Biochemical Pharmacology, Vol. 26, pp. 1179–1180. Pergamon Press, 1977. Printed in Great Britain.

SHORT COMMUNICATION

Effect of an imported fire ant venom component on respiration and oxidative phosphorylation of mitochondria*

(*Received* 17 *July* 1976; *accepted* 20 *October* 1976)

Imported fire ants, *Solenopsis invicta* and *S. richteri*, contain alkaloid-type venoms which are insecticidal, bactericidal and fungicidal [1–3]. Although their potency is quite great, their mode of action has not been investigated to any great extent. The present research was designed to determine the effect of the C_{15}-2,6-methyl piperidine toxin, which is predominant in workers of *S. invicta* [4], on oxidative phosphorylation of mitochondrial preparations from cockroach muscle.

Coxal muscles of female cockroaches were used for mitochondrial preparations. Dissections were made in ice-cold 0.25 M sucrose solution containing 5 mM imidazole, 1 mM EGTA† and 0.05% BSA at pH 7.5, which was based upon the method of Carafoli *et al.* [5] except for reduction in the level of BSA. Fractionation by centrifugation followed the usual preparation of the ATPase system [6]. For the preparation of the mitochondrial fraction, one extra wash was used at 13,000 *g* for 20 min using the same dissection solution, except for omitting the EGTA. The mitochondrial fraction was resuspended in sucrose–imidazole–BSA solution without EGTA. The preparation was held in an ice bath until assay of oxygen uptake which was made within a 24-hr period.

The oxygen consumption of the mitochondrial preparation was determined polarographically at 27 ± 0.5° using a Clark type oxygen electrode (Yellow Springs Instrument Co., Ohio) and a Speedomax H recorder. Calibration was made in an air-saturated medium following the method of Robinson and Cooper [7], assuming an oxygen content of 1120 nmoles O_2 in the 4-ml reaction mixture. Alpha-glycerophosphate (10 mM) was used as the reaction substrate; in addition, the mitochondrial preparation had a protein concentration of 0.17 mg/ml at pH 7.1 to 7.3. ADP was added to bring about rapid respiration. The Respiratory Control Index (RCI) was obtained by comparing the respiratory rate before and after the addition of ADP.

The state III respiratory rate of mitochondrial preparations from cockroach coxal muscle was reduced somewhat at 2.8×10^{-6} M of C_{15}-2,6-methyl piperidine (Table 1), but the most pronounced effect occurs at 4.8×10^{-6} M and stronger concentrations. At these concentrations the Respiratory Control Index (obtained by dividing state III rate, when ADP was added, by state IV rate) becomes 1.0, indicating a pattern of effect similar to that of 2,4-dinitrophenol (DNP) which is a standard uncoupling agent. Uncoupling is characterized by a diminution of the ADP/O ratio and RCI (last column, Table 1), and a stimulation of the state IV respiration rate with no significant change in the state III respiration rate [8]. However, the decrease in the state III respiratory rate at 4.8×10^{-6} M (Table 1) probably indicates a combination of partial inhibition of Mg^{2+} ATPase and/or partial inhibition of electron transport. Koch and Desaiah [9] have shown that mitochondrial Mg^{2+} ATPase from fire ant head preparations was completely inhibited at 3.3×10^{-5} M C_{15}-2,6-methyl piperidine derivatives. At a higher dosage of venom, 8.3

Table 1. C_{15}-2,6-methyl piperidine tested against respiration and oxidative phosphorylation in isolated mitochondria from American cockroach muscle*

Venom dosage	Respiratory rate (nmoles O_2/min) State IV Initial rate	State IV Venom added	State III ADP added (440 nmoles)	Respiratory Control Index (RCI)
Blank	10.8	10.8	43.0	4.0
Acetone (4 μl)	9.8	9.8	38.4	3.9
Venom ($\times 10^{-6}$ M)				
0.6	9.7	9.7	37.0	3.8
1.1	9.9	9.9	36.7	3.7
1.7	9.1	9.7	36.6	3.8
2.8	8.7	11.8	33.3	2.8
3.4	9.7	16.1	30.8	1.9
4.1	9.4	22.8	30.2	1.3
4.8	9.3	20.2	20.2	1.0
5.5	8.6	25.1	25.1	1.0
8.3	9.8	8.9	8.9	1.0
11.0	8.9	5.4	5.4	1.0
DNP (250 nmoles)	10.7	36.2	36.2	1.0

* C_{15}-2,6 methyl piperidine = *cis*-2-methyl-6-*n*-pentadecylpiperidine (sample supplied by Dr. M. S. Blum, University of Georgia, Athens, Ga.). The respiratory rate was determined polarographically at 27 ± 0.5°, pH 7.1 to 7.3, using a Clark-type oxygen electrode. Mitochondrial protein value = 0.174 mg/ml.

* Paper No. 9557, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, St. Paul, MN 55108.

† Ethyleneglycol-bis-(B-aminoethyl ether)-*N*,*N*¹-tetra-acetic acid.

to 11.0×10^{-6} M, the inhibition of the electron transport chain becomes apparent as the state IV respiratory rate was reduced to below the initial rate.

The type of uncoupling effect was also similar to that reported for dicofol, a DDT-related acaricide [10]. DDT, in contrast, does not produce an uncoupling effect, but is a strong inhibitor of oxygen consumption of insects at 10^{-6} M*.

The results indicate a prominent mechanism of action by low concentrations of C_{15}-2,6-methyl piperidine. Further experiments are needed to determine whether an effect *in vivo* could be demonstrated.

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