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Ectopic inhibition of the complexes of the electron transport system by rotenone, piericidin A, demerol and antimycin A

Previous studies from this and other laboratories have shown that in mitochondria or the purified complexes of the electron transport system, DPNH–ubiquinone reductase activity is inhibited by barbiturates, demerol, rotenone and piericidin A; succinate–ubiquinone reductase activity by 2-thenoyltrifluoroacetone; and ubiquinol–cytochrome *c* reductase activity by antimycin A[1–7]. More recently, this laboratory has shown that in DPNH–ubiquinone reductase (complex I) the site of inhibition of amytal, demerol, rotenone and piericidin A is between the flavoprotein and the iron-protein components of complex I[5], and in succinate–ubiquinone reductase the site of 2-thenoyltrifluoroacetone inhibition appears to be between succinate dehydrogenase and ubiquinone–cytochrome *b*[6].

However, recent reports from other laboratories working with whole mitochondria or electron transport particles have indicated multiple inhibition sites for rotenone and piericidin A. Thus FOLKERS, CRANE and co-workers[7] have shown that piericidin A inhibits DPNH oxidase activity of mitochondria at very low levels, and that at higher concentrations the succinate oxidase and cytochrome oxidase activity of mitochondria are also affected. JENG AND CRANE[8] have proposed multiple sites for the inhibition of rotenone and piericidin A in a scheme involving separate pathways from DPNH dehydrogenase and succinate dehydrogenase to cytochrome c_1, to ubiquinone and to cytochrome *b*. PALMER *et al.*[9] have suggested two successive sites for the inhibition of rotenone and piericidin A, one site immediately on the substrate side of cytochrome c_1 and another immediately on the substrate side of ubiquinone. In addition, companion studies from SINGER's laboratory[10,11] have shown that both [^{14}C]rotenone and [^{14}C]piericidin A bind to mitochondrial particles in amounts considerably greater than those necessary for complete inhibition of DPNH oxidase activity. Since the study of multiple inhibition sites in an integrated system of consecutive electron transfer such as electron transport particles is not without complication, we have examined the effect of the above inhibitors on the purified complexes of the electron transport system.

Our results summarized in Table I, show that at high concentration, the above inhibitors can affect electron transfer at regions other than the specific site assigned to each. Of special interest are the partial inhibitions of succinate–ubiquinone reductase and succinate–cytochrome *c* reductase activities by high concentrations of demerol, rotenone and piericidin A; the partial inhibition of cytochrome oxidase activity by both rotenone and piericidin A (the latter is in agreement with HALL *et al.*[7]); the complete inhibition of DPNH–ubiquinone reductase activity by 1 mM thenoyltrifluoroacetone; and the profound inhibitory effect of high concentrations of antimycin A on DPNH–ubiquinone reductase, succinate–ubiquinone reductase and cytochrome *c* oxidase segments of the respiratory chain. Piericidin A inhibition of succinate–ubiquinone reductase activity is not surprising as piericidin A has many structural features (planar ring, nontetrahedral ring nitrogen, oxygens *ortho* and *para* to the ring nitrogen, hydrophobic side chain) in common with barbiturates, which have been shown by CHANCE AND HOLLUNGER[12] to inhibit the succinate oxidase activity of mitochondria. Also, the thenoyltrifluoroacetone inhibition of DPNH–

TABLE I

INHIBITION OF THE COMPLEXES OF THE ELECTRON TRANSPORT SYSTEM BY DEMEROL, ROTENONE, PIERICIDIN A, THENOYLTRIFLUOROACETONE AND ANTIMYCIN A

Assay conditions: Complexes I, II, III and IV were prepared as described previously[1-4]. DPNH–ubiquinone reductase, succinate–ubiquinone reductase (succinate → ubiquinone → ferricyanide), succinate–cytochrome *c* reductase and cytochrome oxidase assays were conducted according to refs. 3, 13 and 14, and the enzyme concentrations in 1 ml of reaction mixture at 38° were respectively 5 μg, 2 μg, 4 μg (reconstituted by premixing equal amounts of II and III) and 0.5 μg. The rates were recorded by Beckman DK-2A fitted with a time-drive attachment set at a drive speed of 24 mm/min, and activities were calculated from absorbance changes during the first minute of the reaction. Rotenone, piericidin A, thenoyltrifluoroacetone and antimycin A were added in a volume of 10–30 μl of ethanol and in each case the results were calculated on the basis of appropriate ethanol controls. Except where a range has been given, variations in inhibition or activation in different experiments were about ± 5%. Complex III was not assayed directly because the substrate QH_2-2 reacts non-enzymatically with cytochrome *c* at a rate rapid enough to complicate detection of small inhibition effects. However, the difference between columns 4 and 5 provides an indication of the effect of inhibitors on complex III (see, for example, the inhibition of antimycin A). A plus sign denotes activation, and roman numerals refer to complexes I, II, III and IV of the electron transport system.

Inhibitor	*Concn.* (μ*M*)	*Percent inhibition or activation*			
		DPNH $\xrightarrow{(I)}$ *Q-1*	*Succinate* $\xrightarrow{(II)}$ *Q*	*Succinate* $\xrightarrow{(II\text{-}III)}$ *cyt. c*	*Cyt. c* $\xrightarrow{(IV)}$ O_2
Demerol	2 000	100	7.0	17	+24
	10 000	100	17	24	+17–30
	20 000	100	32	34	+11
	30 000	100	37	53	+ 8
Rotenone	1	100	7	5	+12
	10	100	—	15	+ 3
	50	100	25	43	25–40
Piericidin A	1	100	5	8	6
	10	100	32	10	32
	20	100	40	27	36
Thenoyltrifluoroacetone	1	2	9	4	+ 3
	10	2	42	28	+ 6
	100	51	70	71	+ 8
	1000	100	90	90	+12
Antimycin A	1	2*	9*	100	8
	10	8*	20–40*	100	5–23
	100	42–56*	63*	100	15–40

* Progressive with time.

ubiquinone reductase activity might be related to the iron-chelating property of this compound. It should be noted, however, that at low concentrations (1 μM or 250 nmoles/mg enzyme) rotenone and piericidin A have very little effect on the electron transfer system of complex III (compare columns 4 and 5 of Table I). Also, when the concentrations of these compounds per mg of enzyme were made comparable to those used by PALMER *et al.*[9], then no inhibition was observed in either the succinate–ubiquinone reductase or the succinate–cytochrome *c* reductase systems. However, since by necessity the electron spin resonance experiments of PALMER *et al.*[9] were conducted at high protein concentration, it is possible that the molar concentration of rotenone and piericidin A (10–30 μM range) had been a factor in the partial inhibitions reported by them near the cytochrome c_1 region.

The above results obtained under defined conditions and with the use of purified, highly active complexes of the electron transport system indicate, therefore, that at high concentrations, demerol, rotenone, piericidin A, thenoyltrifluoroacetone and antimycin A are all capable of inhibiting electron transfer in more than one region of the respiratory chain, and that only at low concentrations of the inhibitors single-site specificity can be achieved.

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