samples were filtered rapidly through Grade 32 glass fiber filters (Schleicher & Schuell) using a Brandel cell harvester (model MP-43RS, Biochemical Research and Development Laboratories, Inc.). Filters were then washed three times with 3 mL of assay buffer containing 1 mM catechol. To minimize non-specific binding of [^{3}H]DA, filters were pre-soaked for 2 hr in cold assay buffer containing 1 mM catechol. Non-specific [^{3}H]DA uptake was determined in triplicate samples in the presence of 10 μM nomifensine. Filters were placed into scintillation vials containing 10 mL of scintillation mixture, and radioactivity was determined by scintillation spectrometry.

To determine whether a loss of high energy substrates was responsible for 3-NP-induced changes in [^{3}H]DA

uptake, a series of experiments determined the effect of 3-NP in the presence of either 1.0 mM ADP plus 1.0 mM P_i or 1.5 mM ATP. As a result of the concern that the addition of ATP to the outside of synaptosomes would not correct a toxin-induced deficiency in ATP (or ADP + P_i), nucleotides were included in the buffer during homogenization of the striatum.

To determine if the generation of ROS contributed to the inhibition of DAT function, another series of experiments examining the effects of malonate and cyanide were conducted in the presence of the spin trap agent PBN (10 mM), which sequesters ROS and forms stable adducts [16]. In this series of experiments, PBN was included in the buffer during the 5-min preincubation with mitochondrial toxin, as well as during the 10-min incubation with [^{3}H]DA.

To determine if the mitochondrial toxin-induced inhibition of [^{3}H]DA uptake was the result of an alteration in the K_m or V_{max} of transport, kinetic studies of [^{3}H]DA uptake were conducted in the absence and presence of mitochondrial inhibitors. To generate saturation isotherms for DA, samples were incubated with [^{3}H]DA (500,000 dpm/sample) isotopically diluted with non-radioactive DA (final DA concentration, 1.0 nM to 5000 μM). Parallel sets of synaptosomal samples were incubated with a range of [^{3}H]DA concentrations in the absence and presence of the IC_{50} of each of the mitochondrial inhibitors, determined from the above experiments. As in the inhibition experiments described above, each of the toxins was dissolved in assay buffer, except for rotenone. Since the IC_{50} of rotenone was utilized, the concentration of DMSO in the buffer in these experiments was 0.02%. To verify that DMSO alone produced no effect on DAT function, the kinetic parameters for [^{3}H]DA uptake were also determined in assay buffer containing 1% DMSO, in the absence of rotenone. Non-specific uptake was determined in triplicate samples in the presence of 10 μM nomifensine.

2.5. Data analysis

Inhibition assays provided the concentration of mitochondrial toxin inhibiting 50% of specific [^{3}H]DA uptake (IC_{50}), as determined by non-linear regression analysis and fitting the data to a one-site model by using the commercially available program GraphPAD-PRIZM (GraphPAD). K_i values were calculated from the IC_{50} values using the Cheng–Prusoff equation $[K_i = IC_{50}/(1 + L/K_m)]$ [17]. Data from saturation assays were used to determine the kinetic parameters, K_m and V_{max}, expressed as means+ SEM, which were determined by non-linear regression, fitting to both one- and two-site models. An *F*-test determined whether the one- or two-site model best fit the data. The simplest model, which significantly fit the data, was chosen for determination of K_m and V_{max} values. Significant differences for the kinetic parameters of [^{3}H]DA uptake between control and mitochondrial toxin-treated synaptosomes were analyzed using paired *t*-tests.

