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Heart Rate Independence of Catecholamine-Induced Myocardial Damage in the Newborn Pig

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ABSTRACT

Neonates undergoing heart surgery are exposed to high levels of circulating catecholamines. Our objective was to determine to what extent epinephrine (E)-related cardiotoxicity can be attributed to induced tachycardia. We assessed left ventricle function by pressure-volume data obtained by conductance catheter/micromanometer technique and correlated it with ultrastructure in newborn piglets (3 to 7 d old). Group A (n = 6) received E (1 μg/kg/min) for 2 h, whereas group B piglets (n = 6) were atrially paced at a rate (220/min) matched to group A. Left ventricle peak systolic pressure and stroke work were significantly higher ($p < 0.05$) in group A. End-systolic elastance increased during E infusion (70%) *versus* no significant change in group B. After 2 h of E infusion, end-systolic elastance was significantly reduced in group A from 9.8 ± 3.5 to 5 ± 2.4 mm Hg/mL ($p < 0.05$). Chamber stiffness index increased during E infusion from 0.36 ± 0.2 to 0.6 ± 0.3 mL^{-1} ($p < 0.05$) and remained elevated (0.58 ± 0.2 mL^{-1}) after E infusion was discontinued *versus* no change in group B. E-induced left ventricle dysfunction was associated with scattered but irreversible ultrastructural changes consisting of sarcolemmal rupture and loss of mitochondrial architecture, whereas only minor reversible changes such as microvesicular lipid accumulation and mitochondrial swelling were seen in group B. We conclude that E cardiotoxicity in the neonate is independent of induced tachycardia. (***Pediatr Res* 36: 49–54, 1994**)

Abbreviations

E, epinephrine
LV, left ventricle
PV, pressure-volume
Ees, end-systolic elastance
NOR, norepinephrine
V_{100}, volume when end-systolic pressure = 100 mm Hg

High plasma levels of endogenous catecholamines exist in neonates having congenital heart disease (1), and administration of catecholamines is frequently required for hemodynamic support preoperatively. The inotropic effect of all β-agonists is mediated through activation of the enzyme adenylate cyclase, resulting in increased intracellular levels of cAMP and enhancement of transarcolemmal Ca^{2+} influx (2). The association between high circulating plasma catecholamines and acute cardiotoxicity has previously been studied (3, 4), and the mechanisms are attributed to impaired intracellular Ca^{2+} handling (5), reduced coronary blood flow, and formation of highly cytotoxic free radicals derived from catecholamine autoxidation (6). Because most β-agonists exert both positive inotropic and chronotropic effects, it is still unclear to what extent catecholamine-induced cardiomyopathy is attributable to tachycardia. Therefore, this study was designed to compare the effects of rapid atrial pacing with those of E on LV function and ultrastructure in newborn piglets. Myocardial performance was assessed by the end-systolic and diastolic PV relationship (7) and correlated with ultrastructural and metabolic recovery by serial ATP measurements before and after administration of E and pacing.

METHODS

Newborn pigs aged 3 to 5 d and weighing 1.6 to 2.8 kg were anesthetized with i.v. sodium pentobarbital (30 mg/kg) after administration of atropine sulfate (0.1 mg/kg). After endotracheal intubation, ventilation was maintained with a fixed-pressure neonatal-pediatric respirator (series 300, Penlon, UK) with an inspired mixture of oxygen and room air (fraction of inspired oxygen = 0.4). A median sternotomy was performed, the pericardium was opened longitudinally, and the edges were retracted with stay sutures. LV pressure was monitored with a high-fidelity 5F micromanometer-tipped pressure trans-

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ducer (PC-350, Millar Instruments, Inc., Houston, TX). A 5F multielectrode conductance catheter (Webster Labs, Baldwin Park, CA) was inserted through the right carotid artery into the LV with the most distal electrode placed at the apex and the most proximal electrode just cephalad to the aortic valve. Proper position of the conductance catheter was confirmed by palpation and at postmortem.

