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Mitochondrial respiratory inhibition by N-methylated β-carboline derivatives structurally resembling *N*-methyl-4-phenylpyridine

(neurotoxins/Parkinson disease/mitochondrial respiration)

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ABSTRACT **Mitochondrial accumulation and respiratory inhibition are critical steps in the actions of *N*-methyl-4-phenylpyridinium ion (MPP^+), the toxic metabolite of the parkinsonism-inducing agent, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. We examined the respiratory characteristics of 2-methylated β-carbolines (2-MeβCs) and 2-methylated 3,4-dihydro-β-carbolines (2-MeDHβCs), which encompass the MPP^+ structure. As indoleamine derivatives, they could have endogenous roles in idiopathic parkinsonism. With rat liver mitochondria, the order for inhibition of NAD^+-linked O_2 consumption (6-min preincubations) was as follows: MPP^+ = 2-methylharmine > 2-methylharmol = 2-methylharmaline >> 2-methylharmalol > 2-methylnorharman > 6-OH-2-methylharmalan >> 2-methylharman. Similar to MPP^+, 2-MeDHβC/2-MeβC inhibition was potentiated by tetraphenylboron and reversed by dinitrophenol, consistent with the involvement of cationic forms. However, the participation of neutral forms was indicated by the 2-MeDHβC/2-MeβC inhibitory time courses, which were unlike MPP^+. The neutral forms probably arise via indolic nitrogen deprotonation because the characteristics of a cationic β-carboline that cannot N-deprotonate, 2,9-dimethylnorharman, mirrored MPP^+ rather than 2-MeβCs. Succinate-supported respiration was also significantly blocked by 2-MeDHβCs/2-MeβCs, but results with tetraphenylboron and 2,9-dimethylnorharman indicated that cationic forms were less important than in the inhibition of NAD^+-linked respiration. We suggest that the relatively potent inhibition by certain 2-MeDHβCs/2-MeβCs involves neutral forms for passive mitochondrial entry and cationic as well as neutral forms that act at several respiratory sites. Respiratory inhibition could reasonably underlie the reported neurotoxicity of 2-MeβCs.**

Interest in *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a street drug contaminant that selectively destroys nigrostriatal cells, has stimulated investigations of environmental or endogenous toxins that might be associated with idiopathic parkinsonism (1, 2). An oxidation product of MPTP, *N*-methyl-4-phenylpyridine (MPP^+), is believed to be the species that exerts neurotoxicity by inhibiting the mitochondrial respiratory chain at site I (NADH dehydrogenase) (3, 4). Intrigued by the structural overlap between MPP^+ and indole-derived β-carboline (βC) compounds that are methylated (quaternized) on the 2-nitrogen (2-MeβCs), we and others (5–9) have hypothesized possible neurotoxic roles for 2-MeβCs in Parkinson disease. Endogenous biosynthetic pathways can be envisioned for 2-MeβC formation from indoleamines or even tryptophan (10).

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Although βCs and their hydrogenated derivatives (3,4-dihyro-βCs; DHβCs) have been studied extensively (see *Discussion*), little is known about the toxic capabilities of their N-methylated analogs. Hoppel *et al.* (8) noted that the βC, 2-methylharmine, was an effective mitochondrial respiratory inhibitor, and we now present results showing that several 2-MeDHβC/2-MeβC isomers with 7-oxygenated substitution are comparable to MPP^+ as inhibitors of glutamate/malate-supported O_2 utilization with and without tetraphenylboron counterion (TPB^-). Additionally, succinate-supported respiration is extensively blocked by the 2-MeDHβCs/2-MeβCs, and TPB^- has a moderate potentiating effect. Both cationic and neutral, apparently deprotonated, forms of 2-MeDHβCs/2-MeβCs may be important in mitochondrial respiratory inhibition. When deprotonation of the indole nitrogen to produce neutral forms is precluded, as for 2,9-dimethylnorharman (2,9-Me_2Nh), the inhibitory characteristics of the dimethylated βC closely mimic MPP^+.

