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Differential Effects of Typical and Atypical Neuroleptics on Mitochondrial Function *In Vitro*

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A series of typical (chlorpromazine, haloperidol and thioridazine) and atypical (risperidone, quetiapine, clozapine and olanzapine) antipsychotics were tested for effects on integrated bioenergetic functions of isolated rat liver mitochondria. Polarographic measurement of oxygen consumption in freshly isolated mitochondria showed that electron transfer activity at respiratory complex I is inhibited by chlorpromazine, haloperidol, risperidone, and quetiapine, but not by clozapine, olanzapine, or thioridazine. Chlorpromazine and thioridazine act as modest uncouplers of oxidative phosphorylation. The typical neuroleptics inhibited NADH-coenzyme Q reductase in freeze-thawed mitochondria, which is a direct measure of complex I enzyme activity. The inhibition of NADH-coenzyme Q reductase activity by the atypicals risperidone and quetiapine was 2-4 fold less than that for the typical neuroleptics. Clozapine and olanzapine had only slight effects on NADH-coenzyme Q reductase activity, even at 200 μM. The relative potencies of these neuroleptic drugs as inhibitors of mitochondrial bioenergetic function is similar to their relative potencies as risk factors in the reported incidence of extrapyramidal symptoms, including tardive dyskinesia (TD). This suggests that compromised bioenergetic function may be involved in the cellular pathology underlying TD.

Key words: Neuroleptics, Mitochondrial bioenergetic function, Extrapyramidal symptoms, Tardive dyskinesia, Electron transport, Respiratory enzyme complex I, Oxidative phosphorylation

INTRODUCTION

Tardive dyskinesia (TD), which is characterized by abnormal, involuntary, repetitive movements of the face, trunk and limbs, is an undesirable and often limiting side-effect of long-term treatment with neuroleptic drugs (Tarsy and Baldessarini, 1984). TD is one of the most important and problematic extrapyramidal movement disorders produced by the chronic administration of classical or typical neuroleptic drugs due to its high prevalence and frequently irreversible course (Tarsy and Baldessarini, 1984). The widespread clinical use of typical neuroleptics continues, however, and significant numbers of patients remain at risk for TD. Additionally, despite the promise of the new atypical antipsychotics with their low, if not negligible incidence of TD to date, it has yet to be determined whether long-term treatment with these drugs might also be associated with untoward extrapyramidal symptoms.

Although currently there is no clear understanding of the pathogenesis of TD, the hypothesis that mitochondrial dysfunction contributes to neuroleptic-induced impairment of striatal energy metabolism as a causative factor is gaining support based on a substantial body of evidence. Clinically, neuroleptic-induced impairment of brain energy metabolism has been demonstrated in human patients symptomatic of TD (Goff *et al.*, 1995). *In vivo*, neuroleptic treatment sufficient to produce TD causes regional changes in glucose utilization in cebus monkey brain (Mitchell *et al.*, 1992), and alterations in mitochondrial morphology in the rat striatum (Roberts *et al.*, 1995). Moreover, vacuous chewing movements, the rodent equivalent of TD, are induced in rats by long term treatment with the typical neuroleptic haloperidol (Andreassen and Jorgensen, 2000), as well as with the mitochondrial toxin, 3-nitropropionic acid (Andreassen and Jorgensen, 1995). *In vitro*, certain classical neuroleptics such as chlorpromazine were shown

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to disrupt mitochondrial membrane integrity as early as 1955 (Grenell *et al.*, 1955; Guth *et al.*, 1964; Gallagher *et al.*, 1965).

More recently, the likely mechanism(s) by which antipsychotic drugs might perturb mitochondrial energy metabolism has been examined. One possibility is that neuroleptics bind selectively to components of the mitochondrial electron transport chain, thereby inhibiting overall respiratory activity. Several studies have demonstrated inhibitory effects of certain neuroleptics on mitochondrial respiratory enzyme complex I. For example, the typical neuroleptics haloperidol, chlorpromazine, and thiothixine, and to a lesser extent the atypical neuroleptic clozapine, were shown to have a direct inhibitory effect on complex I enzyme activity in freeze-thawed preparations of rat brain mitochondria (Burkhardt *et al.*, 1993). Subsequently, it was demonstrated that the extent of inhibition of complex I enzyme activity by certain typical and atypical neuroleptics in homogenates of previously frozen normal human brain samples positively correlates with the incidence of extrapyramidal side effects caused by these agents (Maurer and Moller, 1998). These results were confirmed and extended in a study involving drug-treated sagittal slices of mouse brain (Balijepalli *et al.*, 1999). Furthermore, the classical neuroleptics haloperidol and fluphenazine have been shown to cause a generalized reduction in complex I activity in rat brain in vivo; this effect was not observed for the atypical neuroleptic clozapine (Prince *et al.*, 1997). Taken together, these results suggest that mitochondria are likely targets for neuroleptic-induced cellular damage, and that the effects of neuroleptics on mitochondrial function may underlie the development of TD.

