

# EVALUATION OF ADENYLATE CYCLASE ACTIVITY IN MITRAL VALVE PROLAPSE

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## ABSTRACT

The term mitral valve prolapse (MVP) is used for a particular subset of patients with hyperadrenergic dysautonomia. It occurs when part of a leaflet or both leaflets of the mitral valve extend above the plane of the atrioventricular junction during ventricular systole. The adenylate cyclase activity in MVP dys-autonomia was studied by extraction of enzyme from the erythrocytes from 62 normal subjects and 78 MVP patients. Adenylate cyclase activity in the MVP group was increased compared to that in controls [ $6.51 \pm 0.38$  (mean  $\pm$  S.D.,  $n=78$ ) vs.  $2.76 \pm 0.12$ ,  $n=62$ , U activity/mg erythrocyte protein,  $p<0.05$ ]. The determination of adenylate cyclase activity in combination with echocardiography allows a reliable diagnosis of MVP patients.

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## INTRODUCTION

Mitral valve prolapse (MVP) is primary and is due to an inherited anomaly of the mitral valve leaflets and their supporting chorda tendineae. Among the several forms of hyperadrenergic dysautonomias, there is a particular subset that has been distinguished based upon a distinctive pattern of abnormal cardiovascular autonomic function.<sup>1</sup> No satisfactory term exists for this subtype, but one of the most commonly applied terms is MVP dysautonomia. This term reflects the historical notion that the dysautonomia is somehow associated with findings of MVP. This dysautonomia is characterized subjectively by hyperadrenergic activity, exercise intolerance, asthenia, chest pain, dyspnea, syncope or presyncope, orthostatic palpitations or weakness, migraine, and symptoms of vasospasm.<sup>1,2</sup> Physiologically demonstrable  $\beta$ -adrenergic receptor hypersensitivity contributes to the cardiovascular abnormalities and is related biochemically to supercoupling of isoproterenol stimulated  $\beta$ -adrenergic receptor coupling

which requires the mediation of the stimulatory guanine nucleotide regulatory protein.<sup>2</sup> Previous data suggest different degrees of autonomic involvement in mitral valve prolapse which may be related to the various degrees of arrhythmia which seem to contribute to their symptoms.<sup>3</sup> This subset of hyperadrenergic dysautonomia patients therefore has supercoupled beta 2-adrenergic receptors conferred by an abnormal Gs, whose effects on agonist binding reflect the severity of illness.<sup>2,3</sup> Although MVP is a common valvular abnormality, its diagnostic classification has not been well defined. MVP is considered to be an anatomic entity with specific pathologic tissue characteristics and pathophysiologic consequences.<sup>4</sup> Autonomic nervous system dysfunction has recently been identified in a subset of patients with MVP. These autonomic nervous system abnormalities may correspond, in part, to biochemical alterations in beta-adrenergic receptors.<sup>5,6</sup> It seems that beta-adrenergic receptor hyperactivity contributes to the cardiovascular abnormalities and is related to the supercoupling of the stimulated beta-adrenergic receptors

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to the enzyme adenylate cyclase. The current study addressed the specific hypothesis that the beta-adrenergic receptor supercoupling and supersensitivity observed in this subset of hyperadrenergic dysautonomia is a consequence of a functional abnormality in adenylate cyclase enzyme activity. The purpose of this study was to determine a very sensitive indicator for diagnosing MVP patients. This report may lead to a better understanding for the detection of MVP.

### MATERIALS AND METHODS

#### Materials

Sodium phosphate, guanylyl-5-imidodiphosphate (GppNHp), isoproterenol, Tris-HCl, NaCl, NaF, EDTA, KCl,  $MgCl_2$ , GTP, ATP, NADP,  $CaCl_2$ , phosphoenolpyruvate, dithiothreitol, bovine serum albumin, apyrase, NaOH, nucleotidase, adenosine deaminase were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other reagents and solvents were of analytical grade and purchased from E. Merck, Germany.

#### Patients

Seventy-eight patients between the age of 23 and 53 years with mitral valve prolapse and 62 healthy controls between the age of 27 and 49 years were studied. The normal control group was comprised of asymptomatic individuals who had no clinical evidence of MVP dysautonomia. The patient group had dysautonomic features including orthostatic palpitations, chest pain, and headaches. Each member of this group demonstrated characteristics of MVP dysautonomia. All underwent 24 h ECG recording, 2-D echocardiography and psychological assessment.