METHODS

Desmethylated βCs and DHβCs, MPP^+, TPB^-, and the anhydronium base of 2-methylharmine were obtained commercially. The 2-MeDHβCs and 2-MeβCs other than 2-methylharmine (all in Fig. 1) were synthesized as iodide salts from the desmethyl compounds as described (10). The iodide salt of 2,9-Me_2Nh was from L. Meyerson of Shaman Pharmaceuticals (San Carlos, CA) and also was synthesized in our laboratory by K. Matsubara by sequential reaction of norharman with methyl iodide. The structure of the recrystallized product, a single peak by HPLC, was confirmed by NMR and melting-point comparison (11).

Adult female rats (Harlan–Sprague–Dawley, 250–350 g) were sacrificed by stunning and decapitation. After isolation (12), liver mitochondria were suspended in the pH 7.4-buffered medium (13). O_2 consumption was determined polarographically with a Clark-type electrode (Yellow Springs Instruments) in a 4-ml incubation chamber at 25°C. Concentrations of mitochondrial protein in the chamber were ≈2 mg/ml, determined according to Lowry *et al.* (14). Mitochondria, buffered medium, and substrate (5 mM glutamate/2.5 mM malate, or 20 mM succinate) were preincubated with or without the βCs or pyridines, and ADP (250 μM) was added 15 sec to 12 min later, depending on the experiment. Tet-

Abbreviations: βC, β-carboline; DHβC, 3,4-dihydro-β-carboline; DNP, 2,4-dinitrophenol; 2,9-Me_2Nh, 2,9-dimethylnorharman; MPP^+, *N*-methyl-4-phenylpyridine; 2-MeβC, 2-methyl-β-carboline; 2-MeDHβC, 2-methyl-3,4-dihydro-β-carboline; MPTP, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; THβC, 1,2,3,4-tetrahydro-β-carboline; TPB^-, tetraphenylboron ion.

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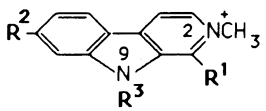
rahydrofuran/H_2O, 20:80 (vol/vol) was used to dissolve stock solutions of 6-OH-2-methylharmalan; the solvent mixture, diluted into the incubations, was shown not to alter the respiratory control ratio. When used, TPB^- (10 μM) was introduced 1 min before inhibitors. For uncoupling experiments, 2,4-dinitrophenol (DNP; 50 μM) was added 1 min after ADP (2 mM). Rotenone (200 nM) was included in succinate-supported respiration measurements. Typical respiratory control ratios were >8 for glutamate/malate as substrate and >5 for succinate as substrate.

RESULTS

IC_{50} Values. Seven 2-MeβCs and 2-MeDHβCs in Fig. 1 were compared with MPP^+ with respect to inhibition of NAD^+-linked (glutamate/malate substrate), ADP-initiated mitochondrial respiration. Small uncoupling effects (increases in state-2 slopes) were observed for 2-methylharmine and 2-methlnorharman at higher concentrations. In Table 1, the IC_{50} values after 6-min preincubation show that 2-methylharmine differed little from MPP^+. Two derivatives of 2-methylharmine, O-demethylated 2-methylharmol and the DHβC analog, 2-methylharmaline, were slightly less effective. The corresponding O-demethylated DHβC derivative, 2-methylharmalol, was a considerably weaker inhibitor than the aforementioned 7-oxygenated compounds or MPP^+. It was followed by the simplest 2-MeβC, 2-methylnorharman, and the only 6-oxygenated compound examined, 6-hydroxy-2-methylharmalan. As the least potent compound tested, 2-methylharman was ≈20–25 times weaker than its 7-methoxy-substituted analog, 2-methylharmine. Sodium iodide (400 μM) had no significant effect on respiration.