To date, most studies investigating neuroleptic-induced impairment of mitochondrial function have only measured the effects of antipsychotic drugs on individual respiratory enzymes in membrane fractions of brain mitochondria. Our approach is quite different and more comprehensive. First, a series of typical and atypical antipsychotic drugs are screened for effects on the integrated bioenergetic functions of isolated, intact rat liver mitochondria using an assay that measures oxygen consumption polarographically. If an effect is seen then individual respiratory enzyme assays are performed in order to pinpoint more precisely the site-specific effects suggested by polarographic data. The power of this approach is to more definitively elucidate the mechanism(s) of drug-induced impairment of holistic mitochondrial bioenergetic function. The results of the study presented in this paper support a relationship between the effects of typical and atypical neuroleptics on mitochondrial function *in vitro* and the known incidence and patterns of TD induced by these drugs, and thereby suggest that the polarographic assay is a useful predictive indicator of adverse extrapyramidal symptoms. It is expected that results from experiments such as those described herein will help advance our understanding of the cellular mechanisms involved in the pathogenesis of TD. Furthermore, this approach of screening drugs for specific effects on cellular bioenergetic function in rat liver mitochondria (which can be prepared easily and in large quantities) should also prove useful in drug development as a means for evaluating the potential for new drugs to induce adverse extrapyramidal side-effects.

MATERIALS AND METHODS

Materials

The typical antipsychotics chlorpromazine, haloperidol, and thioridazine, and the atypical antipsychotics clozapine, quetiapine, risperidone, and olanzapine, were obtained from commercial sources or from manufacturers under a materials transfer agreement. All drugs were prepared at a stock concentration of 25 mM and stored at 4°C for no longer than 6 weeks. Haloperidol, risperidone, clozapine, and quetiapine were dissolved in 100% methanol; chlorpromazine and thioridazine were dissolved in H_2O; olanzapine was dissolved in dimethylsulphoxide.

Isolation of mitochondria

Liver mitochondria were isolated from male CD-1 (Sprague Dawley rats by differential centrifugation as previously described (Modica-Napolitano *et al.*, 1990). Approximately 5 g of tissue were minced and homogenized in STE buffer (250 mM sucrose, 1 mM Tris-HCl, 1 mM EDTA, pH 7.4) and then centrifuged at 600×g for 10 min at 4°C. The supernatant was removed and centrifuged at 8000×g for 10 min at 4°C. The mitochondrial pellet was washed twice by resuspension in STE (250 mM sucrose, 1 mM Tris-HCl, pH 7.4) and then centrifugation at 8000×g. The final pellet was resuspended in ST buffer to a volume of about 25 mg protein/mL. Protein concentration was determined by the method of Lowry, using bovine serum albumin as the standard. The care and use of all animals in this study were in accordance with National Institutes of Health guidelines, and approved by the Institutional Animal Care and Use Committee.

Respiration

Oxygen consumption was measured polarographically with a Clark electrode in a 1 mL water-jacketed chamber maintained at 30°C (Modica-Napolitano *et al.*, 1990) The basic respiratory assay medium consisted of 225 mM sucrose, 10 mM KCl, 1 mM EDTA, 10 mM K_2HPO_4-KH_2PO_4, 5 mM $MgCl_2$ and 10 mM Tris-HCl, pH 7.4. An initial rate of oxygen consumption (state 2 rate) was recorded following the addition of substrate (i.e., 5 mM each glutamate plus malate, or 10 mM succinate) and about 0.6 mg (protein)

intact isolated mitochondria. Two min after addition of mitochondria, ADP (120 nmol) was added and a state 3 rate obtained. After recording a measurable state 4 rate (i.e. rate after ADP is phosphorylated), 80 uM 2, 4 dinitrophenol (DNP) was added to obtain an uncoupled respiratory rate.