Left ventricular volume measurement. The conductance catheter was connected to a model Sigma-5 signal-conditioner processor (Leycom, Oegstgeest, The Netherlands) for continuous and instantaneous LV volume measurements (8). The conductance catheter has eight electrodes spanning a total distance of 3 cm. A constant alternating current (30 μA, 20 kHz) was passed across the distal and proximal electrodes, allowing measurement of impedance across the five successive pairs of electrodes. The sum of the five segment conductances,

$$G(t) = \sum_{n=1}^{5} Gn(t),$$

is linearly related to ventricular volume, V(t), by the following equation:

$$V(t) = (1/\alpha)(L^2/\delta)G(t) - Vc$$

where α is an empirical slope constant for the V(t) − G(t) relation, L is the interelectrode distance, and δ is the resistivity of the blood measured by a cuvette attached to the Sigma-5 system. Vc is the correction volume for the parallel conductance formed by tissue surrounding the LV cavity. To obtain V(t), Vc was determined by a rapid injection of hypertonic saline (1 mL) into the right atrium while ventilation was held at end-expiration and calculated from a least-square regression of the altered end-systolic and end-diastolic volumes. A signal-processing computer (NEC 386) and software designed in our laboratory was used to determine Vc when end-systolic volume was equal to end-diastolic volume (9). Measurement of Vc was repeated three times in each experiment, and the results were averaged to determine Vc. The value obtained was subtracted from the total volume to yield absolute volume.

Experimental protocol. After signal calibration and baseline PV relation measurements, administration of epinephrine chloride (1 μg/kg/min) in group A (n = 6) was started into the external jugular vein (infusion pump model 921, Harvard Apparatus Inc., Millis, MA) and continued for 2 h. In group B (n = 6), the effect of 2 h of atrial pacing on LV function at a rate of 220 beats/min was studied. Two pacing electrodes were sutured to the right atrial wall and connected to a pacemaker (Cordis, Pacer System Analyzers 209B, Cordis Corporation, Miami, FL). After 30 min of E infusion and atrial pacing, PV measurements were performed by transient inferior vena caval occlusion with an adjustable suture passed around the inferior vena cava. The last set of PV relation measurements was performed after E infusion and atrial pacing were discontinued, when the heart rate had returned to baseline level. Transmural LV biopsies for ATP measurements were taken concurrently with the measurements of LV function at the end of the experiment, and myocardial ultrastructure was studied and compared with normal neonatal myocardium. During the course of the experiment, the hematocrit was maintained between 0.30 and 0.35, pH between 7.35 and 7.45, and P_{O_2} and P_{CO_2} between 20 and 33 kPa and 4.6 and 6 kPa, respectively. Serum ionized Ca^{2+}, K^+, and glucose were maintained at normal values. The temperature was maintained at 37°C along the course of the experiment.

Electron microscopy study. At the conclusion of each experiment, the effects of E on myocardial macroscopic structure and ultrastructure were studied and compared with normal neonatal myocardium, as previously described (10). Semiquantitative assessment of ultrastructural changes was performed in a blinded fashion by a single observer with the method described previously (11). Mitochondrial structure change was assessed by assigning an ischemic damage grade of 0 to 4 to the 300 mitochondria in each micrograph from each heart (12), with grade 0 being normal ultrastructure; grade 1, early swelling as manifested by clearing matrix density and separation of cristae; grade 2, more marked swelling without disruption of cristae; grade 3, massive swelling with disruption of cristae; and grade 4, massive swelling with disruption of cristae and loss of integrity of the mitochondrial membrane. The mitochondrial score in each case was determined by averaging the grade of more than 300 mitochondria.

ATP measurements. Transmural LV tissue biopsies were immersed in liquid nitrogen and freeze dried in a tissue lyophilizer; the dried tissue was then stored at −80°C. ATP was measured as previously described (13) using a fluorescence spectrophotometer (650–10s, Perkin-Elmer, Norwalk, CT).

Plasma catecholamine measurements. Plasma E and NOR were measured in venous blood (5 mL) obtained from a peripheral vein before infusion, at 30 min of E infusion, and at the time of the last set of PV relation measurements. The blood samples were rapidly placed in chilled tubes containing EGTA and centrifuged at 4°C for 15 min. Plasma was separated into collecting tubes and frozen at −70°C for subsequent assay (14).

