Pseudomonas aeruginosa Exotoxin A Enhances Automaticity and Potentiates Hypoxic Depression of Isolated Rat Hearts

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Abstract. **The potent virulence factor exotoxin A, produced by *Pseudomonas aeruginosa*, has been reported to suppress the synthesis of the α-subunit of cardiac G_i protein and may have general effects upon synthesis of other myocardial proteins. To determine whether such exotoxin A actions influence specific functional properties of the intact heart, characteristics of isolated perfused hearts obtained from rats receiving injections of exotoxin A 48 hr before sacrifice were compared with those of rats receiving no exotoxin A. Exotoxin A treatment increased the spontaneous beating rates and potentiated the suppressive effects of hypoxia upon heart rate, left ventricular systolic pressure, and rates of ventricular contraction and relaxation. On the other hand, exotoxin A treatment did *not* influence the magnitude or rate of pressure development under control conditions, the positive chronotropic and inotropic responses to isoproterenol, or the negative chronotropic responses to adenosine. Since a specific exotoxin A-induced suppression of myocardial α-subunit of the G_i protein should confer hypersensitivity to isoproterenol and reduced sensitivity to adenosine, the absence of alterations in responses to these interventions suggests that exotoxin A's effect was not confined to specific suppression of this protein. However, net effects of exotoxin A exposure included a pronounced increase in excitability of the hearts and enhanced vulnerability to hypoxic insults.**

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Pseudomonas aeruginosa, a gram-negative opportunistic microorganism, is a leading cause of morbidity and mortality in immunocompromised patients with burns, cancer, and cystic fibrosis (1–3). Exotoxin A, which is one of several extracellular substances produced by *P. aeruginosa*, appears to be one of the most important virulence factors (4–6). Nearly all strains of *P. aeruginosa* contain the gene for exotoxin A (7) and a majority of the strains produce the exotoxin during *in vivo* infection (8–10). The mechanism of action of this exotoxin is identical to that of diphtheria toxin (11) and involves inhibition of protein synthesis by ADP-ribosylation of the peptidyl tRNA translocase, elongation factor 2 (12).

Recently, it was shown that exotoxin A causes time- and concentration-dependent cytotoxic effects on cultured rat heart muscle cells associated with loss of cell potassium, cessation of spontaneous beating, and detachment from the culture dish (13). In other recent studies, exotoxin A was shown to prevent the β-receptor-induced upregulation of G_i protein α-subunits and adenylyl cyclase desensitization in rat heart muscle cells (14). We hypothesized that such influences on myocyte characteristics in general and G_i protein α-subunit synthesis in particular ought to be associated with some cardiac functional deficits that might contribute to the overall clinical deterioration that accompanies exotoxin A exposure.

To address this issue, studies were performed on isolated perfused hearts of rats that had been injected with *P. aeruginosa* exotoxin A 48 hr before sacrifice.

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Various characteristics of these hearts from exotoxin-treated rats were compared with those of nontreated rats. In addition to comparison of control characteristics of these isolated hearts, responses to brief episodes of hypoxia, isoproterenol infusion, and exogenous adenosine infusion were also evaluated. Hypoxia was chosen to test for the ability of the exotoxin A to influence responses to changes in cardiac metabolic status such as might occur in patients with the severe pneumonia induced by Pseudomonas infection. Isoproterenol infusion was chosen to test for the possibility that exotoxin A produced specific alterations in the cardiac β-receptor-mediated responses (generally associated with increases in myocyte cyclic AMP). Adenosine infusion was chosen to test for the possibility that exotoxin A altered the cardiac A_1 purinergic receptor-mediated negative chronotropic responses, generally associated with G_i-mediated alterations in K channels and/or decreases in cyclic AMP (15).

Methods

The exotoxin A used in these experiments was isolated from *P. aeruginosa* strain PA 103 (List Biological Laboratories, Inc.) and was a kind gift from Dr. Barbara Iglewski (University of Rochester, Rochester, New York). The chromatographic procedures employed in purification have been shown by the manufacturer to remove most of the endotoxin contamination from the samples, as verified in one lot by limulus assay indicating 0.0006% endotoxin in the final product. No direct measurement, however, was done on the lot used for the present study; therefore, the possibility of endotoxin contamination cannot be unconditionally eliminated.