Enzyme assays

Rotenone-sensitive NADH-coenzyme Q reductase activity (a measure of electron transport through respiratory complex I) was measured by monitoring the reduction of NADH at 340 nm in the presence of electron acceptor coenzyme Q_1. An aliquot (160 μg protein) of freeze-thawed mitochondria was added to a 2.0 mL assay mix consisting of 23 mM KPO_4 (pH 7.4), 5 mM $MgCl_2$, 2 mM KCN, 5 mg BSA, and 200 μM CoQ_1, at room temperature. 200 uM NADH was added to begin the reaction. Rates were measured in the presence and absence of 5.0 uM rotenone, and the rotenone-sensitive NADH-coenzyme Q reductase rate was calculated by subtracting the rate as measured in the presence of rotenone from the rate obtained in the absence of rotenone. Succinate-cytochrome c reductase activity (a measure of electron transport through respiratory complexes II and III) was determined spectrophotometrically by measuring the rate of increase in absorbance at 550 nm due to the reduction of cytochrome c in the presence of succinate. An aliquot (40 μg) of freeze-thawed mitochondria was added to a cuvette containing 2 mM KCN, 50 mM potassium phosphate (KH_2PO_4/K_2HPO_4) (pH 7.4), and 20 mM succinate at room temperature. The reaction was initiated by adding 1 mg oxidized cytochrome c to the cuvette, and a change in the absorbance was recorded over time. The reaction was terminated by the addition of 5 μg antimycin A; the rate following addition of antimycin A was subtracted as background. Cytochrome c oxidase activity (complex IV) was determined spectrophotometrically by measuring the decrease in absorbance at 550 nm due to the oxidation of cytochrome c. An aliquot (40 μg) of freeze-thawed, rat liver mitochondria were added to a cuvette containing 40 mM potassium phosphate (KH_2PO_4/K_2HPO_4) (pH 7.0) at room temperature. The reaction was initiated by adding 0.7 mg reduced cytochrome c and a linear rate was recorded.

RESULTS

Polarographic measurement of oxygen consumption

Measurements of oxygen consumption in intact, isolated rat liver mitochondria were made in the absence (vehicle alone) and presence of varying concentrations of typical and atypical neuroleptics. Six of the seven drugs were tested over a concentration range of 25-200 μM. Due to its more limited solubility, thioridazine was tested over a lower concentration range of 5-50 μM. As determined polarographically, oxygen consumption in isolated, intact mitochondria is a measure of the rate of repiration. In the presence of ADP the oxygen consumption rate measures oxidative phosphorylation; that is, the process of substrate oxidation (via electron transfer) coupled to ATP synthesis. The initial or state 2 (S2) rate of oxygen consumption, in the presence of oxidizeable substrate but absence of externally added ADP, is a measure of the basal rate of respiration. As shown in Fig. 1, only two of the seven drugs had any significant effect on the S2 respiratory rate. Chlorpromazine induced a dose-dependent stimulation of S2 respiration of approximately 4-5-fold that of control respiration at a drug concentration of 100 μM. At a higher concentration (200 μM), however, the S2 respiratory rate decreased to a value nearly that of the control because respiration is inhibited at the higher concentrations (see below and Figs. 2 and 3). This biphasic effect of chlorpromazine on S2 respiration was similar when either glutamate+malate (NADH-linked) or succinate ($FADH_2$-linked) was used as the respiratory substrate. Thioridazine also induced a dose-dependent stimulation of S2 respiration, to approximately 4-5-fold that of control respiration at a drug concentration of 50 μM, the highest concentration of drug tested. As was the case for chlorpromazine, this effect of thioridazine on S2 respiration was similar when either glutamate+malate or succinate was used as the respiratory substrate. The remaining five drugs-haloperidol, risperidone, clozapine, quetiapine, and olanzapine-had no significant effect on S2 respiratory rates with glutamate+malate as the respiratory substrate. Modest effects were observed for clozapine, olanzapine and quetiapine when succinate was used as the respiratory substrate, but only at concentrations at or above 100 μM.

The state 3 (S3) respiratory rate, induced by addition of ADP, is a measure of "coupled" respiration (i.e., the integrated functions of substrate oxidation via electron transfer linked to ATP synthesis). Fig. 2 shows that chlorpromazine induced a dose-dependent decrease in S3 respiratory rate, with approximately 90% inhibition achieved at the highest concentration of drug tested (200 μM). This effect of chlorpromazine on S3 respiration was similar when either glutamate+malate (NADH-linked respiration) or succinate ($FADH_2$-linked respiration) was used as the respiratory substrate. Three other drugs affected only NADH-linked S3 respiratory rates; the order of potency was risperidone > haloperidol > quetiapine. Clozapine produced a modest effect on S3 (coupled) respiration when either glutamate+malate or succinate was used as the respiratory substrate, reaching approximately 25% inhibition of the S3 respiratory rate at a drug concentration of 200 μM. Olanzapine and thioridazine had only