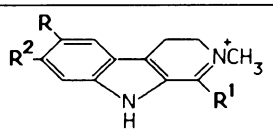
Inhibitory Time Courses and the Effect of TPB^-. The time courses for inhibition of NAD^+-linked respiration by MPP^+, two βCs, 2-methylharmine, and 2-methylharmol, and a DHβC, 2-methylharmaline, are compared in Fig. 2. In agreement with others (15), we observed concentration- and time-dependent inhibition by MPP^+. In Fig. 2 *Upper*, half-maximal inhibition by MPP^+ at 400 μM was ≈2 min, and 100% inhibition was reached by 7.5 min. At 100 μM (Fig. 2 *Lower*), MPP^+ did not display significant inhibition (10%) until 4 min. As already shown by Ramsay *et al.* (16), complete inhibition by 100 μM MPP^+ occurred within 15 sec when TPB^- was present (Fig. 2 *Lower*). TPB^- alone did not alter glutamate/malate-supported O_2 consumption.

Unlike the gradual inhibition with MPP^+, abrupt onset of inhibition occurred with 2-methylharmine, 2-methylharmaline, and 2-methylharmol (400 μM in Fig. 2 *Upper* and 100 μM



2Me-βC

Name	R^1	R^2	R^3
2Me-Norharman	H	H	H
2Me-Harman	CH_3	H	H
2Me-Harmine	CH_3	CH_3O	H
2Me-Harmol	CH_3	HO	H
2,9-diMe-Norharman	H	H	CH_3



2Me-DHβC

Name	R^1	R^2	R
2Me-Harmaline	CH_3	CH_3O	H
2Me-Harmalol	CH_3	HO	H
6HO-2Me-Harmalan	CH_3	H	HO

FIG. 1. 2-MeβCs and 2-MeDHβCs used in this study were iodide salts. 2Me, 2-methyl; 2,9-diMe-Norharman is 2,9-Me_2Nh.

Table 1. Inhibition of NAD^+-linked respiration in rat liver mitochondria by MPP^+, 2-MeβCs, and 2-MeDHβCs

Compound	IC_{50}, μM
MPP^+	171.0 ± 16.5
2-Methylharmine	185.7 ± 20.9
2-Methylharmol	208.7 ± 37.9
2-Methylharmaline	232.0 ± 4.4
2-Methylharmalol	840; 600
2-Methylnorharman	1190; 1100
6-Hydroxy-2-methylharmalan	1790
2-Methylharman	3450; 2250

Compounds were preincubated for 6 min with mitochondria and glutamate/malate substrate as described, and state-3 respiration was initiated with 250 μM ADP. Inhibition data from n = 2–4 separate runs (1 run for 6-hydroxy-2-methylharmalan because of limited sample) with 5–10 concentrations in duplicate were fitted to logarithmic curves by least-squares regression to obtain IC_{50} values (± SEM where shown).

