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# 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE (MPTP) AND 1-METHYL-4-PHENYLPYRIDINE ($MPP^+$) CAUSE RAPID ATP DEPLETION IN ISOLATED HEPATOCYTES

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1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its oxidized metabolite 1-methyl-4-phenylpyridine ($MPP^+$) produce a rapid depletion of intracellular ATP in isolated rat hepatocytes. This effect was dose-dependent and was consistently observed before the onset of toxicity. The monoamine oxidase inhibitor pargyline provided significant protection against MPTP-induced cell death and ATP loss, but had no effect with $MPP^+$. Thus, ATP depletion may play a critical role in MPTP toxicity, possibly via the metabolic production of $MPP^+$. 

In a previous study we compared the toxic effects of 1-methyl-4-phenylpyridine ($MPP^+$) with those of the structurally related herbicide paraquat ($PQ^{++}$) in isolated rat hepatocytes (1). Our results indicated that $MPP^+$ cytotoxicity could not be attributed solely to a mechanism involving generation of reactive oxygen species which has been suggested by several authors (2,3). Other recent reports have shown that $MPP^+$ is actively taken up by liver mitochondria (4) and causes inhibition of NADH-linked substrate oxidation in brain mitochondria (5). Thus, an alternative mechanism of toxicity could involve a block within the respiratory process and subsequent depletion of cellular energy supplies in the form of ATP. In this study we have correlated intracellular ATP content with hepatocyte cell death induced by $MPP^+$ and its parent compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

## MATERIALS AND METHODS

Hepatocytes were isolated from male Sprague-Dawley rats (180-250 g) and incubated in Krebs-Henseleit buffer (pH 7.4) (6). In some experiments, a pretreatment procedure with 1,3,-bis(2-chloroethyl)-1-nitrosourea (BCNU) was used to inhibit glutathione reductase (EC 1.6.4.2) (7). Cell viability was assessed by Trypan blue exclusion (6). Intracellular ATP was measured spectrophotometrically at 340 nm as NADPH formation in the presence of glucose, $NADP^+$, hexokinase and glucose-6-phosphate dehydrogenase (8). The

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possibility of interference by BCNU , pargyline, or MPTP and its metabolites with the enzymatic ATP assay was ruled out by the use of internal standards and measurement of several samples by chemiluminescence (9). Results shown here are typical of at least three separate cell preparations. All chemicals were of reagent grade or higher and obtained commercially, except for the following: MPTP and $MPP^+$ were kindly supplied by Professor Neal Castagnoli, Jr., Department of Pharmaceutical Chemistry, University of California, San Francisco; BCNU was provided by the National Cancer Institute, Bethesda, Maryland; pure $PQ^{++}$ was a gift from Dr. L.L. Smith, C.T.L., I.C.I. (U.K.).

## RESULTS AND DISCUSSION

Both $PQ^{++}$ and $MPP^+$ are toxic to isolated rat hepatocytes pretreated with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), an inhibitor of glutathione reductase, which potentiates oxidative stress by preventing the regeneration of reduced glutathione (GSH) from GSSG (Fig 1B). However, we have previously shown that the BCNU treatment is necessary for early manifestation of the toxic effects of $PQ^{++}$ in hepatocytes, while it does not significantly affect the pattern of cell death induced by $MPP^+$ (1). Measurements of intracellular ATP content during the incubation of BCNU-treated hepatocytes with either $PQ^{++}$ or $MPP^+$ yield markedly different results (Fig 1A): $MPP^+$ completely depletes ATP well before the onset of cell death, while exposure to $PQ^{++}$ only slowly reduces the content of ATP to approxi-

