to ionic influences (*e.g.* Ca^{2+}) and to some uncoupling agents (*e.g.* valinomycin) than intact mitochondria. It is apparent from the findings that the type and concentration of externally added cations and anions may profoundly influence the phosphorylating efficiency of submitochondrial particles and their affinity for ADP and P_i.

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1 S. PAPA, J. M. TAGER, F. GUERRIERI AND E. QUAGLIARIELLO, *Biochim. Biophys. Acta*, 172 (1969) 184.
2 E. RACKER AND L. HORSTMAN, *J. Biol. Chem.*, 242 (1967) 2547.
3 E. RACKER, *Federation Proc.*, 26 (1967) 1335.
4 R. E. BEYER, D. L. CRANKSHAW AND J. M. KUNER, *Biochem. Biophys. Res. Commun.*, 28 (1967) 758.
5 C. P. LEE AND L. ERNSTER, in J. M. TAGER, S. PAPA, E. QUAGLIARIELLO AND E. C. SLATER, *Regulation of Metabolic Processes in Mitochondria*, BBA Library Vol. 7, Elsevier, Amsterdam, 1966, p. 218.
6 A. LOYTER, R. O. CHRISTIANSEN AND E. RACKER, *Biochem. Biophys. Res. Commun.*, 29 (1967) 450.
7 M. HANSEN AND A. L. SMITH, *Biochim. Biophys. Acta*, 81 (1964) 214.
8 I. VALLIN, *Biochim. Biophys. Acta*, 162 (1968) 477.
9 N. E. GOOD, G. D. WINGET, W. WINTER, T. N. CONNOLLY, S. IZAWA AND R. M. M. SINGH, *Biochemistry*, 5 (1966) 467.
10 R. E. MCCARTY AND E. RACKER, *J. Biol. Chem.*, 243 (1968) 129.
11 T. E. CONOVER, R. L. PRAIRIE AND E. RACKER, *J. Biol. Chem.*, 238 (1963) 2831.
12 G. SCHATZ AND E. RACKER, *J. Biol. Chem.*, 241 (1966) 1429.
13 C. MOORE AND B. PRESSMAN, *Biochem. Biophys. Res. Commun.*, 15 (1964) 562.
14 M. MONTAL, B. CHANCE, C. P. LEE AND A. AZZI, *Biochem. Biophys. Res. Commun.*, 34 (1969) 104.
15 R. COCKRELL, *Federation Proc.*, 28 (1969) 1200.
16 P. MITCHELL, *Biol. Rev. Cambridge Phil. Soc.*, 41 (1966) 445.
17 B. C. PRESSMAN, *Federation Proc.*, 27 (1968) 1283.
18 J. B. CHAPPELL AND K. N. HAARHOFF, in E. C. SLATER, Z. KANUIGA AND L. WOJTCZAK, *Biochemistry of Mitochondria*, Academic Press, London, 1967, p. 75.

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BBA 41 139

The effect of some local anesthetic compounds on sarcotubular calcium transport

Local anesthetics have been shown to have varied effects on the contractility and calcium fluxes of skeletal muscle. FEINSTEIN[1] showed that the caffeine-induced contracture of frog skeletal muscle, along with the accompanying calcium efflux, was blocked by procaine and tetracaine. KUPERMAN *et al.*[2] observed that higher concentrations of the same compounds caused a release of calcium from frog skeletal muscle. BIANCHI AND BOLTON[3] and WEISS[4] demonstrated that if the intracellular pH was elevated the local anesthetics themselves were able to induce contracture, thus suggesting that the specific effect of these compounds was a function of the relative concentrations of the charged and uncharged forms.

The effect of the local anesthetics on mammalian skeletal muscle appears to be different. ISAACSON *et al.*[5], working with rat muscle, have reported that procaine mimics the effect of caffeine in that it produces a contracture which is more marked at 37° than at 20°.

Since the release of calcium from the sarcoplasmic reticulum appears to be the crucial link between excitation and contraction it is reasonable to suppose that the local anesthetic compounds are able to interact with the calcium transport system of the sarcoplasmic reticulum[3,6]. In this report we show that dibucaine, tetracaine, procaine, and lidocaine can cause a profound inhibition of active calcium transport by isolated sarcotubular vesicles.

All experiments were performed with a 10000–30000 × *g* centrifugal fraction of rabbit skeletal muscle, prepared as described elsewhere[7]. The rate of accumulation of calcium was measured by the Millipore filtration technique[8]. Incubations were carried out at 25° in a medium containing 100 mM KCl, 20 mM imidazole (pH 7.0), 4 mM $MgCl_2$, 4 mM potassium oxalate, 0.20 mM $^{45}CaCl_2$, 4 mM ATP, and ethylene glycol bis-(β-aminoethylether)-*N*,*N*'-tetraacetic acid (0.24 mM) sufficient to adjust the ionized calcium to a physiological level of approx. 10^{-6} M (ref. 9). Protein concentration, as determined by the method of LOWRY *et al.*[10] was 0.02 mg/ml.

The effects of tetracaine (1.5 mM) and dibucaine (0.35 mM) on the kinetics of calcium uptake are shown in Fig. 1. Illustrated in Fig. 2 are the concentration–response relationships for dibucaine, tetracaine, procaine, and lidocaine. The concentrations required for half-maximal inhibition ranged from about 0.3 mM for dibucaine to 15–20 mM for procaine and lidocaine. These concentrations are not very different from those employed by ANDERSEN AND GRAVENSTEIN[11] to demonstrate inhibition of Na^+ and K^+ transport in erythrocytes. However, they are generally higher than the concentrations reported to block conduction in excitable membranes[12,13].