3. Results

Under control conditions (in the absence of mitochondrial toxin), the rate of [^{3}H]DA uptake into synaptosomes with a fixed concentration of substrate (0.1 μM) ranged from 30 to 40 pmol/min/mg protein. Non-specific [^{3}H]DA uptake comprised only 3–5% of total uptake (data not shown). The addition of either rotenone, cyanide, azide, 3-NP, or malonate to the incubation medium resulted in a concentration-dependent inhibition of [^{3}H]DA uptake (Fig. 1). Hill

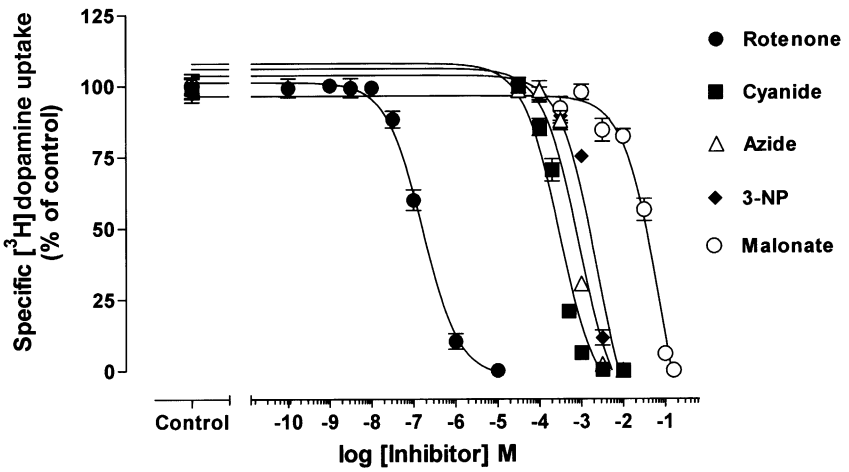

Fig. 1. Concentration–response for mitochondrial toxin-induced inhibition of specific [^{3}H]DA uptake into rat striatal synaptosomes. Synaptosomes were prepared as described in Section 2. Synaptosomes were preincubated at 34° for 5 min with various concentrations of mitochondrial toxins and then for 10 min with [^{3}H]DA (final concentration 0.1 μM). Values (means ± SEM) represent specific [^{3}H]DA uptake as a percentage of control (specific uptake in the absence of mitochondrial toxin) from 4 to 6 independent experiments performed in triplicate. Specific [^{3}H]DA uptake under control conditions was 31.2 ± 3.5 pmol/min/mg protein. In the experiments determining the effect of rotenone, the assay buffer contained 1% DMSO. As such, specific [^{3}H]DA uptake was determined in the presence of 1% DMSO (in the absence of rotenone) as a contemporaneous control (30.6 ± 4.8 pmol/min/mg protein). Non-specific [^{3}H]DA uptake determined in the presence of 10 μM nomifensine was 3–5% of total [^{3}H]DA uptake. K_i values for each compound are presented in Table 1.

coefficients for the inhibition curves ranged from −1.13 to −2.69 (Table 1). The rank order of inhibitory potency of these mitochondrial toxins was rotenone ⩾ cyanide > azide > 3-NP ⩾ malonate (Fig. 1 and Table 1).

Since DMSO was used to initially dissolve rotenone, an additional concern was that DMSO itself may have altered [^{3}H]DA uptake and, thereby, contributed to the higher potency for the rotenone-induced inhibition. However, this was not the case. The highest concentration (1%) of DMSO included in the assay buffer did not alter [^{3}H]DA uptake in the absence of rotenone (Fig. 1, legend). To further determine if DMSO potentiated the inhibitory effect of rotenone, experiments were conducted assessing the effect of

Table 1
Mitochondrial toxin-induced inhibition of [^{3}H]DA uptake into rat striatal synaptosomes

Inhibitor	K_i (μM)	Hill coefficient
Rotenone[a]	0.051 ± 0.012[b]	−2.04
Cyanide	85.9 ± 15.2	−2.16
Azide	216 ± 19.2	−2.46
3-NP	465 ± 25.0	−2.69
Malonate	15,500 ± 3700	−1.13

[a] DMSO was included in the assay buffer of experiments determining the effect of rotenone (see Section 2 and the legend of Fig. 1).

[b] Values are means ± SEM of 4–6 independent experiments performed in triplicate, and were derived from the inhibition curves shown in Fig. 1.