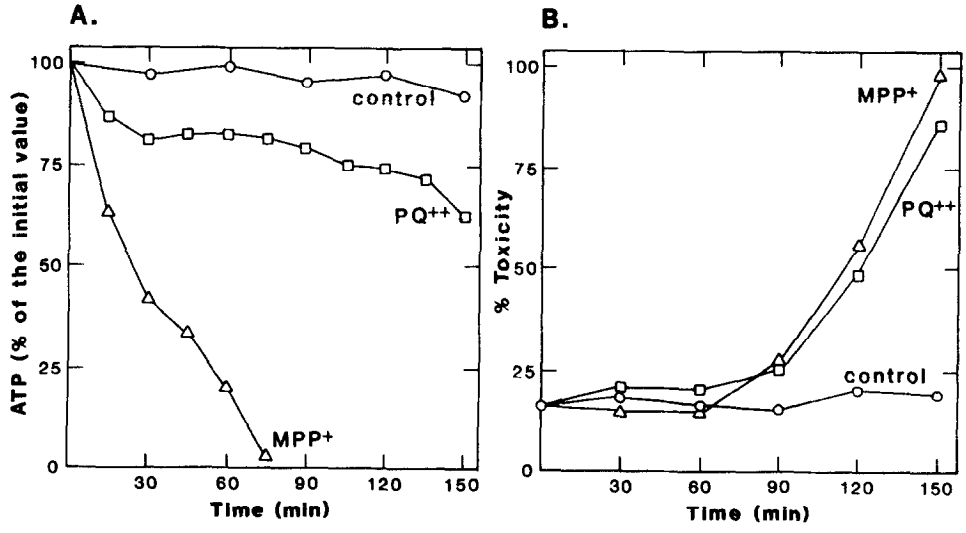


Fig. 1 Intracellular ATP content (A) and cell toxicity (B) after exposure of isolated hepatocytes to 1.5 mM $PQ^{++}$ (□) or 1.5 mM $MPP^+$ (△). Initial value for intracellular ATP was 8-10 nmoles/$10^6$ cells.

mately 60% of the initial value (i.e., to 4-6 nmoles/$10^6$ cells). These data suggest that two toxic mechanisms may occur, but clearly play different roles in the toxicity induced by $PQ^{++}$ and $MPP^+$. Production of reactive oxygen species seems to be a critical toxic event when hepatocytes are exposed to $PQ^{++}$ (1), while depletion of the cellular energy supply could lead to irreversible damage during incubation with $MPP^+$.

The unexpectedly dramatic effect of $MPP^+$ on intracellular ATP prompted a closer investigation of the nature of this toxic event in non-BCNU treated hepatoctyes. $MPP^+$ is the fully oxidized metabolite implicated in the marked neurotoxicity which results from administration of MPTP (10). Oxidation of the parent compound is initiated by type B monoamine oxidase (MAO-B) located on the outer mitochondrial membrane (11). It has been suggested that local production and active uptake of $MPP^+$ by the mitochondria could lead to high intraorganelle concentrations of the metabolite (4) and inhibition of NADH-dependent respiration (5). Both MPTP and $MPP^+$ cause rapid net losses of intracellular ATP in isolated hepatocytes (Fig 2). In both cases the effects are dose-dependent and correlate well with loss of cell viability. The onset of toxicity was invariably seen after complete depletion of the ATP content. The two compounds differ in the duration of initial lag time, but not in the rate of cell death (Fig 2). The more rapid onset of toxicity and ATP depletion following MPTP addition may be explained by a relatively faster uptake into the hepatocytes due to its uncharged, lipophilic structure.

Pargyline is a specific inhibitor of MAO, and can protect against the in vivo toxic effects of MPTP (12). Accordingly, preincubation of hepatocytes with 10 μM pargyline clearly prevents the occurrence of MPTP-induced cell death (Fig 3A). This protection, however, does not seem to be complete, since an increased number of dead cells (approx. 40%) are counted after 3 hours of incubation with higher concentration of MPTP (e.g. 2.0 and 2.5 mM) (data not shown). Similarly, a clear yet incomplete prevention of the rapid depletion of ATP induced by MPTP is observed in cells after prein-