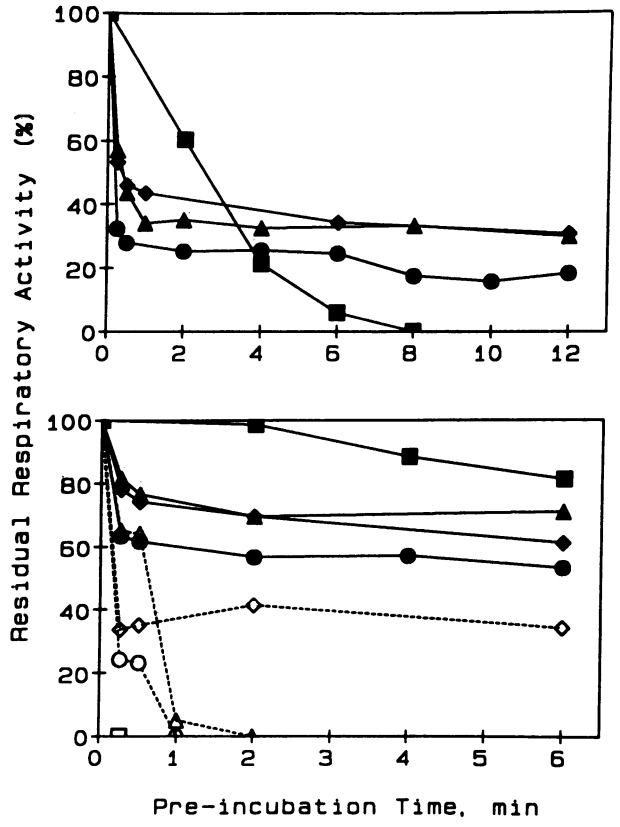


FIG. 2. Time courses of development of inhibition of glutamate/malate-supported, ADP-initiated respiration in rat liver mitochondria by MPP^+ (■), 2-methylharmine (●), 2-methylharmaline (▲), and 2-methylharmol (◆). Results are means of three to five separate experiments. (*Upper*) Percent residual respiratory activity in mitochondria preincubated for the times indicated with 400 μM each of the above compounds. (*Lower*) Percent residual respiratory activity in mitochondria preincubated for the times indicated with 100 μM each of the above compounds, in the absence (filled symbols) and presence (open symbols) of 10 μM TPB^-. □, Zero percent respiratory activity at 15 sec with 100 μM MPP^+/TPB^-.

in Fig. 2 *Lower*). After 30–60 sec, the initial steep inhibitory slopes were followed by distinct concentration-dependent plateaus. Complete inhibition was not reached for any 2-monomethylated compound within the time frame studied. (2-Methylharmalol displayed curves similar to but somewhat less inhibitory than those for the above three 2-methylated compounds; data not shown.) With 10 μM TPB^- (Fig. 2 *Lower*), essentially complete inhibition resulted for 100 μM 2-methylharmine within 1 min and for 100 μM 2-methylharmaline within 2 min. TPB^- had less effect on 2-methylharmol, altering its inhibitory potency at 2 min from ≈25% inhibition to a new plateau of 60–65% inhibition.

For comparison, we examined the inhibitory time courses for desmethylated nonquaternary βCs with and without TPB^- (data not shown). 4-Phenylpyridine, the desmethyl derivative of MPP^+, was also compared. At 100 μM each, harmine, harmol, harman, and 4-phenylpyridine displayed time courses very similar to each other and to their 2-methylated analogs in Fig. 2 *Lower*. The curves had initial rapid bursts reaching 25–35% inhibition within 30–60 sec, followed by a plateau. The inhibitory curve for harmol also showed reversibility, with the plateau at ≈30% inhibition gradually returning to no inhibition by 6 min. Inhibition by desmethyl βCs was not increased or accelerated by TPB^-; indeed, there was a trend toward diminished inhibition with the anion. As noted by others (17), TPB^- did not change the inhibition by 4-phenylpyridine.

Inhibitory Time Course for 2,9-Me_2Nh. The development of inhibition of NAD^+-linked respiration by 2,9-Me_2Nh, a cationic βC that is unable to N-deprotonate to a neutral form, was unlike the abrupt inhibitory onset-plateau curves of the 2-MeDHβCs/2-MeβCs or their desmethyl analogs. Instead, it displayed a gradual time course (Fig. 3) very similar to MPP^+ in Fig. 2. At 400 μM 2,9-Me_2Nh, 50% of maximal inhibition was achieved after ≈1-min preincubation, and 100% inhibition was reached at 4 min. At 100 μM, 50% of maximal inhibition occurred in 5 min, and 100% inhibition was achieved by 12 min. The IC_{50} for the 2,9-dimethyl compound after 6-min preincubation was estimated at 90–100 μM. As with MPP^+, coincubation of TPB^- caused complete respiratory inhibition by 100 μM 2,9-Me_2Nh within 15 sec (Fig. 3).

