Toxicology Letters, 12 (1982) 165–170
Elsevier Biomedical Press

DISODIUM TETRACHLOROPALLADATE (Na_2PdCl_4), AN INHIBITOR OF RAT LIVER MITOCHONDRIAL ELECTRON TRANSPORT

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(Received November 19th, 1981)
(Accepted February 22nd, 1982)

SUMMARY

The effects of Na_2PdCl_4 were studied on isolated rat liver mitochondrial electron transport and oxidative phosphorylation in vitro. Significant reductions in ADP-stimulated respiration were observed with increasing Na_2PdCl_4 concentrations with both succinate and NADH-linked substrate oxidations. Concentration necessary for half-maximal inhibition of oxygen uptake (EC_{50}) for an NADH-linked substrate system was 18 μM while with succinate as substrate the EC_{50} was 15 μM. At 64 μM both systems were inhibited maximally at 60 and 80%, respectively. At concentrations of Na_2PdCl_4 sufficient to inhibit acceptor-stimulated oxygen uptake, there was a concomitant decrease in the rate of ADP phosphorylation as measured by proton absorption. Uncoupling agents had no effect on Na_2PdCl_4 inhibited mitochondria. Mg-ATPase activity and phosphate acceptor limited (State 4) respiratory activity were not stimulated by any Na_2PdCl_4 concentration used in these investigations. Data from these experiments indicate that Na_2PdCl_4 inhibits the mitochondrial respiratory chain in vitro.

INTRODUCTION

Palladium metal catalysts are used in an increasing number of industrial processes. One of the more important uses of palladium metal catalysts is in conjunction with platinum in automobile catalytic converters. Various palladium salts have been shown to be skin and eye irritants, cardiotoxins and angiotoxic agents. Target organ effects from lethal doses have been found in the liver, kidney, heart, bone marrow and hematopoietic systems [1].

Abbreviations: ACR, acceptor control; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; GMM, 5 mM glutamate, 1 mM malate, 1 mM malonate; RLM, rat liver mitochondria.

0378-4274/82/0000–0000/$02.75

The effect of various palladium salts on rat liver mixed-function oxidase activity has been studied. It was found that intraperitoneal injection of $Pd(NO_3)_2$ increased hexobarbital-induced sleeping time in rats in vivo and generally decreased aminopyrine demethylase activity and the cytochrome P-450 content of isolated liver microsomes. $Pd(NO_3)_2$ was also found to inhibit aminopyrine demethylase acitivity in vitro [2].

The effect of $PdSO_4$ on in vitro succinate-dependent respiration of rat liver slices has also been studied [3]. These investigators found that high concentrations of the palladium salt (10^{-3}M) were necessary to observe 50% reduction in control oxygen uptake activity.

Na_2PdCl_4 is an important intermediate in the refining of palladium metal, and represents the specific salt for which there is the greatest potential for occupational exposure [4]. The $PdCl_4^{=}$ ion is known to interact with biological membranes as measured by electron paramagnetic resonance spectroscopy [5]. The mitochondrion is a complex membrane-bound, multi-enzyme system whose toxic responses to xenobiotics represent an in vitro model of membrane and protein interaction effects.

The objective of the present study is to investigate the interaction of Na_2PdCl_4 with isolated electron transport and energy metabolism to further define mechanisms of potential toxic sequelae from palladium exposure.

METHODS

RLM from male and female Sprague–Dawley rats (300–500 g), obtained from Charles River Breeding Laboratories (Wilmington, MA) were isolated by standard methods [6], using 0.25 M sucrose, wich was 1 mM ethylene glycol bis (β-aminoethyl ether)-*N*-*N*′,1-tetraacetic acid (EGTA) and 2.5 mM sodium *N*-2-hydroxyethyl-piperazine-*N*-ethanesulfonic acid (Na-HEPES), pH 7.4. The animals were fed standard laboratory chow (Ralston Purino Co., St. Louis, MO) and water ad lib. Prior to killing, the animals were given water only (12 h). The final mitochondrial suspension (50–60 mg protein/ml), was stored as a stock suspension on ice. Protein was estimated according to Lowry et al. [7]. All chemicals and reagents unless otherwise stated were analytical grade obtained from the Sigma Chemical Co (St. Louis, MO).