Data analysis. The ECG, LV pressure, and volume data were recorded on ½-inch magnetic tape (pr 280, Ampex, Redwood City, CA) and simultaneously displayed on a precalibrated digital X-Y oscilloscope (2090, Nicolet, Madison, WI). The three signals were digitized by an analog-to-digital convertor (DT 2621, Data Translator, Boston, MA) at a sample frequency of 333 Hz or every 3 ms through the cardiac cycle. The obtained PV loops were analyzed including one or two steady state beats and six to eight subsequent cycles in the first 3 s after caval occlusion. End-systole was defined by the points of maximal pressure–absolute volume ratio for each cardiac cycle. End-systolic PV relation was generated from a

least-square linear regression of these points with the following expression:

$$Pes = Ees\ (Ves - Vo)$$

relating end-systolic pressure (Pes) to end-systolic volume (Ves) with the slope, or Ees, and the volume axis intercept (Vo). To describe the position of the end-systolic PV relation, we calculated the V_{100} (15). End-diastole was taken at the time corresponding to the peak of the R wave of the ECG. The end-diastolic PV relation points during caval occlusion were exponentially fitted by the following equation:

$$Ped = A + Be^{kVed}$$

where Ped is end-diastolic pressure, Ved is end-diastolic volume, A and B are fitting parameters, e is the base of the natural logarithm, and k is the slope constant considered as the chamber elastic stiffness (16).

Left ventricular net stroke work (SW) was calculated as the integral of LV pressure and volume for the entire cardiac cycle as described by the following expression:

$$SW = \int P \cdot dV$$

Statistical analysis. All data are presented as mean ± SD. A series of repeated hemodynamic variables was subjected to analysis of variance. To compare the hemodynamic variables between groups, analysis of covariance was performed by using the SAS statistical package (17). The level of significance was considered $p < 0.05$. All animals received humane care in accordance with the guidelines of The University of Toronto Animal Care Committee.

RESULTS

Table 1 shows the mean hemodynamic variables and systolic indices at baseline, 30 min later, and after 2 h of E administration and atrial pacing. The administration of E resulted in a significant ($p < 0.05$) increase in LV end-systolic pressure, in heart rate from 130 ± 14 to 220 ± 24 beats/min ($p < 0.05$), in cardiac output ($p < 0.05$), and in stroke work ($p < 0.05$), and also resulted in a leftward shift of the end-systolic PV relationship with an increase in contractility (Ees increased from 9.8 ± 3.5 to 16 ± 6 mm Hg/mL ($p < 0.05$). However, after E was discontinued, there was a significant ($p < 0.05$) reduction in Ees and cardiac output compared with baseline. The reduction in LV function was associated with a significant increase in V_{100} ($p < 0.05$). In contrast, atrial pacing resulted in a significant increase in cardiac output, but no significant changes in Ees or V_{100} were demonstrated in the pacing group along the course of the experiment.

Comparison of the diastolic data (Table 2) demonstrated a significant ($p < 0.05$) increase in k during infusion of E but not during atrial pacing. After E infusion was discontinued, end-diastolic pressure and volume significantly increased ($p < 0.05$). The exponentially fitted curve of the end-diastolic PV relation demonstrated a rightward and upward shift with a significant increase in k in group A (0.58 ± 0.2 mL^{-1}) compared with baseline ($p < 0.05$). In contrast, there was no change in k compared with baseline value in the pacing group.

ATP measurements. After 2 h of E administration, there was a significant reduction in ATP in group A compared with baseline (Fig. 1). In contrast, there was no change in ATP level after 2 h of rapid atrial pacing.

Electron microscopic study. Ultrastructural analysis of the myocardium after administration of E demonstrated scattered but irreversible changes consisting of disruption of the sarcolemma, mitochondrial swelling with loss of internal architecture, and deposition of amorphous granules (Fig. 2*A* and *B*). In contrast, in the pacing group a lesser degree of ultrastructural change was demonstrated. Lipid droplet accumulation and mild mitochondrial swelling were seen; both are indicative of reversible tissue damage. The mitochondrial score in the E group was significantly higher (3.2 ± 0.7) than that in the pacing group (0.8 ± 0.5) ($p < 0.05$).