Adult, male Sprague-Dawley rats were used for these experiments. Preliminary studies indicated that the 48-hr LD_{50} for the exotoxin A in these rats was 33 μg/kg ip. In the subsequent experiments, exotoxin A was injected (intraperitoneally) 48 hr before the experiments at one of two dose levels: 7 μg/kg ($n = 6$) or 22 μg/kg ($n = 8$). (The actual number of rats used for data analysis in the latter group was actually five and not eight, since one died before the experiment began and hearts of two others developed irreversible fibrillation during the initial phase of the experiment.) At the time of sacrifice, these exotoxin A-treated animals appeared somewhat sluggish but otherwise healthy. Animals used for the control group received no pretreatment.

On the day of the experiment, rats received heparin (2.5 mg ip) and ~20 min later were anesthetized with sodium pentobarbital (35 mg/kg ip). Hearts were removed rapidly and perfused at 32°C via the aortic stump in Langendorff-fashion with modified Krebs-Henseleit solution containing (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 3.0, $MgSO_4$ 1.2, Na-EDTA 0.5, and glucose 10.0. In addition, the solution contained heparin, 100 units/liter, and insulin, 10 units/liter, and was bubbled with 95% O_2 and 5% CO_2. Perfusate flow rate was maintained constant by a perfusion pump and perfusion pressure was measured from a side arm in the perfusion line at heart level. Flow was set so as to produce an initial pressure of ~80 mm Hg and averaged 16 ml/min/g wet wt. Heart weight was determined at the end of each experiment.

Atrial tissue was trimmed from the heart to reduce spontaneous beating rate and allow easy access to the ventricular cavities. Spontaneous beating rates were established by atrioventricular nodal or ventricular pacemakers. A fluid-filled balloon attached to a pressure transducer was inserted through the mitral valve into the left ventricle to measure ventricular pressures. The balloon volume was adjusted to achieve an end-diastolic pressure of 15 mm Hg. A drain was placed in the left ventricle alongside the balloon to prevent fluid from interstitial space or thebesian drainage from accumulating in the chamber. Oxygen content of samples of coronary venous effluent collected anaerobically from a cannula inserted into the right ventricle via the pulmonary outflow tract was measured along with that of inflow samples collected from the arterial perfusion line. These values along with coronary flow rate were used for determination of myocardial oxygen consumption. A thermistor probe placed in the right ventricle via the tricuspid valve provided a feedback signal used to adjust the temperature of the perfusate.

Measured or calculated variables included heart rate, left ventricular diastolic and systolic pressure, left ventricular positive and negative dP/dt, coronary vascular resistance, myocardial oxygen consumption, and adenosine and inosine concentration of the coronary effluent. These procedures have all been used for previous studies from this laboratory (16, 17).

After the initial set-up manipulations were completed, the hearts were allowed to equilibrate for 25 min. At the end of this period, control values for all variables were determined. Subsequent experimental manipulations were identical for all preparations and included (i) a 6-min episode of perfusion under hypoxic conditions (perfusate equilibrated with 30% O_2, 5% CO_2, and 65% N_2), (ii) a 15-min re-equilibration period under normoxic conditions, (iii) a 6-min infusion of perfusate containing 10 nM isoproterenol, (iv) a 30-min re-equilibration period with normal perfusate, and, finally, (v) a 6-min infusion of perfusate containing 10 μM adenosine. (These conditions were chosen because they produce submaximal and fully reversible responses by the preparations. Therefore, any increases or decreases in sensitivity to these interventions would be detected. Full "concentration-response" characteristics to each intervention were not determined because of the deterioration of the preparations evoked by severe hypoxia or high concentrations of isoproterenol.)

Data are expressed throughout as mean ± SE. Responses of the hearts from the rats treated with exotoxin A (both low and high dose) were statistically compared with those of nontreated rats using either Students paired t test or unpaired t test with Bonferroni's correction for multiple comparisons or factorial analysis of variance. Significant differences were declared for P-values < 0.05.

Results

Body weights and hearts weights of the rats exposed to exotoxin A at either the low dose ($n = 6$; 461 ± 21 g and 1.46 ± 0.10 g, respectively) or the high dose ($n = 5$; 466 ± 40 g and 1.38 ± 0.11 g, respectively) were not significantly different from each other or from those of the nontreated rats ($n = 11$; 465 ± 26 g and 1.29 ± 0.14 g, respectively).