ACR and phosphate acceptor ratios (ADP:O) were determined by the method of Estabrook [8]. Respiration measurements were carried out by a previously described method [9] at 25 °C in a closed water-jacketed reaction vessel equipped with a Clark oxygen electrode (Gilson 5/6 pH recording oxygraph, Gilson Medical Electronics, Madison, WI). The respiration medium contained in a 1.7 ml total volume 80 mM NaCl, 10 mM $MgCl_2$, 3 mM Na-HEPES, pH 7.4, and 4.5 mM sodium phosphate. GMM or 10 mM succinate were used as substrates in individual experiments. State 3 (ADP-stimulated) respiration was initiated by the addition of freshly prepared ADP

in limiting amounts (150–300 nmol). Respiration was uncoupled from phosphorylation with CCCP at a final concentration of 1 μM. Disodium tetrachloropalladate, obtained from Aldrich Chemical Co. (Milwaukee, WI) was prepared freshly as a stock solution in distilled water. The stock solution was protected from light and used within 3 h. All additions were made into the injection port of the water-jacketed reaction cell using calibrated μl syringes.

ATPase activity was assayed by the determination of proton ejection following additions of CCCP or Na_2PdCl_4 [10]. Incubation before assay was for 2 min in a pH 7.4 medium containing 80 mM NaCl, 10 mM $MgCl_2$, 10 mM sodium succinate, 3 mM Na-HEPES, 4.5 mM sodium phosphate and 5–6.5 mg RLM protein. ATP, 0.25 mM, was then added in a total volume of 10 μl. After 2 min either CCCP or Na_2PdCl_4 was added in 10–20 μl amounts.

Hydrogen ion movements were followed after additions using the pH recording channel of the Gilson pH 5/6 oxygraph. A Sensorex combination gel electrode (West Minister, CA) was used to monitor pH changes. Observed pH changes after an experiment were titrated in the direction of change with known μl additions of standardized HCl. In the ATPase studies reported here, activity was estimated from initial rates and actual pH changes never exceeded 0.5 pH units. pH changes were found to be linear over the reaction period studied (5 min).

ADP-stimulated proton absorption measurements were performed following the addition of 400 nmol ADP. The reaction medium contained in a final volume of 1.7 ml, 80 mM NaCl, 10 mM $MgCl_2$, 10 mM sodium succinate 3 mM Na-HEPES, pH 7.4 and 5–6 mg RLM protein. Hydrogen ion movements were followed using the pH recording system described above. Temperature was 25 °C.

The equality of all sample population variances were tested using a variance ratio technique. Observed values were tested for independence using Student's two-tailed *t*-test [11].

RESULTS

The observed control values for the ACR and ADP:O ratios as well as the ADP- and CCCP-stimulated oxygen consumption rates presented in Table I are consistent with high-quality preparations of intact RLM. The ACRs of 4.2 and 5.0 for succinate and GMM, respectively, demonstrate that tight coupling of oxidation with phosphorylation existed. The ADP:O ratios of 1.7 and 2.7 for succinate and GMM substrates, flavoprotein- and NAD^+-linked oxidations, respectively, were consistent with mitochondrial preparations in which all three coupling sites were intact.

It was observed that ADP:O ratios were not affected by any of the Na $PdCl_4$ concentrations used in this study. The effect of increasing Na_2PdCl_4 concentrations on ADP-stimulated respiration was studied in order to observe whether inhibition was substrate specific and to determine the relative effective concentrations

TABLE I

RLM RESPIRATORY ACTIVITIES, ACCEPTOR CONTROL AND ADP:O RATIOS

Substrate	Activity[a]				
	State 3	State 4	CCCP	ACR[b]	ADP:O[c]
Succinate[d]	99.1 ± 8.6	23.6 ± 4.3	121 ± 11.8	4.2 ± 0.3	1.72 ± 0.2
GMM[e]	64.2 ± 5.4	13.0 ± 3.3	71.9 ± 7.9	5.0 ± 0.5	2.65 ± 0.3

[a] ng-atoms oxygen consumed/min/mg protein, ± SD (5 determinations).
[b] Acceptor control ratio, expressed as the ratio state 3/state 4.
[c] Expressed as the ratio, nmol of ADP phosphorylated/ngatoms oxygen consumed during state 3 respiratory jump.
[d] 10 mM sodium succinate.
[e] 5 mM glutamate, 1 mM malate and 1 mM maleate.

necessary for half maximal inhibitions of state 3 respiration (EC_{50}). We also studied the effect of a post Na_2PdCl_4, post ADP respiratory jump addition of CCCP on respiratory rates. In this way we attempted to elucidate whether the observed inhibition was at the level of the respiratory chain, the coupling mechanism or both. The results of these experiments are reported in Figs. 1 and 2 which demonstrate respective EC_{50}s of about 18 μM for GMM-linked oxidation and 15 μM for succinate-linked oxidation. In both cases there was no reversal of the observed inhibition by an addition of CCCP.