Uncoupling by DNP During Inhibition of Glutamate/Malate-Supported Respiration. Qualitative comparison of the effect of DNP (50 μM) on the inhibition of NAD^+-linked state-3 mitochondrial respiration by 2-methylharmol, MPP^+, and 2,9-Me_2Nh (400 μM each) is represented in the polarographic measurements in Fig. 4. The control tracing with no inhibitor

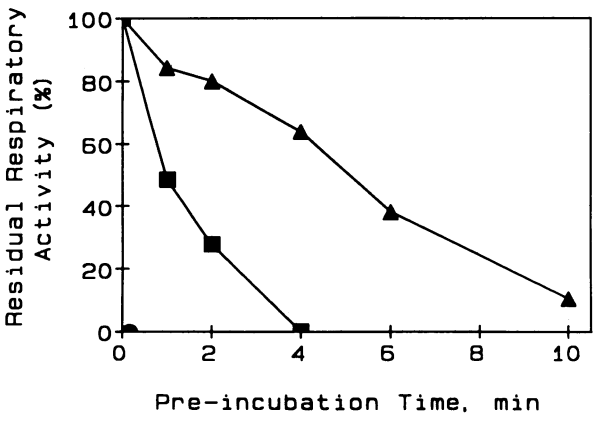


FIG. 3. Time courses of development of inhibition of glutamate/malate-supported O_2 respiration in rat liver mitochondria by 2,9-Me_2Nh at 400 μM (■), 100 μM (▲), and 100 μM with 10 μM TPB^- (●; 0 residual activity at 15 sec). Residual respiratory activities are means of three to four separate experiments.

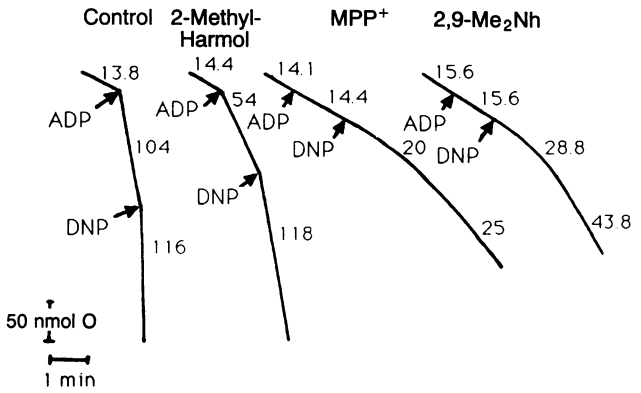


FIG. 4. Representative polarographic measurements of glutamate/malate-supported respiration in rat liver mitochondria at 25°C recorded with a Clark O_2 electrode. Preincubation of substrate and buffer (control tracing) with 400 μM 2-methylharmol, 400 μM MPP^+, or 400 μM 2,9-Me_2Nh was for 6 min before ADP (2 mM) addition. Numbers at right of four tracings are O_2 consumption in nmol of O/min per mg of protein.

shows the immediate onset of DNP uncoupling on ADP-induced state-3 slope. After 2-methylharmol preincubation, addition of DNP also caused a prompt and abrupt increase in O_2 consumption during inhibited state 3. Similar sharp onsets of uncoupling by DNP were observed after 2-methylharmine and 2-methylharmaline preincubations (data not shown). In contrast, DNP reversal of the inhibited state 3 in mitochondria preincubated with MPP^+ proceeded in a reproducibly slow and gradual manner as shown. After 2,9-Me_2Nh preincubation, the effect of DNP on ADP-initiated state 3 consistently resembled the slow uncoupling pattern with MPP^+ rather than the abrupt reversal seen during inhibition by 2-methylharmol, 2-methylharmine, and 2-methylharmaline.