#### Membrane preparation

Twenty milliliters of whole blood was added to tubes containing 0.1 mM EDTA, mixed on a mechanical stirrer, and transported on ice. The blood was mixed with thirty milliliters of ice buffer (5 mM sodium phosphate at pH 7.4 and 50 mM NaCl at 10°C). The mixture was centrifuged at 3000 g at 10°C for 20 min and the supernatant was discarded. The erythrocyte pellets were resuspended and centrifuged four more times with phosphate-saline buffer for 20 min at 5000 g and 10°C. Membranes were collected by centrifugation, and the final membrane pellets were stored at -20°C in aliquots until enzyme activity was measured.

#### Adenylate cyclase activity

Measurement of adenylate cyclase activity was performed according to the procedure described by Wiegand et al., with minor modifications.<sup>7</sup> A volume of 5  $\mu$ L water, 0.1 mM NaF, 0.001 mM GppNHp, and 0.01 mM isoproterenol was added to each reaction tube and maintained at 4°C. Afterwards 50  $\mu$ L of reaction mixture (50 mM Tris acetate, pH 7.2, 30 mM KCl, 5 mM  $MgCl_2$ , 30 mM phosphoenol

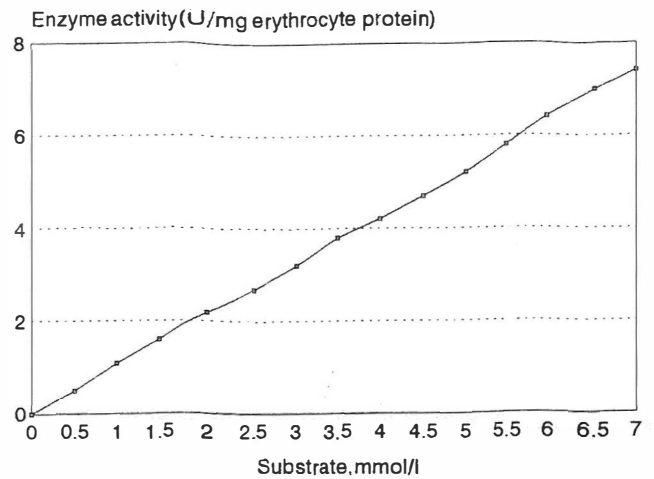


Fig. 1. Calibration curve for measurement of adenylate cyclase activity. Each point is the result of 10-12 tests, each assayed in duplicate with a *p* value of less than 0.05.

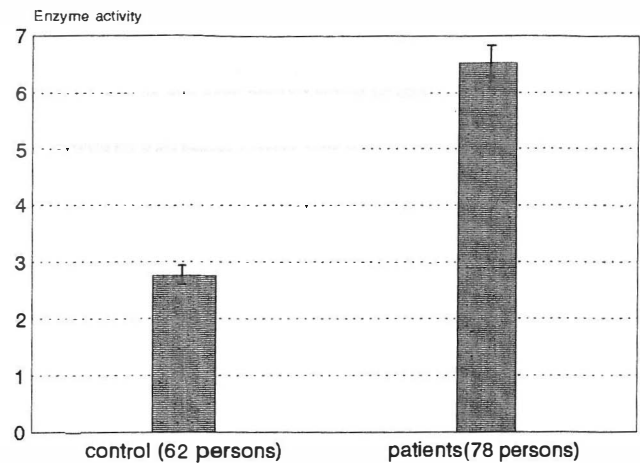


Fig. 2. Adenylate cyclase activity in normal volunteers and MVP patients. Student's *t*-test performed on the absolute values for control and MVP patients. Each column represents the mean value. Bars indicate  $\pm$  S.D. Each assayed in duplicate with a *p* value of less than 0.05.