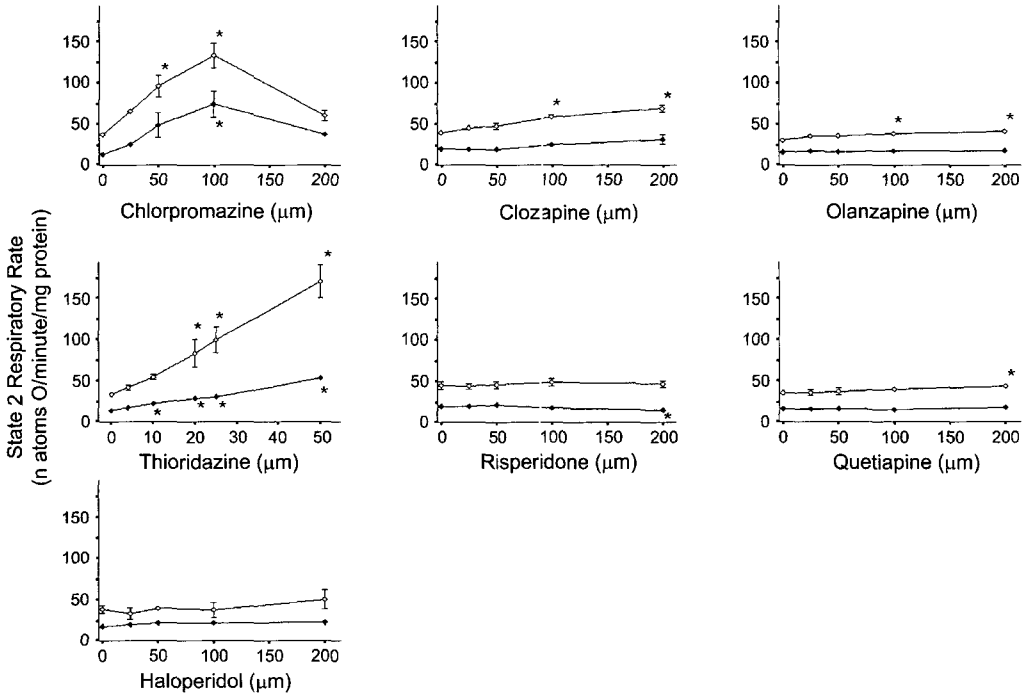

Fig. 1. Effect of typical neuroleptics (chlorpromazine, haloperidol and thioridazine) and atypical neuroleptics (risperidone, quetiapine, clozapine and olanzapine) on state 2 respiration in isolated rat liver mitochondria. The rate of O_2 consumption was determined polarographically in intact mitochondria after addition of either glutamate+malate (◆) or succinate (◇) as the respiratory substrate. Values are the mean±S.E. of three separate experiments. Asterisk (*) indicates $p<0.05$; i.e., data point is significantly different from control according to repeated measures analysis of variance.

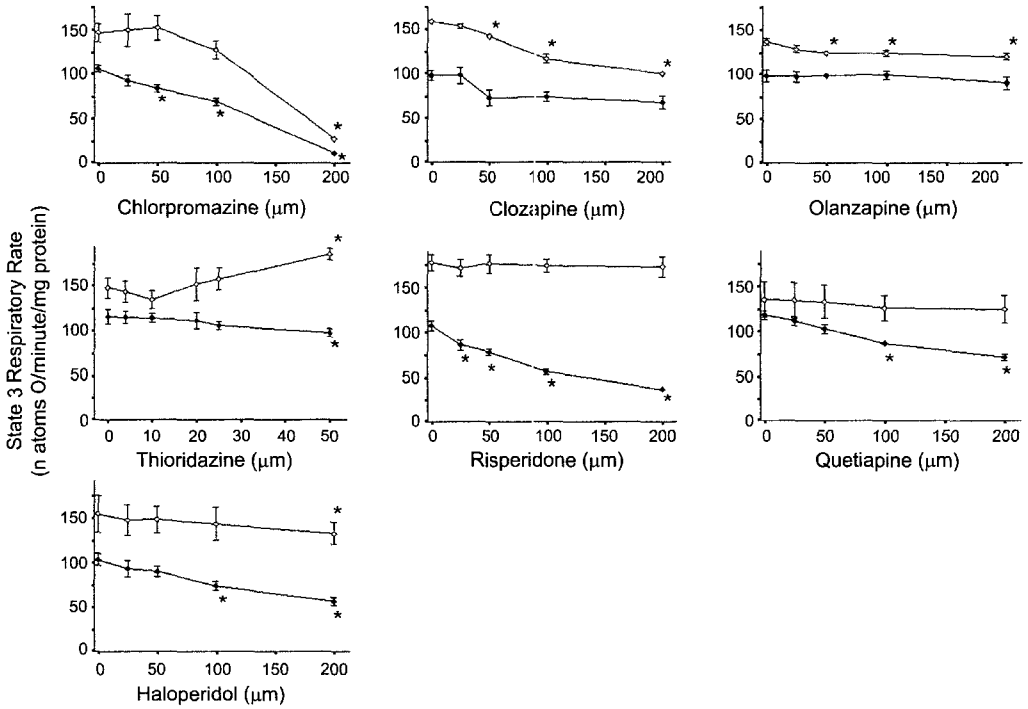

Fig. 2. Effect of neuroleptics on state 3 respiration in isolated rat liver mitochondria. The rate of O_2 consumption was determined polarographically in intact mitochondria after addition of either glutamate+malate (◆) or succinate (◇) as the respiratory substrate, followed by addition of 120 nmol ADP. Values are the mean±S.E. of three separate experiments. Asterisk (*) indicates $p<0.05$; i.e., data point is significantly different from control according to repeated measures analysis of variance.