The ability of Na_2PdCl_4 to reduce the rate of ADP phosphorylation was studied by recording the rate of proton absorption during ADP phosphorylation. At a concentration of 29 μM Na_2PdCl_4 with succinate as substrate, the proton absorption rate for phosphorylation of 400 nmol of ADP (5–6.5 mg protein) was 115 ± 32 nmol H^+ absorbed/min. The control proton absorption rate was 350 ± 47 nmol H^+ absorbed/min. This represents a decrease in proton absorption rate of over 70% by 29 μM Na_2PdCl_4 ($P < 0.01$). This agrees well with the inhibition of state 3 activity seen at this same concentration (Fig. 2).

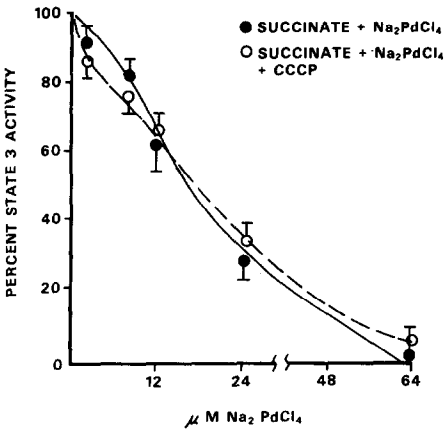
The direct correlation between proton ejection and phosphate release from ATP hydrolysis by the RLM ATPase has been reported by others. Thus this parameter may be exploited as an indicator of ATPase activity [10]. We observed control

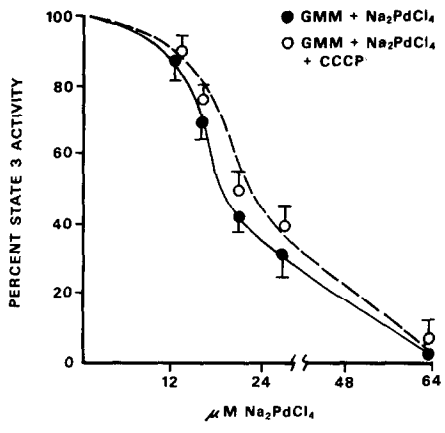
TABLE II

THE EFFECT OF Na_2PdCl_4 ON ADP-STIMULATED PROTON ABSORPTION

Additions	Rate of proton absorption[a]
None	350 ± 47
29 μM Na_2PdCl_4	115 ± 32[b]

[a] nmol H^+ absorbed/min/mg protein ± SD (5 determinations).
[b] $P < 0.01$ (Student's t-test).





Figs. 1 and 2. Effect of increasing Na_2PdCl_4 concentration on ADP-stimulated respiration and lack of revesibility by 1 μM CCCP expressed as percent of maximal inhibition (see text). Fig. 1. Substrate: GMM; Fig. 2. Substrate: 10 mM succinate.

proton ejection rates in the presence of CCCP of 49 ± 7 nmol H^+ ejected/min/mg protein, which agrees well with published rates for this system [10], however, no stimulation of proton ejection was seen by any of the concentrations of Na_2PdCl_4 used in this study.

DISCUSSION

The results presented showed Na_2PdCl_4 to be a relatively potent inhibitor of mitochondrial electron transport, while having no effect of the RLM Mg-ATPase or direct uncoupling activity. These effects were concentration-dependent. At the highest concentration tested (64 μM), state 3 respiration rates with both succinate and GMM were inhibited to 20 and 40% of control values, respectively. The concomitant inhibition of CCCP-stimulated respiration (i.e., lack of reversal) with state 3 respiration (Figs. 1 and 2) suggests that Na_2PdCl_4 is acting as a respiratory chain inhibitor and has little observed direct effect on the coupling mechanism. From the inhibitor sensitivities of the substrates utilized, one cannot conjure a locus of the respiratory chain for primary inhibition, however, the sigmoidal curves present in Fig. 1 (GMM substrate) indicate probable multiple site effects.

Further, we observed that simultaneous to the inhibition of the electron transport chain, Na_2PdCl_4 suppressed the rate of accumulation of protons during ADP phosphorylation, which was consistent with the inhibition of oxygen consumption. In addition, the inability of Na_2PdCl_4 to stimulate state 4 respiration or ATPase activity gives credibility to a chain inhibition mode of action for this compound.

In conclusion, the data presented herein demonstrated multiphasic inhibitor

interaction with the RLM electron transport system yielding primary inhibition of respiration without observed interaction with the mechanics of the coupling mechanism. However, because these investigations were conducted in vitro, the physiologic significance of the reported findings remains to be established.

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