Effect of Exotoxin A upon Isolated Heart Characteristics under Control Conditions. Characteristics of the isolated hearts from the two exotoxin-treated groups and the untreated group determined at the end of the 25-min equilibration period are indicated in Table I. Treatment of the rats with exotoxin A of either dose resulted in a significant increase in the spontaneous beating rate of the isolated heart preparations as compared with the untreated group. Other measured variables of the exotoxin A-treated groups under these initial control conditions, however, were not different from those of the untreated group.

Although the spontaneous beating rate was usually quite regular in most preparations, extra beats occasionally occurred during the course of the experiment and spontaneous episodes of reversible ventricular fibrillation occurred in two of the six hearts from rats treated with the low dose of exotoxin A and in two of the five hearts from rats treated with the high dose of exotoxin A. No such episodes were observed in hearts from untreated rats. While this study did not specifically address the excitability status of the hearts, these observations suggest that exotoxin exposure may predispose the hearts to arrhythmias.

This preparation is not optimal for determining influences on coronary vascular resistance. The high perfusate flow delivered at a constant rate maximizes oxygen delivery and contractile capability of the heart but may blunt vascular responsiveness. In addition, the strong left ventricular isovolumic contractions result in substantial extravascular compressive forces that also may influence the vascular resistance. Because of these complications, the small hypoxia-, isoproterenol-, and adenosine-induced changes in vascular resistance of these preparations will not be considered further.

Responses to Metabolic Challenges. The effects of 6 min of hypoxia or isoproterenol upon the mechanical characteristics of the isolated hearts from the two exotoxin-treated groups of rats and the untreated group of rats are shown in Figure 1. As can be seen, hypoxia generally depresses the functional characteristics of the isolated hearts, whereas isoproterenol enhances these characteristics. Because there were no significant differences between the responses of the hearts from rats treated with the low and high doses of exotoxin A, these data were combined for further analysis to compare with the untreated group.

Responses to Hypoxia. The time-dependent responses of the exotoxin A-treated and untreated groups to a 6-min episode of perfusion under hypoxic conditions are shown in Figure 2. Hypoxia significantly decreased the spontaneous beating rate of hearts from the exotoxin A-treated rats, but had no significant effect upon that of hearts from the untreated rats (upper left panel). The slope of the linear regression of the data from the exotoxin A-treated group (−9.0 ± 3.1 bpm/min of hypoxia) was significantly greater than that of the nontreated group (−0.6 ± 2.3 bpm/min of hypoxia). Hypoxia decreased the left ventricular systolic pressure development in both groups, with the hearts of the exotoxin A-treated rats being significantly more depressed by the hypoxia (−6.1 ± 1.3 mm Hg/min of hypoxia) than those of the untreated rats (−2.7 ± 0.8 mm Hg/min of hypoxia) (upper right panel). The maximum rates of contraction (+dP/dt, lower left panel)

Table I. Control Characteristics after Initial Equilibration Period of Isolated Perfused Hearts of Rats[a]

	Untreated ($n = 11$)	Low dose exotoxin A ($n = 6$)	High dose exotoxin A ($n = 5$)
Heart rate (bpm)	180 ± 25	264 ± 14[b]	261 ± 21[b]
Left ventricular systolic pressure (mm Hg)	154 ± 6	152 ± 11	156 ± 4
Left ventricular +dP/dt (mm Hg/sec)	1545 ± 124	1600 ± 164	1675 ± 170
Left ventricular −dP/dt (mm Hg/sec)	−1115 ± 71	−1260 ± 120	−1300 ± 129
O_2 consumption (μl/min/g)	110 ± 11	139 ± 9	128 ± 19
Coronary vascular resistance (mmHg/ml/min/g)	5.2 ± 0.3	4.6 ± 0.5	4.6 ± 0.6

[a] Rats were either untreated or injected with a low dose (7 μg/kg) or high dose (22 μg/kg) of *Pseudomonas aeruginosa* exotoxin A 48 hr before the experiment.
[b] $P < 0.05$ as compared with untreated group (unpaired t test with Bonferroni correction).