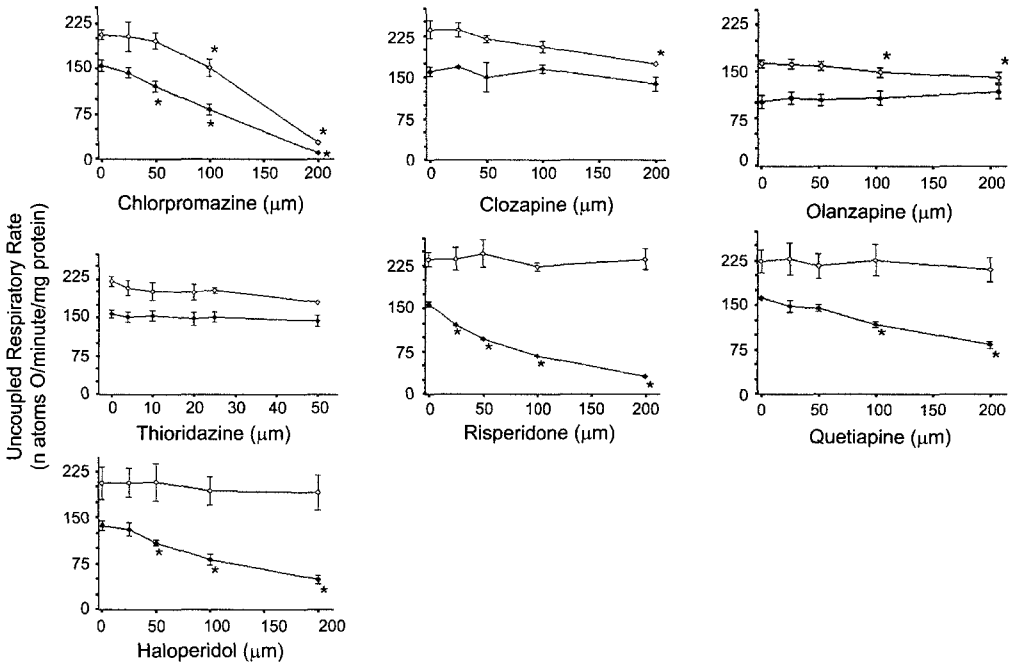

Fig. 3. Effect of neuroleptics on uncoupler-stimulated respiration in isolated rat liver mitochondria. The rate of O_2 consumption was determined polarographically in intact mitochondria in the presence of 80 μM 2,4-dinitrophenol, using either glutamate+malate (◆) or succinate (◇) as the respiratory substrate. Values are the mean±S.E. of three separate experiments. Asterisk (*) indicates $p<0.05$; i.e., data point is significantly different from control according to repeated measures analysis of variance.

slight effects on either glutamate+malate or succinate-linked S3 respiratory rates at the highest concentrations tested.

The uncoupler-stimulated respiratory rate, induced by addition of the proton ionophore 2,4-dinitrophenol (2,4 DNP), is essentially a measure of overall electron transfer activity that is no longer "coupled" to ATP synthesis. Fig. 3 shows that among all seven drugs tested, chlorpromazine had the most pronounced inhibitory effect on uncoupler-stimulated respiratory rate. This drug produced a dose-dependent decrease in uncoupler-stimulated respiration, with approximately 90% inhibition achieved at the highest concentration (200 μM). The effect was similar when either glutamate+malate or succinate was used as the respiratory substrate. Three drugs affected glutamate+malate-linked but not succinate-linked uncoupler-stimulated respiratory rates suggesting an effect on respiratory complex I only (not II, III, or IV). The order of potency for these was risperidone > haloperidol > quetiapine. Clozapine had a modest inhibitory effect on uncoupler-stimulated respiration at the highest drug concentration tested (200 μM) when succinate was used as the substrate, but this effect was not observed when glutamate+malate was used as the substrate. Thioridazine and olanzapine had only slight effects on uncoupler-stimulated respiration with either respiratory substrate.

Enzyme assays

The effect of both typical and atypical neuroleptics on electron transfer using frozen preparations of rat liver mitochondria was investigated next. Assays were chosen such that only specific, limited segments of the respiratory chain were engaged in electron transfer activity at any one time. For example, NADH-coenzyme Q reductase activity is a measure of the rate of electron transfer from the oxidizeable substrate NADH, through complex I, to coenzyme Q. Succinate-cytochrome c reductase activity is a measure of electron transfer from an oxidizable $FADH_2$-linked substrate (succinate), through complex II and III, to cytochrome c. Cytochrome c oxidase activity measures the rate of complex IV directly by monitoring its rate of oxidation of reduced cytochrome c.

