

EFFECT OF SELECTED PERFLUORO-COMPOUNDS ON MITOCHONDRIAL β -OXIDATION

SUMMARY

It was hypothesized that the perfluorochemicals may be structural mimics of long chain fatty acids and thereby be competitive inhibitors of mitochondrial fatty acid oxidation. Such inhibition may occur at the stage of formation of the CoA or carnitine esters (depletion of cytosolic CoASH, inhibition of fatty acyl CoA synthetase [thiokinase], or inhibition of carnitine acyltransferase I), transport of the carnitine ester across the inner mitochondrial membrane, interference with the formation of the CoA ester within the mitochondrial matrix (depletion of matrix CoASH or inhibition of carnitine acyltransferase II), or inhibition of the mitochondrial β -oxidation enzymes themselves (alternate electron acceptor complex II of the electron transport chain). To test this, we first asked the general question of whether the fluorochemicals inhibit the oxidation of palmitoylcarnitine, which is a measure of all steps beginning with the transport of the carnitine ester across the inner mitochondrial membrane. Thus, this is a measure of the effect of the fluorochemicals on all steps that occur within the mitochondrion, but does not reflect any potential effect of the compounds on the extramitochondrial formation of the CoASH or carnitine esters. Eight fluorochemical compounds were selected for this study (N-EtFOSE, FC-129 (PFOSAA), FX-12, FC-95 (PFOS), PFOSA, FC-143 (PFOA), FC-228, and CMPD-8 (M556)).

MATERIAL AND METHODS

Rat liver mitochondria were isolated by differential centrifugation and incubated in a medium containing 200 mM mannitol, 10 mM sucrose, 5 mM HEPES (pH=7.4), 1 mM EGTA, 2 μ M oligomycin, and 10 mM KH_2PO_4 . Mitochondria were added at 0.8 - 1 mg protein/ml. All of the test compounds except FX-228 were added at a concentration of 50 nmol/mg mitochondrial protein. FX-228 was added at 50 μ g/ mg mitochondrial protein. Palmitoylcarnitine was added to a final concentration of 40 μ M. Glutamate + malate (final concentration of 5 mM, each) and succinate + rotenone (final concentration, 5 mM and 2 μ M, respectively) were pre-mixed and added as indicated. 2,4-Dinitrophenol was added at a final concentration of 40 μ M. Mitochondrial respiration was measured with a Clark-type electrode.

RESULTS

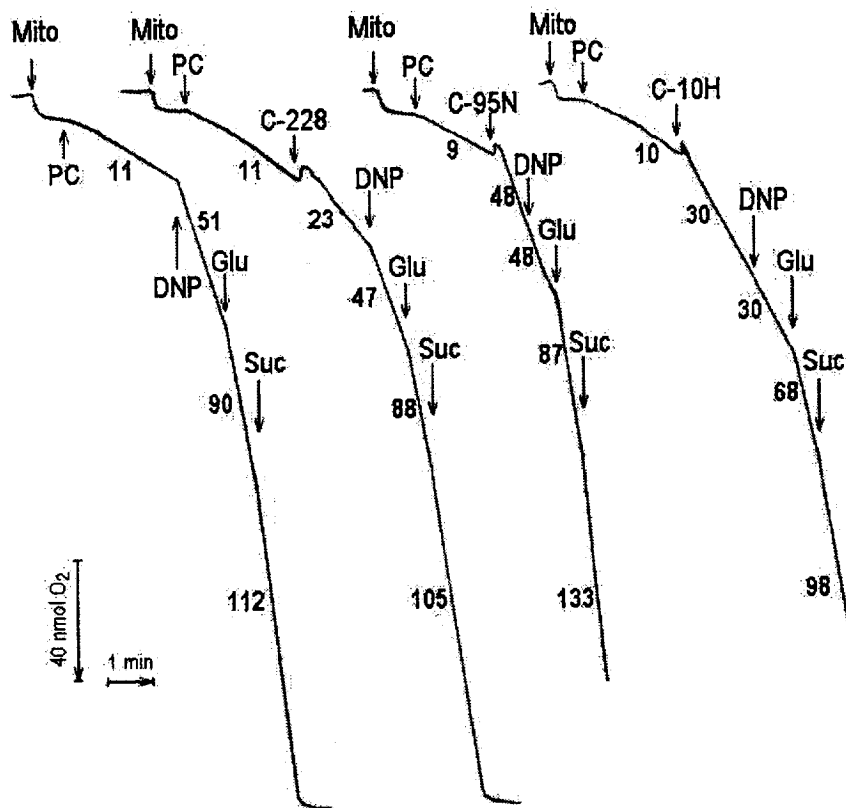


Figure 1. The effect of representative perfluorochemical compounds on respiration of rat liver mitochondria oxidizing palmitoylcarnitine. Medium composition and additions see Methods. Mitochondrial protein content was 1 mg/ml. Numbers near the curves indicate the rate of respiration, $\text{nmol O}_2 \times \text{min}^{-1} \times \text{mg}^{-1} \text{ protein}$. Abbreviations: Mito, mitochondria; PC, palmitoylcarnitine; DNP, 2,4-dinitrophenol; C-228, FX-228; Glu, glutamate + malate; Suc, succinate + rotenone; C-95N, PFOSA; C-10H, FC-129 (PFOSAA). For explanations, please see the text.

