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ON THE MECHANISM OF ACTION OF ANESTHETICS

DIRECT INHIBITION OF MITOCHONDRIAL F_1-ATPase BY *n*-BUTANOL AND TETRACAINE

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Summary

The concentrations of *n*-butanol and tetracaine required for 50% inhibition of the ATPase activity of F_1 particles isolated from bovine heart mitochondria were 160 mM and 1.1 mM, respectively. The results are offered as evidence that the physiological effects of these anesthetics may be due to direct interaction with membrane proteins rather than with the lipids.

The question of whether anesthetics affect the activities of membrane enzymes by direct interaction or through their effects on the lipid bilayer remains unresolved. Richards et al. [1] have argued that the nerve blocking action of *n*-alkanols must result from their direct interaction with neural proteins rather than with the lipids. Lenaz et al. [2—4], on the other hand, contend that the enzyme inhibitory actions of anesthetics are caused indirectly by the perturbation of the lipid bilayer environment of membrane proteins. Other workers (notably Lee [5] and Trudell [6]) have likewise proposed lipid perturbation models, whereas Eyring et al. [7] have argued in favor of a direct interaction between anesthetics and proteins.

Lenaz et al. [2—4] studied the ATPase activity of bovine heart mitochondria as a function of the concentrations of *n*-butanol and other general anesthetics. Their mechanistic proposals were based largely on the finding that similar concentrations of anesthetics which gave physically observable changes in the

lipid properties of mitochondrial membranes also caused inhibition of mitochondrial ATPase activity [4].

We reasoned that since the mitochondrial ATPase activity resides on F_1, the inner membrane-associated 'headpieces', a clear test to differentiate between direct and indirect actions of anesthetics on this enzyme should be possible. F_1 can be readily isolated in an enzymically active but water soluble, lipid free form. If lipid is obligatorily involved in ATPase inhibition by anesthetics, then no inhibition should be found with F_1 alone. We prepared F_1 and found that it is indeed inhibited, not only by *n*-butanol but also by the common local anesthetic tetracaine.

F_1 was prepared by the method of Beechey et al. [8] from sonic submitochondrial particles [9] derived from bovine heart mitochondria [10]. The ATP regenerating system of Pullman et al. [11] was used to measure ATPase activity. The assay mixture consisted of 50 mM Tris acetate (pH 7.5), 4 mM $MgCl_2$, 2.5 mM phospho*enol*pyruvate, 3.5 mM NADH, 0.5 mM ATP, 0.4 μM rotenone, 5.2 μg lactate dehydrogenase, 4.5 μg pyruvate kinase, and aliquot of F_1 preparation, anesthetic and water, to a final volume of 0.1 ml. The enzyme reaction was initiated by the final addition of either ATP or F_1. In the former case, the F_1 was preincubated for 2 min in the presence of anesthetics. The same results were obtained with both orders of addition, except for a brief (15 s) lag time when F_1 was added last. The enzyme reaction was followed spectrophotometrically at 340 nm in experiments involving *n*-butanol, but at 355 nm when tetracaine was present in order to avoid the strong ultraviolet absorbance of the latter compound.

All biochemical reagents were obtained from Sigma.

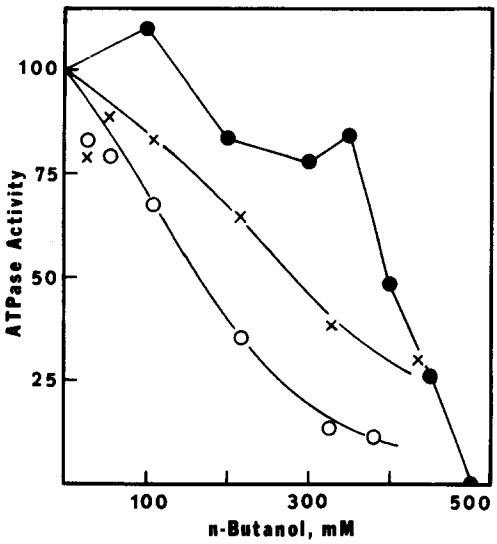
The ATPase activity of F_1 is given as a function of the *n*-butanol concentration in Fig. 1. This figure also includes the data of Lenaz et al. [4] for the inhibition of mitochondrial ATPase by *n*-butanol. It can be seen that *n*-butanol inhibits the extracted, lipid free F_1-ATPase at the same or lower concentrations as it inhibits membrane bound mitochondrial ATPase. 160 mM butanol gave 50% of inhibition at 25°C.