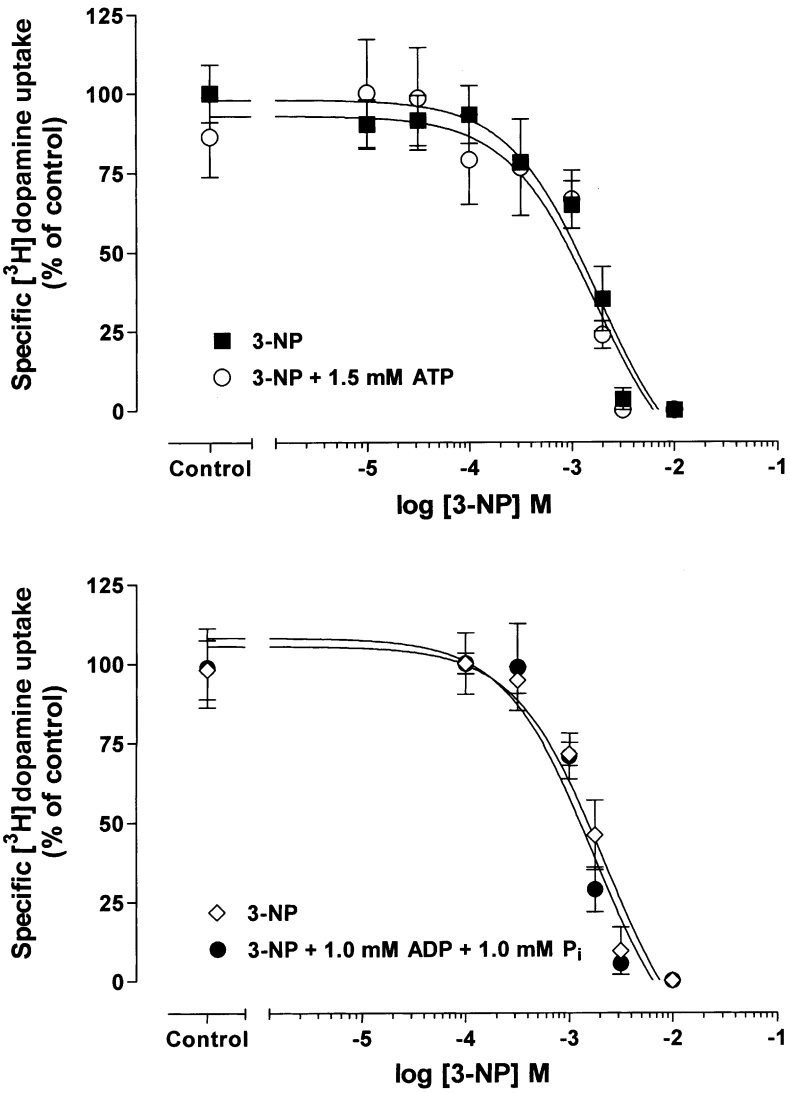

Fig. 2. Effect of high energy phosphates on 3-NP-induced inhibition of specific [^{3}H]DA uptake into rat striatal synaptosomes. This series of experiments was conduced in a fashion identical to that shown in Fig. 1, except that a parallel set of samples was exposed to either 1.5 mM ATP (top panel) or 1.0 mM ADP + 1.0 mM P_i (bottom panel) during the preparation of the synaptosomes. Values (means ± SEM) represent specific [^{3}H]DA uptake as a percentage of the control for 4–6 independent experiments performed in triplicate. In the absence of 3-NP, [^{3}H]DA uptake in control and ATP-treated synaptosomes was not different (32.1 ± 2.0 and 32.4 ± 4.7 pmol/min/mg protein, respectively, top panel). Also, in the absence of 3-NP, [^{3}H]DA uptake in control and ADP + P_i treated synaptosomes was not different (36.2 ± 3.4 and 36.5 ± 4.6 pmol/min/mg protein, respectively, bottom panel). Non-specific [^{3}H]DA uptake determined in the presence of 10 μM nomifensine was 3–5% of the total uptake.

cyanide in the absence and presence of DMSO. DMSO did not alter the cyanide concentration–response curve for inhibition of [^{3}H]DA uptake (cyanide, $K_i = 79 \pm 7.0$ μM; cyanide + DMSO, $K_i = 97 \pm 6.0$ μM). These results show that DMSO did not potentiate the effect of a second mitochondrial toxin cyanide, and, moreover, allow for direct potency comparisons between rotenone and the other mitochondrial inhibitors.