CARVALHO[14] has recently reported that tetracaine, but not procaine, could displace a fraction of the bound calcium from sarcotubular membranes. These obser-

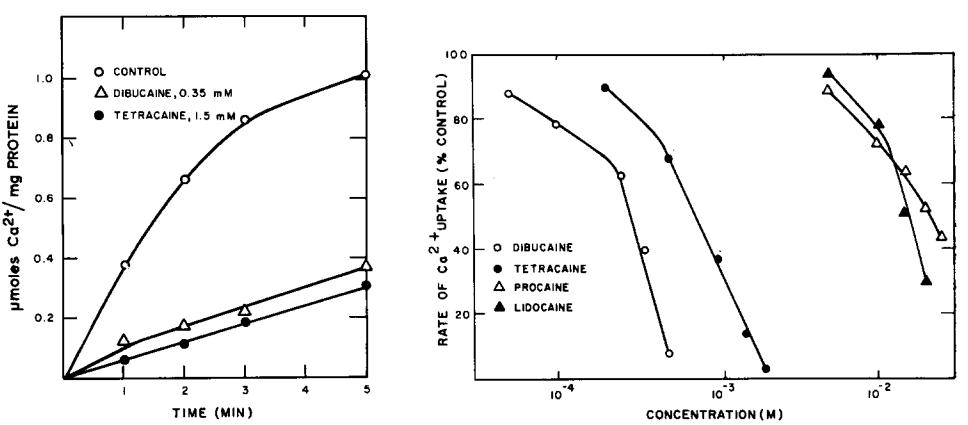

Fig. 1. The effect of dibucaine and tetracaine on the rate of calcium accumulation by sarcotubular vesicles from skeletal muscle. The reaction was started by the addition of ATP (4 mM) at time 0. See text for experimental conditions.

Fig. 2. The relationship between inhibition and concentration for the four local anesthetic compounds studied. Experimental conditions were identical to those in Fig. 1.

vations are not directly comparable to our own because of the rather considerable differences in experimental procedure. However, the failure of CARVALHO to observe any effect of procaine may simply reflect the fact that it is relatively inactive in the concentration range (2–5 mM) which he studied.

BIANCHI AND BOLTON[3] (see also ref. 4), on the basis of studies with intact frog muscles, have suggested that it is the uncharged form of the drug which interferes with the sequestration of calcium by the sarcoplasmic reticulum. While this hypothesis may be valid, other factors must be involved since, as shown in Fig. 2, there was no apparent relationship between inhibitory activity and pK. Thus dibucaine (pK 8.54) and tetracaine (pK 8.49) were much more active than procaine (pK 8.95) and lidocaine (pK 7.85). At pH 7.0 about 15% of the lidocaine and 1% of the procaine exist as the free base as compared with about 5% of the dibucaine and tetracaine. It is probable that differences in lipid solubility of the free base are also of importance[15].

The data provide evidence that the contracture-producing effects of the local anesthetic compounds are related to an inhibition of sarcotubular calcium transport. More detailed studies are in progress on the mechanism of inhibition. With more information becoming available on the biochemistry of the sarcotubular membranes[16,17] this system may prove to be extremely useful for studying the molecular interaction between anesthetic compounds and biological membranes.

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1 M. B. FEINSTEIN, *J. Gen. Physiol.*, 47 (1963) 151.
2 A. S. KUPERMAN, B. T. ALTURA AND J. A. CHEZAR, *Nature*, 217 (1968) 673.
3 C. P. BIANCHI AND T. C. BOLTON, *J. Pharmacol. Exptl. Therap.*, 157 (1967) 388.
4 G. B. WEISS, *J. Pharmacol. Exptl. Therap.*, 160 (1968) 148.
5 A. ISAACSON, S. R. TAYLOR AND M. J. HINKES, *Federation Proc.*, 27 (1968) 443.
6 C. P. BIANCHI, *Federation Proc.*, 27 (1968) 126.
7 F. FUCHS, E. W. GERTZ AND F. N. BRIGGS, *J. Gen. Physiol.*, 52 (1968) 955.
8 A. MARTONOSI AND R. FERETOS, *J. Biol. Chem.*, 239 (1964) 648.
9 S. CHABEREK AND A. E. MARTELL, *Organic Sequestering Agents*, John Wiley, New York, 1959.
10 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. L. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
11 N. B. ANDERSEN AND J. S. GRAVENSTEIN, *J. Pharmacol. Exptl. Therap.*, 147 (1965) 40.
12 J. C. SKOU, *Acta Pharmacol. Toxicol.*, 10 (1954) 281.
13 J. M. RITCHIE AND B. R. RITCHIE, *Science*, 162 (1968) 1394.
14 A. P. CARVALHO, *J. Gen. Physiol.*, 52 (1968) 622.
15 C. P. BIANCHI AND G. E. STROBEL, *Trans. N.Y. Acad. Sci.*, 30 (1968) 1082.
16 A. MARTONOSI, J. DONLEY AND R. A. HALPIN, *J. Biol. Chem.*, 243 (1968) 61.
17 B. P. YU, F. D. DEMARTINIS AND E. J. MASORO, *J. Lipid Res.*, 9 (1968) 492.

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