Plasma catecholamine measurements. Table 3 shows the measurements of circulating plasma E and NOR levels in both groups before, at 30 min, and after the infusion of E and atrial pacing were discontinued (simultaneously with the last set of PV measurements). At baseline, plasma E levels were similar in both groups. However, during and after infusion of E, the levels were significantly higher in

Table 1. *Comparison of mean hemodynamic variables and contractile indices between pacing and high-dose E groups**

	Epinephrine			Pacing		
	Before	30 min	After	Before	30 min	After
ESP (mm Hg)	60 ± 8.6	110 ± 19†	56 ± 9.6	68 ± 9.6	72 ± 12	67 ± 12
SV (mL)	5 ± 2.4	4.5 ± 2.8	4 ± 1.2	5.4 ± 1.2	4.8 ± 1.4	5 ± 2.4
SW (erg · 10^3)‡	200 ± 25	310 ± 35†	160 ± 18†	210 ± 18	240 ± 20	185 ± 20
CO (mL/min)	800 ± 130	1150 ± 240†	640 ± 140†	700 ± 120	1000 ± 320†	680 ± 160
Ees (mm Hg/mL)	9.8 ± 3.5	16 ± 6†§	5 ± 2.4†	8.2 ± 2	9.6 ± 3.3	7.4 ± 2.4
V_{100} (mL)	4 ± 3	3 ± 2.2	8 ± 2.4†§	4.3 ± 2.4	4 ± 1.4	5 ± 1.9

* All data are expressed as mean ± SD. ESP, end-systolic pressure; SV, stroke volume; SW, stroke work; CO, cardiac output.
† $p < 0.05$ compared with baseline.
‡ 1 erg= 10^{-7} J.
§ $p < 0.05$ compared with pacing.

Table 2. *Comparison of diastolic data between pacing and E groups**

	Epinephrine			Pacing		
	Before	30 min	After	Before	30 min	After
EDP (mm Hg)	4 ± 2.8	5 ± 3	7 ± 2.4†	3 ± 1.2	2.5 ± 2	4 ± 2.4
EDV (mL)	9.4 ± 2.4	8 ± 2.2	13 ± 2.2†	10 ± 1	9 ± 1.2	10 ± 2.4
k (mL^{-1})	0.36 ± 0.2	0.6 ± 0.3†‡	0.58 ± 0.2†‡	0.4 ± 0.2	0.36 ± 0.1	0.4 ± 0.2

* All data are expressed as mean ± SD. EDP, end-diastolic pressure; EDV, end-diastolic volume; k, chamber stiffness index.
† $p < 0.05$ compared with baseline.
‡ $p < 0.05$ compared with pacing.

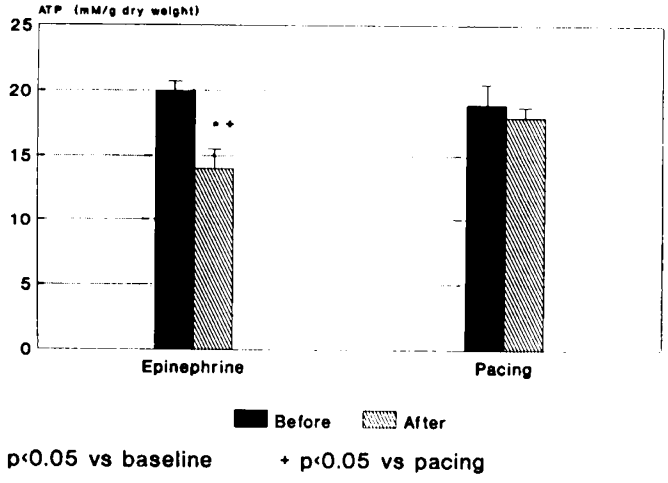

Figure 1. Myocardial ATP levels from LV biopsies before and after E infusion and rapid atrial pacing demonstrating a significant reduction of ATP stores only in the E group.

the E group, indicating that rapid atrial pacing was not associated with high levels of plasma circulating catecholamines.

DISCUSSION

We have shown in the present study that administration of high-dose E in the anesthetized intact neonatal heart resulted in LV dysfunction that was highly correlated with the development of myocardial ultrastructural changes and reduction in ATP stores. However, these abnormalities were not tachycardia induced in a comparable group of neonates paced at heart rate and time interval matching those of E-treated newborns.