The effect of antipsychotics on NADH-coenzyme Q reductase activity is shown in Fig. 4. Each of the typical neuroleptics, chlorpromazine, haloperidol and thioridazine, induced a dose-dependent inhibition of mitochondrial respiratory enzyme complex I activity, with an IC_{50} at a drug concentration of approximately 35 μM. A dose-dependent inhibition of complex I electron transfer was also observed for the atypical neuroleptics risperidone and quetiapine, however the 1/2 maximal inhibitory concentration of each of these drugs (65 μM and 125 μM, respectively) was approximately 2-4 fold greater than that for the typical neuroleptics. At 200 μM, the highest concentration of drug tested, the atypical antipsychotics clozapine and olanzapine had only a comparatively modest effect on mitochondrial respiratory enzyme complex I (30% and

12% inhibition, respectively).

According to the data presented in Fig. 5, chlorpromazine had the greatest effect on succinate-cytochrome c reductase activity, causing 60% inhibition of electron transfer activity at the highest concentration tested (200 μM). Both thioridazine and clozapine had a more modest

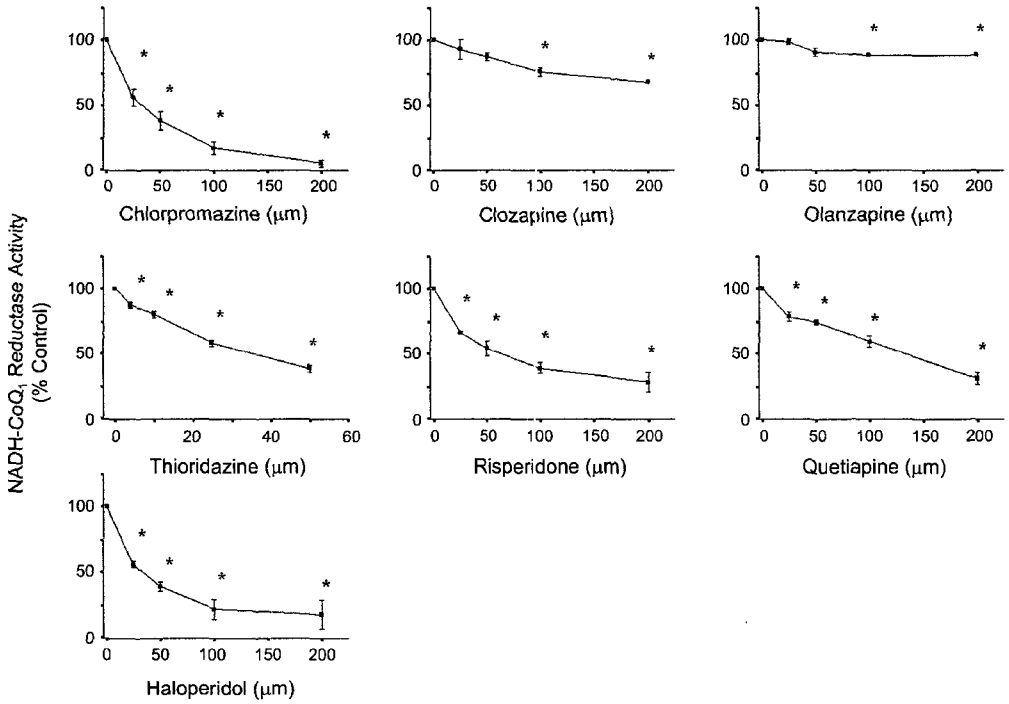

Fig. 4. Effect of neuroleptics on NADH-coenzyme Q reductase activity in freeze-thawed preparations of rat liver mitochondria. Values are the mean±S.E. of three separate experiments. Asterisk (*) indicates $p < 0.05$; i.e., data point is significantly different from control according to repeated measures analysis of variance.