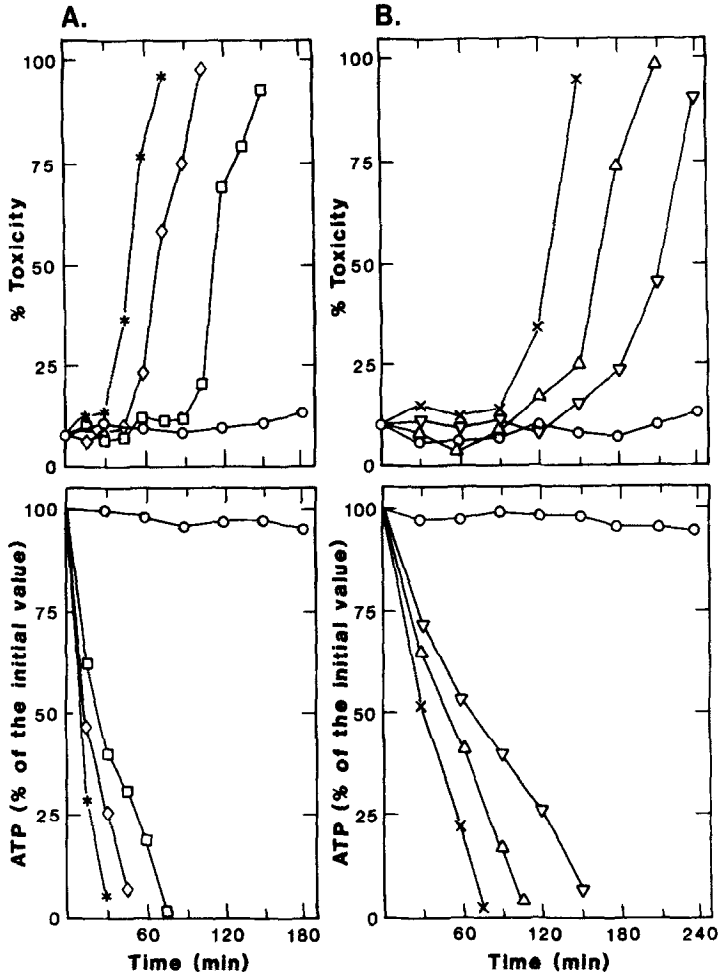


Fig. 2 Cell viability and ATP depletion during incubation of hepatocytes with different concentrations of MPTP (A) or $MPP^+$ (B). Trypan blue uptake was measured in the absence (o) or presence of 0.5 mM (□), 1.5 mM (◇), 2.5 mM (*) MPTP, or 0.75 mM (▽), 1.5 mM (△), 2.5 mM (x) $MPP^+$.

cubation with pargyline (Fig 3A). The specific nature of the protection obtained by inhibiting MAO is supported by the absence of any effect of pargyline on cell toxicity and ATP loss in hepatocytes exposed to $MPP^+$ (Fig 3B).

In summary, these results show that incubation of isolated hepatocytes with either MPTP or $MPP^+$ causes a rapid, dose-dependent decrease in intracellular ATP, which consistently precedes cell death. The protective action exerted by pargyline with MPTP further indicates the critical role of MAO-

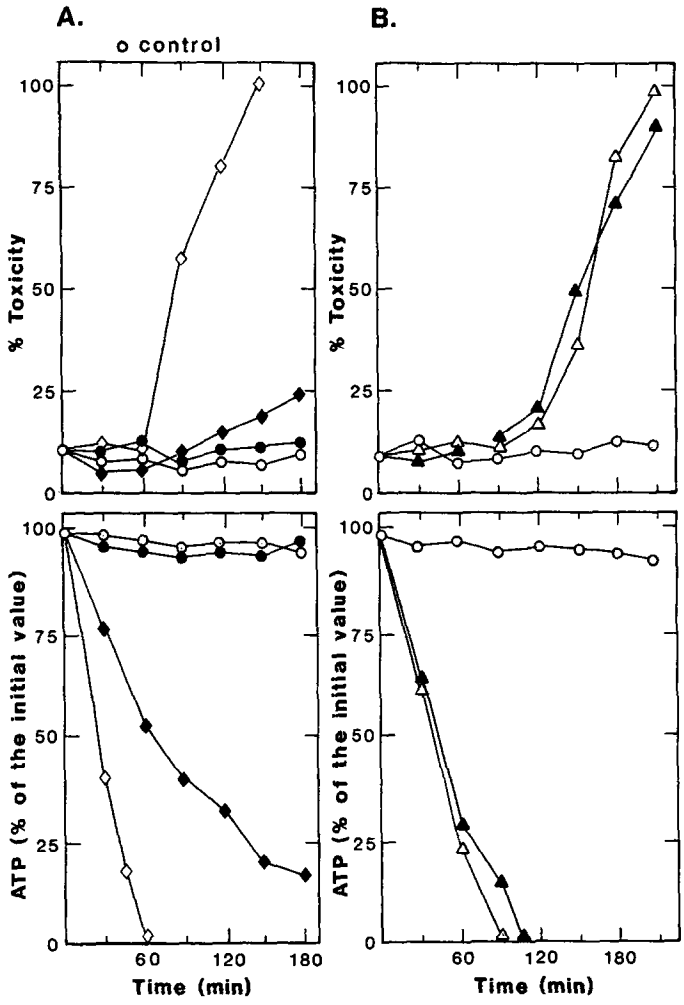


Fig. 3 Effect of pargyline (filled symbols) on cell toxicity and ATP depletion induced by (A) 1.5 mM MPTP (◇) or (B) 1.5 mM $MPP^+$ (△).

mediated metabolism in the cytotoxicity of this compound, and suggests that an oxidized species such as $MPP^+$ plays a key role.

Much attention has recently been focused on the possibility of a free radical mediated toxic mechanism for these compounds. Evidence has been presented for GSH loss after exposure to MPTP *in vivo* (13) and for the protective effect of antioxidants *in vivo* (14). The results described here do not exclude the relevance of these findings. Indeed, production of reactive oxygen species could be expected during the metabolic conversion of MPTP to $MPP^+$ in dopaminergic neurons (15). Nevertheless, we demonstrate the drama-

tic occurrence of another toxic event in our cellular system, which may play an even more important role in the toxicity of MPTP and $MPP^+$. Impairment of ATP production can affect a variety of cell functions, including critical electrolyte homeostatic processes. We believe that this is likely to be a generally valid toxic effect since it does not involve properties unique to our cellular system. This concept is supported by results obtained with brain mitochondria (5), but certainly needs to be confirmed by additional experimental evidence.

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