PV indices were derived using the conductance catheter to measure instantaneous LV volume. The validity and usefulness of this technique for assessment of cardiac contractility has been previously established in adult animals (7) and in newborn lambs (18). We used an open chest model with an open pericardium to assess LV function, although it has been shown that this may influence LV diastolic PV relation under physiologic condi-

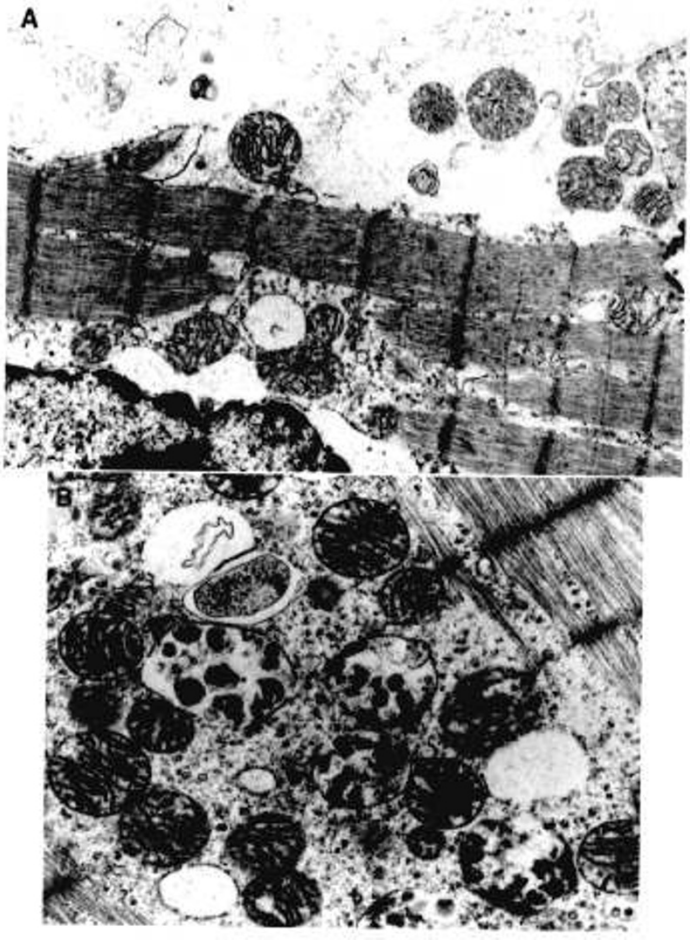

Figure 2. *A*, Electron micrograph of myocardium from an E-treated piglet showing rupture of the sarcolemma, mitochondrial swelling, and loss of the internal architecture (×7000). *B*, Intramitochondrial deposition of dense (calcium) granules (×12 000).

Table 3. *Plasma E and NOR levels (pg/L) before, at 30 min of E administration and pacing, and at the time of late PV relation measurements**

	Epinephrine group		Pacing group	
	E	NOR	E	NOR
Before	0.072 ± 0.014	0.024 ± 0.0096	0.074 ± 0.019	0.020 ± 0.0096
30 min	0.88 ± 0.7†‡	0.228 ± 0.048†	0.098 ± 0.015	0.045 ± 0.028
After	0.28 ± 0.06†‡	0.060 ± 0.024	0.080 ± 0.010	0.036 ± 0.012

* All data are presented as mean ± SD.
† $p < 0.01$ compared with baseline.
‡ $p < 0.05$ compared with pacing group.

tions (19). However, the rationale for doing this was to obtain transmural ventricular biopsies on completion of the last function study.

Recently, Applegate *et al.* (20) have shown that the conductance catheter accurately measures absolute volumes at steady state but can underestimate the slope and position of the end-systolic PV relation when it is determined by caval occlusion. However, the end-systolic PV relation accurately measures the direction and magnitude of change in LV systolic function. In addition, we have used the volume axis value at P_{100} (V_{100}) as a variable describing the position of the slope of the end-systolic PV relation in the normal operating range of the LV, thus avoiding the problems of interpretation of V_0 (volume when end-systolic pressure = 0 mm Hg) in a range where curvilinearity of the slope may occur (15).

The upward shift of end-diastolic PV relation after E infusion withdrawal indicates a significant increase in chamber stiffness related to persistent myofilament cross-linkage (21). We and others (22) assume that the high intracellular Ca^{2+} levels in proximity to the contractile apparatus and inadequate ATP to facilitate cross-bridge detachment account for the increased chamber stiffness.