The effect of tetracaine or F_1 is given in Fig. 2. We see here that this local anesthetic strongly inhibits F_1 at low concentrations, but at higher concentrations a plateau of residual activity is reached at about 45% of the original activity. 1.1 mM gave 50% inhibition at both 25 and 35°C.

Control experiments were carried out at 25°C in the presence and absence of *n*-butanol or tetracaine, using ADP in place of ATP and F_1, to make sure that the ATPase step rather than the ATP regenerating system was rate limiting under all conditions. We found that *n*-butanol, but not tetracaine, partially inhibited the regenerating system. Under all conditions, however, the rate of ATP regeneration was 4- to 10-times that of ATP hydrolysis.

We do not have direct evidence on whether the inhibitory reactions are reversible, but reversibility is suggested by the observation that a constant reaction rate was achieved rapidly (within 15 s) after mixing the F_1 with the anesthetic-containing assay solution, and this rate remained constant for 90 s or longer.

Our findings show that *n*-butanol and tetracaine inhibit F_1 directly, without the mediation of lipid. Chlorpromazine also inhibits F_1, as reported by



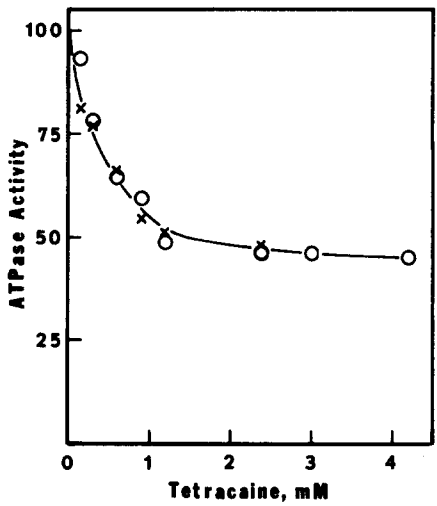


Fig. 1. Inhibition of F_1-ATPase activity by *n*-butanol. The percent activity in the presence of *n*-butanol as compared to that in its absence is plotted versus the *n*-butanol concentration. Each point represents the average of three to five determinations. Symbols: o———o, F_1 at 25°C; X———X, F_1 at 35°C. •———•, ATPase activity of bovine heart mitochondria at 36°C; taken from Lenaz et al. [4].

Fig. 2. Inhibition of F_1-ATPase activity as a function of tetracaine concentration. Symbols: o———o, 25°C; X———X, 35°C.

Penefsky et al. [12], with 0.04 mM giving 50% inhibition at 30°C. Our results do no disagree with those of Lenaz et al. [2—4], who showed that *n*-butanol and other nonionic anesthetics inhibit membrane bound mitochondrial ATPase, but our observations do do provide a simpler explanation to their data. It is rather difficult to comprehend how anesthetic molecules dissolved in the lipid bilayer could affect the extrinsic F_1 protein, unless it were through an indirect effect mediated by the F_0 hydrophobic membrane component. Now we can see that the anesthetic molecules dissolved in the lipid bilayer probably have nothing to do with the enzyme inhibition, since the inhibition of the oligomycin insensitive ATPase activity is entirely accounted for by direct anesthetic-F_1 interactions.

The temperature dependencies of *n*-butanol and tetracaine inhibition of F_1 are quite different, and also differ from what we have found with other systems, viz., the succinate oxidase [13] and cytochrome *c* oxidase activities (Chazotte, B. and Vanderkooi, G., unpublished results) of submitochondrial particles. For the latter activities, the inhibitory potency of all anesthetics tested increased with temperature, but *n*-butanol inhibition of F_1 decreases with temperature, while the tetracaine inhibition of F_1 appears to be insensitive to temperature over the limited range investigated. This peculiar temperature dependence might be related to the cold lability of F_1 [11]. Another unexpected observation was that roughly 45% of the F_1-ATPase activity appears to be insensitive to inhibition by tetracaine. This may mean that tetracaine induces a conformational change to a less active rather than inactive

state. By contrast, elevated concentrations of *n*-butanol gave complete inactivation. These points will be investigated in greater detail in the future.

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