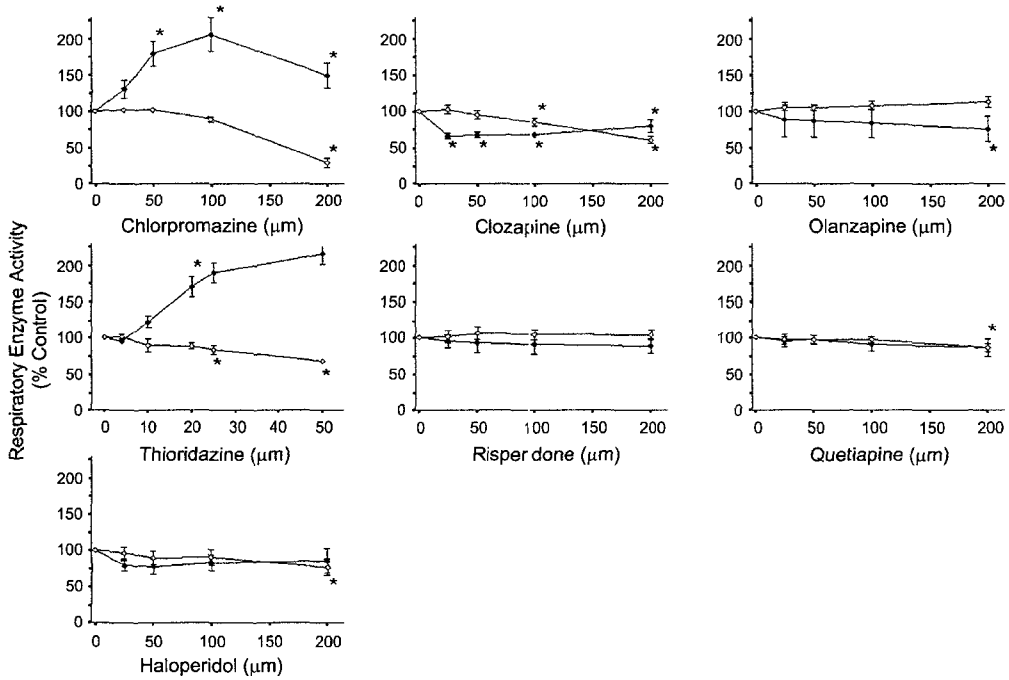

Fig. 5. Effect of neuroleptics on cytochrome c oxidase activity (◆) and succinate-cytochrome c reductase activity (◇) in freeze-thawed preparations of rat liver mitochondria. Values are the mean±S.E. of three separate experiments. Asterisk (*) indicates $p<0.05$; i.e., data point is significantly different from control according to repeated measures analysis of variance.

effect, causing approximately 25% inhibition of electron transfer activity at the highest concentration of each drug tested (50 μM and 200 μM, respectively). There was virtually no effect on succinate-cytochrome c reductase by any of the remaining drugs. Additionally, none of the drugs (except clozapine to some extent) caused significant inhibition of cytochrome c oxidase activity. The apparent stimulation of cytochrome c oxidase activity induced by chlorpromazine and thioridazine is thought to be due to non-specific chemical oxidation of reduced cytochrome c by the drugs.

DISCUSSION

The few recent studies that have compared the direct effects of certain typical vs. atypical neuroleptics on mitochondrial function have done so by examining the effects of these drugs on individual respiratory enzyme complexes in mitochondrial membrane fractions (reviewed under Introduction). In contrast, this study employed polarographic determination of oxygen consumption in well-coupled, functionally intact mitochondria as a measure of the integrated bioenergetic function of the organelle, which includes the processes of electron transfer, ATP synthesis, ATP/ADP translocation, substrate transport and phosphate transport. Polarographic screening of the drug effects on mitochondrial function therefore has the potential to yield much more information regarding possible toxicity of these compounds than that which can be ascertained by assaying respiratory enzymes alone. For example, in this study the stimulation of the S2 respiratory rates induced by chlorpromazine and thioridazine suggest that within the range of concentrations tested these drugs act as partial uncouplers of oxidative phosphorylation. Uncouplers alter the membrane permeability to protons, causing dissipation of the H^+ gradient and stimulating respiration unproductively (i.e., without concomitant synthesis of ATP). This increase in the metabolic rate induced by uncouplers can have a number of additional deleterious consequences such as increased production of reactive oxygen species (Rand, 1994; Loft *et al.*, 1994), alterations in the steady state ratios of ATP/ADP and NAD(P)H/NAD(P), and disruption of calcium homeostasis.

The results of this study also show a significant inhibition of S3 respiration by chlorpromazine when either glutamate+malate or succinate was used as the respiratory substrate (Fig. 3). Because S3 respiration is coupled to ATP synthesis, chronic inhibition of this rate might compromise cellular energy status and the ability to maintain a normal plasma membrane potential is likely to be affected. The cascade effect of gradual depolarization could include disinhibition of glutamate-sensitive NMDA channels (via Mg^{++} release), Ca^{++} influx and cell death. Partial depolarization to threshold could also lead to increased firing and excitotoxic cell death. (Beal 1995). The data also demonstrate that S3 and uncoupler-stimulated respiratory rates are inhibited by chlorpromazine to a similar extent (Fig. 2 and 3). This rules out site-specific inhibition of adenine nucleotide translocase and/or F_oF_1ATPase activities by the drug. Furthermore, the inhibition of both NADH-CoQ and succinate-cytochrome c reductase activities by chlorpromazine, and the previously described uncoupling effects, suggest that the toxic effects of this drug might be caused by a general perturbation of mitochondrial membranes. Interestingly, damaging effects by chlorpromazine on membrane integrity have been reported previously (Byczkowski and Borysewicz, 1979).