Since high concentrations of several of the mitochondrial toxins were used to generate complete concentration–response curves for inhibition of [^{3}H]DA uptake, the effect of inulin (100 μM to 125 mM) was determined to assess the effect of high osmolality in the assay buffer. High concentrations of inulin produced no effect on [^{3}H]DA uptake into striatal synaptosomes (data not shown).

To gain insight into the possible mechanism(s) of mitochondrial toxin-induced inhibition of [^{3}H]DA uptake, several experimental approaches were taken. To determine whether the inhibition of [^{3}H]DA uptake was due to diminished energy production, the effect of inclusion of ATP or of ADP plus P_i during synaptosomal preparation was assessed. Neither the addition of 1.5 mM ATP nor the addition of 1.0 mM ADP plus 1.0 mM P_i during synaptosomal preparation altered the 3-NP-induced inhibition

Table 2
Effects of mitochondrial toxins on the kinetic parameters of [^{3}H]DA uptake into rat striatal synaptosomes

Inhibitor	V_{max} (pmol/min/mg)	K_m (nM)
Control	39.7 ± 3.6	39 ± 2.4
Rotenone	11.8 ± 2.1*	33 ± 2.4
Control	55.4 ± 18.9	39 ± 15
Cyanide	11.7 ± 2.5*	44 ± 14
Control	41.5 ± 13.4	49 ± 14
Azide	13.2 ± 5.0*	30 ± 7
Control	52.0 ± 13.0	50 ± 20
3-NP	20.3 ± 4.1*	30 ± 3
Control	46.9 ± 3.3	55 ± 16
Malonate	14.2 ± 0.7**	36 ± 5

Control refers to uptake in the absence of mitochondrial toxins. The mitochondrial toxins were dissolved in assay buffer, except for rotenone which was dissolved in 1% DMSO. DMSO (1%) alone did not alter either the K_m or V_{max}, in the absence of rotenone (control, $K_m = 53 \pm 17$ nM, $V_{max} = 38.9 \pm 2.6$ pmol/min/mg; DMSO, $K_m = 44 \pm 16$ nM, $V_{max} = 34.2 \pm 2.5$ pmol/min/mg). Values are presented as means ± SEM of 4–6 independent experiments performed in triplicate.

* Significantly different compared with control (paired t-test): $^*P < 0.001$.