Inhibition of Succinate-Dependent Mitochondrial Respiration by 2-MeDHβCs/2-MeβCs, and 2,9-Me_2Nh. In contrast to reports showing negligible inhibition by MPP^+ (3, 4), 2-methylharmine, 2-methylharmaline, and 2-methylharmol (400 μM each) inhibited succinate-dependent ADP-initiated mitochondrial O_2 consumption by 60–65% (Table 2). As with NAD^+-linked respiration, indications are that inhibition of succinate-linked respiration by 2-methylharmine (100 μM)

Table 2. Inhibition of succinate-linked respiration in rat liver mitochondria by methylated βCs and DHβCs

Compound	Preincubation time, min	State-3 O_2 utilization, nmol of O/min per mg of protein
Control (no inhibitor)	5	141.3 ± 10.5
2-Methylharmine (400 μM)	5	54.5 ± 1.2*
2-Methylharmol (400 μM)	5	57.4 ± 1.1*
2-Methylharmaline (400 μM)	5	54.8 ± 5.5*
2,9-Me_2Nh (400 μM)	5	80.1 ± 4.7*
2,9-Me_2Nh (100 μM)	5	112.8 ± 3.2†
2-Methylharmine (100 μM)	5	81.8; 76.2
2-Methylharmine (100 μM) + TPB^-	5	59.2; 55.8
2-Methylharmine (100 μM)	0.5	90.0; 88.5
2-Methylharmine (100 μM) + TPB^-	0.5	69.5; 68.5

Values are means ± SEM. Compounds were preincubated with mitochondria containing 20 mM succinate and 200 nM rotenone as described, and state-3 respiration was initiated with ADP (250 μM) after 5 or 0.5 min. TPB^- = 10 μM. n = 4 except where duplicate values are shown.

*$P < 0.001$ compared to control.

†$P < 0.01$ compared to control.

has a rapid onset and an inhibitory plateau because it did not appreciably differ at 0.5 min and 5 min (37% and 44% inhibition, respectively). Furthermore, in replicate analyses, TPB^- potentiated the inhibition of succinate-supported respiration seen with 100 μM 2-methylharmine by one-third at either preincubation time. Inhibition by 2,9-Me_2Nh was clearly less than that obtained with the 2-monomethyl compounds. At 400 μM and 5-min preincubation times, inhibition by 2,9-Me_2Nh of succinate-supported respiration was equivalent to that obtained with 100 μM 2-methylharmine.

DISCUSSION

The intraneuronal toxic action of MPTP-derived MPP^+ is generally considered to be energized sequestration by mitochondria, inhibition of oxidation of substrates for mitochondrial NADH dehydrogenases at site I, ATP depletion, and cessation of cellular energy-dependent processes (1). Because 2-MeβCs are essentially nitrogen-bridged MPP^+ structures (5, 10), they might degenerate nigrostriatal and other neurons by a similar mechanism. Our results confirm that selected 2-MeβCs and 2-MeDHβCs compare favorably with MPP^+ as inhibitors of mitochondrial respiration. Thus, respiratory inhibition may underlie the neurotoxicity of 2-MeβCs and 2-MeDHβCs observed in PC-12 cell cultures (18) and *in vivo* (9, 19).

The significance of these studies depends on the supposition that methylated βCs and DHβCs exist in mammalian tissues. Several 1,2,3,4-tetrahydro-βCs (THβCs) have been detected *in vivo*, apparently forming from cellular (Pictet-Spengler) condensations of indoleamines or tryptophan with aldehydes or α-keto acids (20, 21). Dietary sources for THβCs are possible as well. There is convincing GC/MS and HPLC evidence for the desmethylated βCs, harman and norharman, in rat tissues (22, 23). Such βCs could arise from environmental sources (24), although it is equally plausible that they are metabolic dehydrogenation products of endogenous THβCs (25). Endogenous βCs oxygenated on the 7 position have not yet been reported, but their occurrence would not be improbable because 7-hydroxylation of administered THβCs has been reported in rodents (26, 27). THβCs and βCs have not yet been investigated as substrates for N-methylation. However, 2-methylated THβC has been measured *in vivo* (28), and related heterocyclics are substrates for *N*-methyl transferases (29, 30).