DISCUSSION

The efficiency of β -oxidation in rat liver mitochondria can be estimated by measuring the rate of oxygen consumption in the presence of palmitoylcarnitine (PC). This substrate is imported into the mitochondrial matrix over the acylcarnitine transporter and subsequently oxidized through the β -oxidation pathway in the matrix of mitochondria. Inside the matrix space, palmitoylcarnitine is converted in palmitoyl-CoA by carnitine acyltransferase II and then oxidized by molecular oxygen with the participation of two complexes of respiratory chain, Complex I and Complex III. Under conditions of uncoupled respiration (following the addition of DNP), the maximal rate of mitochondrial respiration in the presence of palmitoylcarnitine is a

direct measure of the activity of the β -oxidation multienzyme complex and/or the rate of palmitoylcarnitine (PC) penetration into mitochondrial matrix. Hence, if a compound inhibits the transport of PC and/or the β -oxidation enzymes, it will decrease the rate of uncoupled respiration. None of the perfluorochemicals tested decreased the rate of PC-supported respiration in the presence of DNP, indicating that none of the compounds inhibited either the transport or β -oxidation of fatty acids by isolated rat liver mitochondria *in vitro*.

A compound may also inhibit Complex I or Complex III directly, which may complicate the interpretation of the data. However, this complication can be revealed by comparing the rates of palmitoylcarnitine oxidation by uncoupled mitochondria to that of other substrates of Complex I and Complex III, such as glutamate/malate or succinate.

The above mentioned considerations were taken into account in designing our experimental procedure, as illustrated in figure 1. First, we estimated the maximal rate of PC oxidation by uncoupled mitochondria by measuring the maximal rate of PC-supported uncoupled respiration (the uncoupling was achieved by adding 2,4-DNP at a concentration that induced the maximal rate of respiration in the presence of glutamate+malate). The additions of glutamate+malate (Complex I -dependent substrates) and succinate (Complex III substrate) yielded the values for the maximum uninhibited rates of Complex I and Complex III dependent respiration, respectively (Fig. 1, curve a). The other three curves of figure 1 illustrate three possible scenarios of effects on the uncoupling efficiencies of the compounds of interest:

- *curve b* - If a compound (such as FX-228) does not inhibit PC oxidation, but it is a less efficient uncoupler of oxidative phosphorylation than 2,4-DNP, then the addition of the latter would stimulate the coupled respiration (prior to adding DNP), whereas the respiration rate in the presence of 2,4-DNP plus FX-228 is nearly identical to that in the presence of DNP alone (compare with curve a);
- *curve c* - If a compound such as the amide of FC-95 (PFOSA) is more efficient than DNP at uncoupling mitochondrial oxidative phosphorylation, it produces the maximal rate of respiration when added to mitochondria by itself. This rate cannot be further stimulated by adding DNP (compare the respiration rates before and after the adding DNP);
- *curve d* - The FC-129 (PFOSAA)-stimulated rate of respiration is also insensitive to DNP, but the rate of oxygen consumption is only about half of the maximal rate (30 nmol/min/mg after DNP addition compared to 47-51 nmol/min/mg for curves a, b, & c). Furthermore, the respiration rate was also suppressed following the additions of glutamate+malate (Glu) and succinate+rotenone (Suc), which indicates that FC-129 (PFOSAA) is an inhibitor of Complex I (and possibly Complex III) of the respiratory chain, rather than an inhibitor of PC oxidation.

These experiments were all repeated in triplicate for all eight perfluorochemical compounds. For N-EtFOSE, PFOS, PFOA, and M556 the effects were qualitatively similar to those illustrated for

FX-228 (curve b) for all the compounds. FX-12 at 50 μM is a fairly strong uncoupler of oxidative phosphorylation and affected the respiration similar to that caused by PFOSA (curve c).

CONCLUSIONS

In all of the experiments, the chosen concentrations of the compounds were either equal to or several-fold higher than what earlier work demonstrated to be maximal uncoupling concentrations (reference report on mitochondrial bioenergetics). Because all the compounds were practically without effect on maximal rates of PC oxidation, we conclude that none of the eight perfluorochemicals tested altered the rate of oxidation of palmitoylcarnitine by isolated rat liver mitochondria *in vitro*.