ADENYL CYCLASE ACTIVITY IN THE CHRONICALLY DENERVATED CAT HEART

Burton E. Sobel*, Peter J. Dempsey, and Theodore Cooper

Cardiology Branch
National Heart Institute
Bethesda, Maryland 20014

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Denervation supersensitivity, the enhanced responsiveness of denervated tissue to neuro-transmitter substances, is a well recognized biologic phenomenon. In the heart, the loss of binding sites for norepinephrine (the adrenergic nerve terminals) appears to be a major determinant of denervation supersensitivity (Cooper et al., 1967; Trendelenburg, 1966), althoug other alterations such as changes in the postsynaptic myoneural junction (Dempsey and Cooper, 1968) or in the synthesis of specific enzymes (Yellin, 1967) may be partly responsible for the phenomenon. Recent work has suggested that 3',5'-adenosinemonophosphate (cyclic-AMP) is intimately related to the beta adrenergic receptor mechanism, and that synthesis and degradation of this compound are regulated by adenyl cyclase and phosphodiesterase respectively (Sutherland et al., 1965). Since heart muscle demonstrates predominantly beta adrenergic effects and since altered adenyl cyclase or phosphodiesterase activity might play a role in myocardial denervation supersensitivity, an investigation of the activity of these enzymes in extrinsically denervated cat myocardium was undertaken. Although denervated tissue manifested catecholamine depletion and physiologic evidence of supersensitivity, activity of adenyl cyclase and phosphodiesterase remained normal.

Materials and methods: Tritiated adenosine-5' triphosphate (ATP) was obtained from Schwarz BioResearch; cyclic-AMP, ATP, and crystalline bovine serum albumin from Sigma;

*Present address: Department of Medicine, University of California at San Diego, San Diego, California 92037

Dowex (Bio Rad AG-50W X-4, 200-400 mesh) from Cal Biochem; theophylline and epinephrine bitartrate from K & K Laboratories; and BBOT from Packard.

Extrinsic denervation of the hearts of male cats (2.3 to 3.5 kg) was performed by mediastinal neural ablation under halothane anestheria (Cooper et al., 1961). Animals were sacrificed nine to forty-five days postoperatively when one-gram portions of apical left ventricular myocardium were rapidly obtained and immersed in 0.25 M sucrose at 0.4°C. Control tissue was studied simultaneously.

Myocardial catecholamines were determined fluorometrically (Crout et al., 1961) and protein was determined by the Lowry procedure (1951).

Adenyl cyclase activity was measured by the method of Krishna et al. (1968; 1966). The tissue was minced, passed through a tissue press (1 mm holes), homogenized in 15 volumes of magnesium sulfate, .001 M; glycylglycine pH 7.5, .002 M, in a VirTis "45" and subsequently in a Dounce homogenizer, filtered through a single layer of 60 mesh cheesecloth, centrifuged at 2000 g for fifteen minutes, and the twice washed residue resuspended (protein concentration 8 mg/ml). Adenyl cyclase activity was determined in a medium containing Tris HCl, pH 7.3, 4×10^{-2} M; $MgCl_2$, 3.3×10^{-3} M; ^{3}H-ATP, 2.0×10^{-3} M, 50 μc/μmole; theophylline, 1.0×10^{-2} M; and 100 μl of tissue particle suspension (final volume = 0.6 ml. T = 30°C.). Sodium fluoride, 1.0×10^{-2} M, or epinephrine bitartrate, 1.0×10^{-4} M, were added as activators. Reactions were terminated at two-minute intervals by the addition of 0.5 mg of carrier cyclic-AMP and immersion in a boiling water bath for three minutes. Heat denatured enzyme samples were run as blanks. Tritiated cyclic-AMP was separated by chromatography on Dowex-50 H+ and barium zinc precipitation exactly as described by Krishna et al. (1968). Aliquots of the final supernatant fraction were counted in a BBOT phosphor mixture in a Packard liquid scintillation spectrometer and used to calculate recovery ($A_{M_{260}}$ of cyclic-AMP = 14.3×10^3)

Phosphodiesterase activity was assayed by rate of hydrolysis of cyclic 3',5'-AMP. A 100 μl aliquot of the initial tissue homogenate (0.6 mg protein) was added to a reaction

mixture containing Tris HCl, pH 7.4, 4×10^{-2} M; $MgCl_2$, 2×10^{-3} M; and cyclic 3',5'-AMP, 4×10^{-4} M in a final volume of 1 ml. Incubations were carried out at 37°C. and terminated by addition of 1 ml of 2% $ZnSO_4$ and 1 ml of 1.8% $Ba(OH)_2$. Rate of hydrolysis was determined by measuring A_{260} of the supernatant fraction after centrifugation at 4000 X g for ten minutes.

