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The Relation between Calcium Ion Transport and Adenylate Cyclase Activity in Myocardial Sarcoplasmic-Reticulum Preparations

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The regulation of Ca^{2+} concentration in relation to myofibrils plays a central role in the production of contraction and relaxation in cardiac muscle (Langer, 1968). The calcium pump of the sarcoplasmic reticulum is a major factor producing relaxation by removing Ca^{2+} from the region of the myofibrils, and may secondarily produce changes in Ca^{2+} release on activation leading to alterations in myocardial contraction. Cyclic AMP (adenosine 3':5'-cyclic monophosphate) has been shown to stimulate the calcium pump (Epstein *et al.*, 1970; Shinebourne & White, 1970), and variations in the production of cyclic AMP by adenylate cyclase may form the basis of a regulating mechanism.

We have measured Ca^{2+} uptake (Gertz *et al.*, 1967) and adenylate cyclase activity (Krishna *et al.*, 1968) in the same preparations of fresh sarcoplasmic reticulum from canine myocardium. Ca^{2+} uptake was measured in the presence of oxalate and imidazole in duplicate at 1 min intervals, and the mean uptake over 5 min was used in the calculation. Under these con-

ditions Ca^{2+} is known to cross the membrane physically to storage sites inside the vesicle (Hasselbach & Makinose, 1963). The determinations were repeated in the presence of 1 mM-noradrenaline. Adenylate cyclase activity was enhanced by adding 10 mM-NaF and was calculated from the mean gradient of duplicate values at 5 and 10 min. The absence of mitochondrial contamination was confirmed by electron microscopy and by the absence of succinate dehydrogenase activity.

Noradrenaline was shown to increase adenylate cyclase activity significantly, even in the presence of F^- . The molar ratio of Ca^{2+} transported to cyclic AMP formed averaged 189 in controls and 257 in the presence of noradrenaline. On the basis of the adenylate cyclase activity and the time at which the rate of Ca^{2+} uptake reached equilibrium, the maximal density of β -adrenergic receptor sites in the sarcoplasmic-reticulum membrane was calculated to be $1/25000 \text{ sq. } \text{\AA}$. The enhancement of F^- stimulation of adenylate cyclase by noradrenaline and the independent increase in activity produced by glucagon (Entman *et al.*, 1969) suggest that adenylate cyclase activity in the sarcoplasmic-reticulum membrane can be regulated in several different ways.

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