The IC_{50} for the inhibition of NAD^+-linked respiration by 2-methylharmine in rat liver mitochondria (Table 1) agrees with that obtained by Hoppel *et al.* (8) in a study of substituted MPP^+ derivatives. Because 2-methylharmine inhibition of NADH oxidation in inner mitochondrial membrane was also demonstrated (8, 31), the respiratory effects of 2-MeDHβCs/2-MeβCs must be due, in part, to site I inhibition. However, that mitochondrial benzodiazepine receptors may mediate inhibition of respiratory control is of interest (32), and simple βCs are relatively potent benzodiazepine receptor ligands (33). Whether respiratory inhibition by 2-methylated analogs is linked to mitochondrial benzodiazepine receptors remains open to inquiry.

The inhibitory time courses with MPP^+ reflect energy-dependent mitochondrial concentration of the cation in response to the transmembrane potential (16, 34–36). In contrast, the time courses for 2-methylharmine, 2-methylharmaline, and 2-methylharmol are inconsistent with a purely cationic mechanism. Instead, 2-MeDHβC/2-MeβC inhibition is best rationalized on the basis of neutral (lipophilic) forms that diffuse passively into mitochondria, similarly to their neutral desmethyl analogs. The most probable route to neutral 2-MeβCs from the quaternary methylated compounds in aqueous media is shown in Fig. 5. Cationic 2-MeβCs (or 2-MeDHβCs) (I) are in equilibrium with the neutral anhydronium base forms (II) via indole deprotonation–reprotonation. For 2-methylharmol (R = OH), the 7-hydroxyl could deprotonate to provide an additional neutral (quinoidal) form. Additionally, nucleophilic water addition at the 1 position of the cationic 2-MeβCs and 2-MeDHβCs followed by proton loss could yield uncharged carbinolamines.

With pKa values >8.5, 2-MeDHβCs/2-MeβCs would not be extensively N-deprotonated in the buffer used. However, spectrophotometric studies with 2-methylnorharman, 2-methylharman, and related quaternary alkaloids indicate some formation of the respective anhydronium base at neutral pH (37, 38). The deprotonated forms would rapidly partition across the mitochondrial membrane, a step limited only by the rate of proton transfer, presumably at the mitochondrial-membrane surface. Reprotonation would then be expected within the mitochondria; consequently, the observed inhibitory plateaus might represent the point at which reprotonated (cationic) and neutral forms equilibrate inside and outside the mitochondria. Indeed, the most compelling evidence that the mitochondrial inhibitory behavior of the 2-monomethylated compounds depends upon indole nitrogen deprotonation is provided by 2,9-Me_2Nh, a cationic βC that cannot form a neutral anhydronium base by this route. As is apparent, the characteristics of inhibition of NAD^+-linked respiration by 2,9-Me_2Nh clearly differ from 2-monomethylated βCs but are closely akin to MPP^+.

While the evidence strongly indicates participation of neutral forms, the intermediacy of cationic quaternary forms is nevertheless required to explain the strong augmenting effect of TPB^- on the inhibition by 2-MeβCs of NAD^+-linked respiration. TPB^- fails to facilitate mitochondrial entry of neutral inhibitors like the desmethylated βCs or 4-phenylpyridine, but it would promote entry of the predominant cationic 2-MeDHβC/2-MeβC forms and, equally important, could facilitate cationic 2-MeβC action within the mitochondrial membrane by favoring permeation into the hydrophobic sites of respiratory inhibition (site I and others). Studies indicate that TPB^- enhances inhibition by MPP^+ of NAD^+-linked respiration in this manner (16, 35, 36, 39). Parenthetically, direct inhibition by neutral 2-MeβC forms cannot be ruled out because their mitochondrial concentrations may be increased by TPB^- through mass action. The prominent upcoupling of 2-MeDHβC/2-MeβC inhibition by DNP reinforces the interpretation of key roles within mitochondria for cationic 2-methylated forms because DNP characteristically fails to reverse the inhibition by neutral inhibitors (4). However, the abrupt onset of DNP uncoupling with the 2-MeDHβC/2-MeβC inhibition distinguishes a different cation-based mechanism from the slow uncoupling seen during inhibition by MPP^+ or 2,9-Me_2Nh.