Inhibition of both coupled (S3) and uncoupler-stimulated respiration with glutamate/malate as the respiratory substrate indicate that haloperidol, risperidone and quetiapine inhibit electron transport activity at respiratory enzyme complex I. This was confirmed using site-specific enzyme assays, which showed that NADH-CoQ reductase activity was also inhibited by these drugs. In addition to direct effects on electron transport activity and oxidative phosphorylation in coupled mitochondria, inhibition of complex I activity results in oxygen free radical formation which may lead to further damage to mitochondrial respiratory enzyme complexes and/or mitochondrial DNA, lipid peroxidation, and possibly, opening of the mitochondrial permeability transition pore (Beal *et al.*, 1993; Schapira, 1998). This latter effect and consequent release of cytochrome c to the cytosol is known to induce a series of pre-apoptotic events that lead ultimately to programmed cell death (Green and Reed, 1998). Interestingly, striatal neurodegeneration caused by excitotoxic mechanisms and oxidative stress has been implicated in the development of TD (Andreassen and Jorgensen, 2000).

The mean prevalence of neuroleptic induced TD is 20-35% (Chouinard *et al.*, 1980); its incidence seems to increase with age at onset and duration of treatment. The incidence of TD also varies depending on the type of drug used for treatment. For example, it is well established that the first generation or "typical" neuroleptics such as haloperidol and chlorpromazine are associated with a much greater risk for the development of TD than the newer or "atypical" drugs (reviewed in Glazer, 2000). In a prospective double-blind study assessing neuroleptic induced TD liability in schizophrenic patients, it was concluded that the TD incidence (rate/year) was approximately 12-fold higher in haloperidol treated patients than in olanzapine treated patients (Beasley *et al.*, 1997). Additional studies demonstrate that TD and/or other adverse extrapyramidal symptoms (EPS) are more likely to develop in patients treated with haloperidol vs. risperidone (Jeste *et al.*, 1999; Lemmens *et al.*, 1999), quetiapine (Sussman, 2002), or

olanzapine (Schillevoort *et al.*, 2001). Results of our study demonstrate that the typical neuroleptics chlorpromazine, haloperidol, and thioridazine have significantly greater toxic effects on mitochondrial bioenergetic function than any of the atypical neuroleptics tested. Thus, the relative ranking for TD risk between the "typical" and "atypical" neuroleptic groups is consistent with their relative potencies for mitochondrial toxicity.

The results of our study indicate the order of potency for mitochondrial toxicity by atypical antipsychotics is risperidone > quetiapine ≫ clozapine > olanzapine. Few comparative data are available to definitively rank order TD liability among the newer atypical neuroleptics. One review comparing the EPS profiles of patients treated with atypical neuroleptics in several different studies suggests that the EPS rates for clozapine and olanzapine are not significantly different from placebo, whereas risperidone, induces dose-related EPS above 6 mg/day (Casey, 1996). Another review suggests that the EPS risk for risperidone treatment is greater than that for quetiapine (Sussman, 2002). Yet another tentatively ranks the relative risk for adverse EPS as risperidone > olanzapine > quetiapine > clozapine (Tarsy *et al.*, 2002). The apparent discrepancies in specific rank order are likely the result of comparing data from several different studies conducted at different times and sites and under different protocols.

The results of this study are important for several reasons. First, in general, the relative potency of neuroleptic drugs to compromise mitochondrial bioenergetic function is similar to their relative potencies as risk factors in the reported incidence of EPS. This suggests that the analytical screen used in this study might be useful as a predictive indicator of adverse extrapyramidal symptoms and TD liability for the newer antipsychotic drugs or for new drugs in development. Assessment of TD liability based on potencies for mitochondrial toxicities would necessarily take into account the clinically relevant dosage for each drug. Second, the consistency in results obtained in previous studies, which examined the effect of certain neuroleptics on mitochondrial function in human, rat and mouse brain, and in this study, which examined the same in rat liver, are attributable to the fact that the mechanisms of oxidative phosphorylation are "generic" to mammalian mitochondria. Therefore rat liver is a convenient and cost effective source of mitochondria for the screening of drug effects on bioenergetic function. Third, identification of the specific bioenergetic defects induced by neuroleptic treatment suggests the possibility of beneficial therapeutic interventions. For example, clinical administration of an alternative respiratory substrate (such as hydroxybutyrate) or vitamin (such as coenzyme Q) might be used to bypass or ameliorate complex I inhibition by neuroleptics. These approaches have been tried in the treatment of mitochondrial myopathies with some measure of success. Fourth, the results strengthen the hypothesis that mitochondrial dysfunction may be involved in the pathogenesis of neuroleptic-induced TD. Specificity of effects for certain regions of the CNS may be related to receptor-mediated uptake of the drugs, which exposes mitochondria in certain cells to very high concentrations of neuroleptics. Finally, the knowledge gained from this type of study will be useful in the design and development of a new generation of antipsychotics that exhibit enhanced clinical efficacy and fewer adverse side effects than many of those in current use.

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