The effect of heart rate on cardiac contractility has been extensively studied in isolated hearts (23) and in intact anesthetized and conscious adult animals (24). The results varied among species and from one study to another. Previous studies in the conscious dog have shown that increased heart rate induced an increase in LV contractility (25). Higgins *et al.* (26) found that increasing the frequency of contraction of the normal heart of the conscious dog caused reduction in LV change in pressure per unit time, but they observed the opposite effect in the presence of anesthesia. Suga and Sagawa (27), using an isolated ejecting canine heart, demonstrated only a slight and inconsistent increase in the slope of the end-systolic PV relation in response to an increase in paced heart rate. Maughan *et al.* (28) showed in an isolated, ejecting canine heart preparation a significant effect of heart rate on Ees over a heart rate range of 60 to 120 beats/min. However, between 120 and 180 beats/min there was little change in Ees, and at 200 beats/min Ees increased only slightly. This is similar to what we found in untreated newborn pigs in which baseline heart rate ranged between 130 and 150 beats/min, and any increase in heart rate beyond that point would contribute very little to the contractile state. Our results differ from those of a previous study done on conscious neonatal lambs (29). This study demonstrated a substantial increase in LV contractility during rapid atrial pacing. The different results in our study may be attributable to different species, the use of LV change in pressure per unit time and LV change in volume per unit time rather than the PV relationship, a wider range of paced heart rates, or the effect of anesthesia. The absence of force-frequency relationship in the neonatal heart may be related to a greater proportion of noncontractile relative to contractile tissue, a greater dependence on transsarcolemmal Ca^{2+} flux, and immaturity of the sarcoplasmic reticulum. In addition, the neonatal heart has a limited ability to increase the cardiac output because of diminished ventricular diastolic compliance and a fixed stroke volume. Furthermore, the neonatal heart is functioning at near maximum capacity because of high demand, and resting systolic indexes are much higher in comparison with those of adults (10).

The reduction in Ees after 2 h of treatment with E occurred despite higher levels of catecholamine and increased preload. In this model, the measured plasma levels of E and NOR were comparable with those obtained from newborns with congenital heart defects before surgery (30). Catecholamine injury has recently been delineated in cultures of adult cardiac muscle (31). The authors demonstrated that cAMP-mediated Ca^{2+} overload is the primary mechanism responsible for catecholamine toxicity and that there is a concentration-dependent decrease in cardiocyte viability. In addition, administration of high-dose catecholamines alters the Na^+-Ca^{2+} exchange system in the sarcolemma and sarcoplasmic reticulum, thereby affecting the ability of the cell to sequestrate the elevated intracellular Ca^{2+} (32). A number of different biochemical abnormalities have been described after administration of E. There is increased oxygen consumption by the left ventricular myocardium because of a higher external work performance. Increased stimulation of oxidative metabolism results in a mismatch between oxygen demand and delivery such that demand exceeds supply (33). Another aspect is the depression of myocardial oxidation of FFA with resultant accumulation of lipid droplets in the sarcomere and a concentration-dependent decrease in cardiocyte RNA and protein synthetic activity in the sarcomere (31).

This study shows that E toxicity is not related to tachycardia per se, inasmuch as rapid (paced) heart rate alone resulted in only mild and reversible ultrastructural changes not associated with any reduction of LV function or ATP stores. There is, however, evidence that the effects of heart rate on LV function are mediated through alteration in Ca^{2+} release and sequestration, and the inotropic effects observed in other studies may be related to accumulation of the Ca^{2+} entering the cell with each beat (34). We assume that pacing-induced tachycardia does not result in substantial Ca^{2+} overload as occurs with E. However, further study is required to correlate intracellular Ca^{2+} measurements and myocardial function after rapid atrial pacing. In agreement with a previous study in adult dogs (35), rapid (paced) heart rate was not associated with an increase in circulating catecholamines, a finding that may partially explain the absence of major ultrastructural changes in the pacing group.

In summary, after administration of high-dose E in the newborn heart, the withdrawal of E is associated with a decrease in LV systolic performance and maintenance of increased chamber stiffness. These changes are associ-

ated with irreversible ultrastructural abnormalities and are only partially related to the effect of tachycardia.

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