pyruvate, 4.0 mM ATP, 0.03 mM GTP, 5.0 mM dithiothreitol, 0.05% bovine serum albumin, 0.3 mM theophylline, 2.0 mg/mL pyruvate kinase) was added to each reaction tube. Finally, 50  $\mu$ L of erythrocyte membrane suspension (70-90  $\mu$ g protein) was added to each tube and the reaction was initiated by placing the tubes in a water bath at 37°C. After 60 min at 37°C the reaction was stopped by the addition of 100  $\mu$ L of 60 mM NaOH. The reaction mixture was heated for 10 min at 100°C. A volume of 50  $\mu$ L of reaction product was added to 200  $\mu$ L of cleaning reaction mix (100 mM Tris-HCl, pH 7.50, 8 mM  $MgCl_2$ , 3 mM  $CaCl_2$ , 0.03 units/mL nucleotidase, 0.3 units/mL apyrase, and 0.2 mg/mL adenosine deaminase). After 60 min at 37°C the reaction was terminated by heating at 100°C for 10 min. A volume of 500  $\mu$ L of

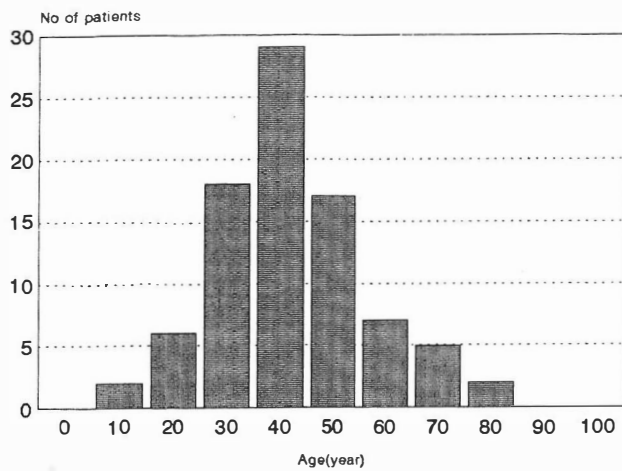


Fig. 3. Mitral valve prolapse symptoms are plotted against patient age in years. Student's t-test performed on the absolute values for control and MVP patients. Each point is the result of 10-12 experiments. Each assayed in duplicate with a  $p$  value of less than 0.05.

cAMP mix was added to the product. After 60 min at 37 °C, 500  $\mu$ L of 2-amino-2-methyl-1-propanol buffer (100 mM, pH 7.2) was added to the reaction mix and the final concentration of NADPH was measured fluorometrically (Cecil model CE 1020).

#### Statistical analysis

Student's t-test was used for between group comparison of results, and  $p < 0.05$  was considered to indicate statistical significance.

### RESULTS

The calibration curve for measurement of adenylate cyclase activity is shown (Fig. 1). The measuring range of substrate concentration extended from 0.0-5.0 mmol/L. Fig. 2 presents a summary of adenylate cyclase activities of the erythrocyte membranes in normal control and MVP patients. Adenylate cyclase activity in the MVP dysautonomia patient group was increased compared to that in controls [ $6.51 \pm 0.38$  (mean  $\pm$  S.D.,  $n = 78$ ) vs.  $2.76 \pm 0.12$ ,  $n = 62$ ,  $p < 0.05$ , U activity/mg erythrocyte protein]. As shown in Fig. 3 the greatest proportion of patients become symptomatic during the third decade.

### DISCUSSION

Results obtained from this paper, like those of other authors<sup>1,2</sup> suggest that the adenylate cyclase activity assay can be used in MVP patients and confirm other reports<sup>4,5</sup> that adenylate cyclase, the enzyme that converts ATP to cAMP, plays a fundamental role in adrenergic signal transduction. Adenylate cyclase activity is generally assayed by measuring radiolabeled cAMP generated from [ $\alpha$ -<sup>32</sup>P] ATP.

Although sensitive, the radioactive approach is costly and time consuming. In this present study, a nonradioactive enzymatic fluorometric method for adenylate cyclase activity was used. Adenylate cyclase activity in MVP patients was increased compared to normal volunteers (Fig. 2). The fluorometric measurement of cAMP, described herein, is based on the principle that AMP, generated from the cleavage of the 3, 5-phosphodiester linkage of cAMP, will stimulate glycogen phosphorylase activity. The cleaning step in the fluorometric cAMP assay removes all endogenous adenine nucleotides which would otherwise substantially increase the blank. It is essential for assay sensitivity. Although patients with MVP may become symptomatic at any age, including the pediatric age group, in the present study the symptom onset in the greater proportion of patients occurs during the third decade (Fig. 3). These findings provide evidence that there is enhanced activation of adenylate cyclase activity in MVP patients. Therefore the determination of adenylate cyclase activity in combination with echocardiography allows for reliable diagnosis of MVP patients.

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