Results and discussion: Particulate fractions from denervated cat myocardium accumulated cyclic-AMP at the same rate as comparable fractions from normal tissue (Table 1).

TABLE 1

Cyclic-AMP Accumulation by Denervated and Control Cat Heart Homogenates

Experiment		Basal	Epinephrine (10^{-4} M)	NaF (10^{-2} M)
1				
	Denervated	385	2461	4044
	Control	362	2367	4175
2				
	Denervated	371	2130	3765
	Control	354	2093	3689
3				
	Denervated	309	1878	3891
	Control	326	1962	3764

Results are CPM accumulated in four minutes by homogenates containing 0.8 mg protein. Values are corrected for heat denatured enzyme blanks and are averages of duplicate determinations.

In addition, the increment in adenyl cyclase activity in the presence of fluoride or epinephrine was normal. The time course of the reaction was the same for denervated tissue and normal myocardium. When fluoride was present in the incubation medium, both preparations accumulated cyclic-AMP linearly for four minutes (approximately 80 picomoles/mg protein/minute). Subsequently, there was a similar decrease of rate in both, probably because of phosphodiesterase activity in the particulate fraction used.

The rate of degradation of cyclic-AMP, a measure of the activity of phosphodiesterase, was the same in homogenate derived from denervated and from normal cat myocardium. Both preparations hydrolyzed cyclic-AMP at a rate of 10 nmoles/mg protein/minute and the reactions were linear for at least 30 minutes. Differences between control and experimental preparations were consistently less than five percent.

Myocardial catecholamine concentration was measured in six denervated and six normal hearts. The concentration in right ventricular samples was $0.104 \pm .04$ μg/g. In normal cat myocardium, the concentration was 1.866 ± 0.031 μg/g. In addition, whole hearts denervated in the same manner and studied by means of an isolated perfusion system have been shown to be supersensitive to norepinephrine and unresponsive to tyramine (Dempsey and Cooper, 1968). This evidence substantiates that the surgical procedure led to virtually complete interruption of adrenergic nerve supply.

The data presented suggest that myocardial production and degradation of cyclic-AMP are not altered by denervation. Since total adenyl cyclase activity, activation of the enzyme by catecholamines, and phosphodiesterase activity in vitro remain normal, it appears that denervated myocardium retains a normal capacity to produce cyclic-AMP in response to adrenergi neuro-transmitters and to degrade cyclic-AMP at a normal rate. Thus, it appears unlikely that altered metabolism of cyclic-AMP contributes to the observed supersensitivity of the denervated heart to exogenous catecholamines.

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REFERENCES

Brodie, B. B., Davies, J. I., Hynie, S., Krishna, G. and Weiss, B., Pharm. Rev. 18, 273 (1966).

Cooper, T., Gilbert, Jr., J. W., Bloodwell, R. D. and Crout, J. R., Circulation Res. 9, 275 (1961).

Cooper, T., Willman, V. L. and Hanlon, C. R., in "Factors Influencing Myocardial Contractility," Tanz, R. D., Kaveler, F. and Roberts, J., eds., Academic Press, New York, 1967.

Crout, J. R., Creveling, C. R. and Udenfriend, S., J. Pharm. Exper. Ther. 132, 269 (1961).

Dempsey, P. J. and Cooper, T., Am. J. Physiol. In press (1968).

Krishna, G., Weiss, B. and Brodie, B. B., J. Pharm. Exper. Ther. In press (1968).

Lowry, D. H., Rosebrough, N. J., Farr, A. L. and Randall, P. J., J. Biol. Chem. 238, 3899 (1951).

Sutherland, E. W., Øye, I. and Butcher, R. W., Recent Progr. Hormone Res. 21, 623 (1965).

Trendelenburg, U., Pharmacol. Rev. 18, 629 (1966).

Yellin, H., Exper. Neurol. 19, 92 (1967).