** Significantly different compared with control (paired t-test): $^{**}P < 0.05$.

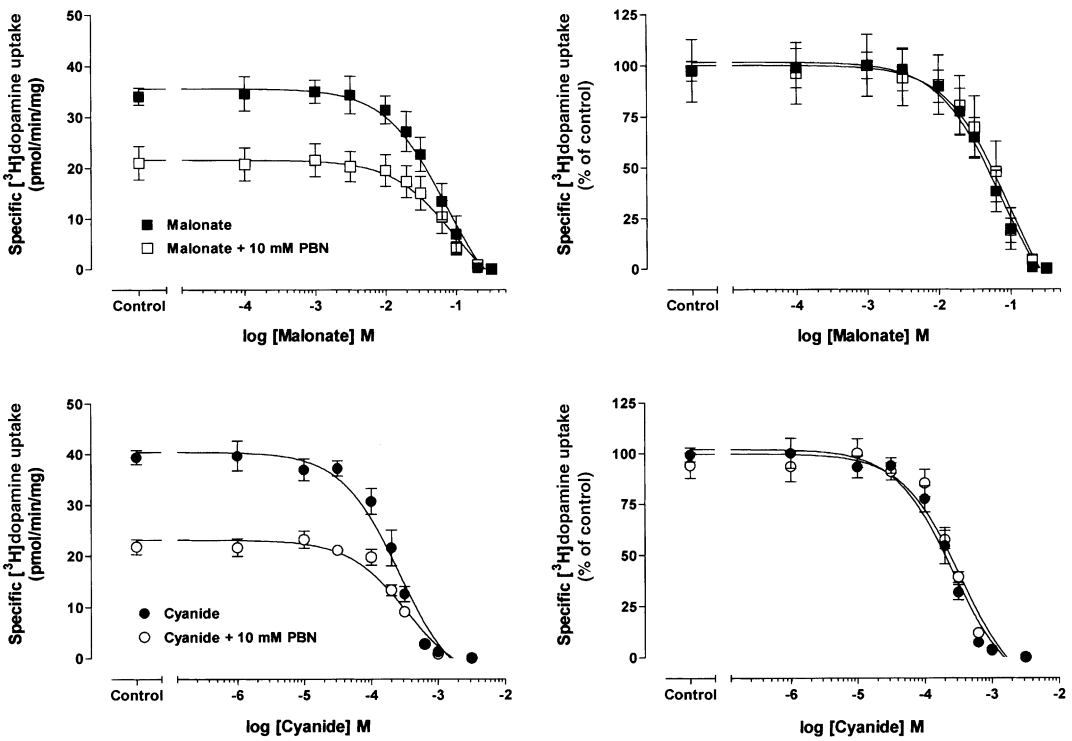

Fig. 3. Concentration-dependent effect of malonate (top panels) and cyanide (bottom panels) on the inhibition of specific [^{3}H]DA uptake into rat striatal synaptosomes in the absence and presence of 10 mM PBN. Values in the left panels (means ± SEM) represent specific [^{3}H]DA uptake as pmol/min/mg in 4–6 independent experiments performed in triplicate. Values in the right panels represent specific [^{3}H]DA uptake as a percentage of control (i.e. uptake in the presence of PBN, but in the absence of mitochondrial toxin). This series of experiments was conducted in a fashion identical to that shown in Fig. 1, except that a parallel set of samples was exposed to 10 mM PBN during incubation with toxin and subsequently with [^{3}H]DA. PBN did not alter the inhibitory effect of malonate or cyanide, under conditions in which PBN alone inhibited DAT by ~40%. Non-specific [^{3}H]DA uptake determined in the presence of 10 μM nomifensine comprised 3–5% of the total uptake.

of [^{3}H]DA uptake (3-NP, $K_i = 540 \pm 40$ μM; 3-NP + ATP, $K_i = 490 \pm 50$ μM; 3-NP + ADP + P_i, $K_i = 530 \pm 20$ μM; Fig. 2).

The ability of PBN (10 mM), a ROS scavenger, to prevent mitochondrial toxin-induced inhibition of [^{3}H]DA uptake was also assessed. At this concentration, PBN inhibited [^{3}H]DA uptake ~40% in the absence of mitochondrial toxin (see Fig. 3). Nevertheless, the concentration–response curves for both malonate- and cyanide-induced inhibition of [^{3}H]DA uptake into the striatal synaptosomes were not altered by PBN (malonate, $K_i = 18.7 \pm 1.6$ mM; malonate + PBN, $K_i = 22.6 \pm 0.26$ mM; cyanide, $K_i = 79 \pm 7.0$ μM; cyanide + PBN, $K_i = 89 \pm 6.0$ μM; Fig. 3).

Table 2 shows that exposure of the rat striatal synaptosomes to the IC_{50} concentration of each of the mitochondrial toxins resulted in a significant decrease in V_{max}, without altering the K_m for [^{3}H]DA uptake. The amount of inhibition was of similar magnitude (range 62–79%) for all of the toxins examined.

4. Discussion

Administration of the mitochondrial toxins malonate and 3-NP has been shown recently to increase extracellular DA concentrations in the striatum [10,11] and to enhance DA turnover [1]. Although there are several processes that could account for these observations, the present results indicate that inhibition of DAT may be one mechanism that contributes to the observed effect. In this study, five different structurally unrelated, mitochondrial toxins that inhibit Complexes I, II, and IV were evaluated. For each toxin tested, inhibition of synaptosomal DA uptake was found to be concentration-dependent. Although all of the toxins inhibited [^{3}H]DA uptake, there was a large variation in their inhibitory potencies, the rank order being rotenone $\gg$ cyanide $>$ azide $>$ 3-NP $\gg$ malonate. The negative Hill coefficients reflect the steep concentration–response relationships, indicating that once an inhibitory concentration of toxin is reached, only a small increment in concentration is required for complete inhibition of DAT function. Similarly, a steep concentration–response relationship between mitochondrial poisons and striatal damage has been observed (Maragos *et al.*, unpublished observations).