A further dissimilarity between oxygenated 2-MeDHβCs/2-MeβCs and MPP^+ is the prominent inhibitory action of the former on site II (and/or site III) respiration, in contrast to the reportedly negligible effect of the pyridinium ion. Our

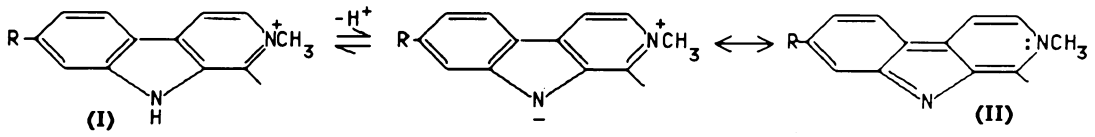


FIG. 5. Scheme showing indole N-deprotonation of a 2-MeβC cationic form (I) to the neutral anhydronium base form (II).

data indicate that 2-MeDHβCs/2-MeβCs inhibit succinate-supported and NAD^+-linked respiration to equivalent extents. Of interest is that TPB^- appears less effective in augmenting 2-methylharmine inhibition of succinate-dependent respiration than of NAD^+-linked respiration (Table 2 vs. Fig. 2). This and the fact that the cationic 2,9-Me_2Nh is less inhibitory than the 2-MeβCs indicate that neutral 2-MeDHβCs/2-MeβC forms may be more important inhibitors at sites II/III than cationic forms.

It is noteworthy that replacing the indole proton with a methyl group effectively converts 2-methylnorharman from a weak inhibitor of NAD^+-linked respiration to one that apparently surpasses MPP^+. Other results suggest that 2,9-Me_2Nh is nearly as toxic as MPP^+ in PC-12 cell cultures (40). The dopaminergic toxicity of 2,9-Me_2Nh *in vivo* would not approach that of MPP^+, however, because the βC is not an avid substrate for the dopamine transport system (IC_{50} for inhibition of synaptosomal uptake of [^{3}H]dopamine by 2,9-Me_2Nh is ≈60 μM; G.D., unpublished work). Also, because methylation of the indole nitrogen is doubtful in animal cells, the findings with 2,9-Me_2Nh do not bear directly on the question of endogenous factors. Nevertheless, they do point to possibilities for synthetic 2,9-disubstituted βC analogs that may have appreciable neurotoxicity.

Summarizing, under certain conditions, 7-oxygenated 2-MeDHβCs/2-MeβCs are comparable with MPP^+ as inhibitors of NAD^+-linked mitochondrial respiration, and the effects of TPB^- and DNP on the respective inhibitions are also qualitatively similar. However, results indicate that the detailed mechanisms for 2-MeDHβCs/2-MeβCs and MPP^+ differ markedly. We surmise that neutral anhydronium base forms of the 2-MeDHβCs/2-MeβCs are predominantly responsible for passive mitochondrial entry, and cationic forms participate prominently as inhibitors of site I respiration. Moreover, both the cationic and neutral forms of the 2-methylated compounds inhibit other mitochondrial sites, rather than mainly site I, like MPP^+. Continuous exposure to 2-MeDHβCs/2-MeβCs over decades could, perhaps, promote premature dopamine deficiency and the onset of parkinsonism by cumulatively compromising energy production in nigrostriatal mitochondria.

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