In the current study, each of the mitochondrial toxins decreased V_{max}, without a significant change in K_m, indicative of a noncompetitive mechanism of toxin-induced inhibition of DAT function. The noncompetitive nature of toxin-induced inhibition was anticipated, because even though these toxins are unrelated structurally, they all inhibit important energy-producing mitochondrial enzymes [14], which suggests a common mechanism underlying the observed attenuation of DAT function.

ATP derived from oxidative phosphorylation is generally accepted to provide the fuel for the maintenance of cellular ionic gradients that are essential for a variety of homeostatic processes including neurotransmitter uptake [18]. Diminished production of ATP and the consequent disruption of these gradients following mitochondrial poisoning would thus be expected to inhibit [^{3}H]DA uptake. Although ATP levels were not measured in the current study, the observation that the 3-NP-induced decrease in [^{3}H]DA uptake was not prevented by addition of either ADP + P_i or ATP argues against decreased energy production as the principal cause of inhibition of the DAT by 3-NP.

Another mechanism whereby mitochondrial toxins could decrease DAT function involves the generation of ROS. ROS can alter DAT function and inhibit DA uptake [19]. ROS could be generated *in situ* by the mitochondria *via* (i) inhibition of electron transport [20,21], (ii) activation of EEA receptors [22,23] or (iii) metabolism of DA [24,25]. Our findings that the spin trap agent PBN was unable to attenuate either malonate- or cyanide-induced inhibition of DAT function suggests that the production of ROS, at least by these two toxins, may not be the mechanism responsible for inhibition of the DAT. Future studies with other classes of antioxidants and mitochondrial toxins will be necessary to confirm and extend this interpretation. Due to the inhibitory effects of PBN itself on DAT function in the absence of mitochondrial toxins, the possibility cannot be ruled out that there exists a subpopulation of DA uptake sites, which are sensitive to the effects of ROS.

The current findings are also consistent with and extend the findings of an earlier study [26], demonstrating decreased DAT function induced by rotenone and a single concentration of either cyanide or the complex III inhibitor, antimycin A. In the latter study, the DAT was more sensitive to these mitochondrial toxins than either the γ-aminobutyric acid or norepinephrine uptake transporters. The authors speculated that the DAT might either require a higher ionic gradient and, hence, a greater energy demand, than the other neurotransmitter transporters or that a deficiency in energy metabolism exists in striatal dopaminergic nerve terminals, which only manifests itself under conditions of impaired energy production. However, the present negative results, showing that neither high energy phosphates nor the free radical spin trap attenuated the toxin-induced inhibition of the DAT, suggest that these toxins may be interacting at a site other than the substrate binding site, i.e. noncompetitive inhibition of the DAT.

Mitochondrial toxin-induced inhibition of DAT function would likely result in elevated synaptic DA concentrations that could, under certain circumstances, contribute to neuronal damage. For instance, administration of the illicit drug methamphetamine causes a large increase in extracellular DA concentrations [27,28] and degeneration of dopaminergic terminals in the striatum [29,30]. Interestingly, administration of methamphetamine has been shown recently to produce an acute reduction in synaptic DA uptake [31], possibly resulting from oxidative damage

[19,32]. Intraparenchymal injections of malonate and systemic administration of 3-NP also cause striatal damage that can be attenuated by removal of dopaminergic input to the striatum [6,7]. Like methamphetamine, infusion of malonate is also associated with increased extracellular DA concentrations [10,11]. The data presented herein support the hypothesis that these mitochondrial toxins, through inhibition of DAT function, lead to greatly increased levels of extracellular DA, which thereby contributes to mitochondrial toxin-induced cell death.

Acknowledgments

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