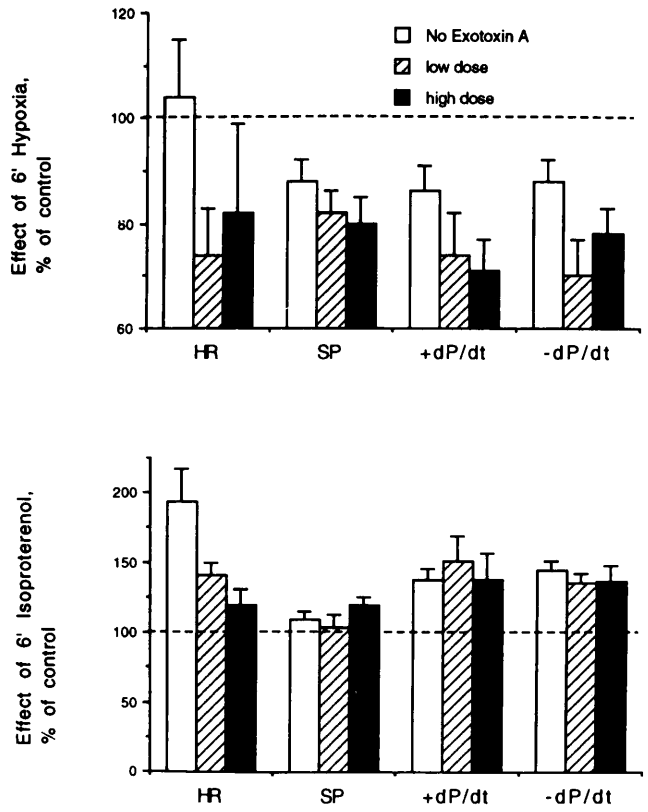

Figure 1. Mechanical characteristics of isolated perfused rat hearts at the end of 6 min of perfusion with hypoxic solution (30% oxygen) (top panel) or with solution containing 10 μM isoproterenol (bottom panel) as percentage of control responses. Rats received either no treatment or an intraperitoneal injection of *Pseudomonas aeruginosa* exotoxin A 48 hr before the experiment. Exotoxin A dose was either 7 μg/kg (low dose, $n = 6$) or 22 μg/kg (high dose, $n = 5$).

and relaxation (−dP/dt, lower right panel) in both groups were also significantly decreased by the hypoxic conditions and comparison of the slopes obtained by linear regression analysis indicated that the hypoxia-dependent decrease in these rates of contraction and relaxation were significantly more pronounced in hearts of the exotoxin A-treated rats (−89 ± 19 and −64 ± 15 mm Hg/sec/min of hypoxia, respectively) than those of untreated rats (−38 ± 14 and −24 ± 9 mm Hg/sec/min of hypoxia, respectively).

Responses to Isoproterenol. The time-dependent responses of the isolated hearts from exotoxin A-treated and untreated rats to a 6-min infusion of isoproterenol are shown in Figure 3. In both groups, these responses included significant increases in heart rate, left ventricular systolic pressure and maximal rates of contraction (+dP/dt) and relaxation (−dP/dt). Aside from the difference in the heart rates observed during this protocol, there were no other significant differences in any of these other measured variables between the exotoxin A-treated and the untreated groups. Myocardial oxygen consumption of the hearts from the exotoxin A-treated rats rose during the isoproterenol infusion from 99 ± 14 μl of O_2/min/g to a maximum after 5 min of 210 ± 18 μl of O_2/min/g, which were not different from the values obtained in the untreated group (111 ± 8 and 173 ± 18 μl of O_2/min/g, respectively).

Responses to Adenosine Infusion. Adenosine significantly slowed the spontaneous beating rate of the hearts in all preparations. As shown in Table II, treatment with exotoxin A had no significant effect upon the hearts' negative chronotropic responses to the infused adenosine when compared with responses of untreated hearts.

Adenosine and Inosine Concentrations in Coronary Effluent during Experimental Manipulations. The adenosine and inosine concentrations in coronary effluent of isolated hearts has been shown previously to decrease during the course of an experiment (17). This decrease is evident in Table III in the values obtained during the consecutive control periods in both groups. However, there were no significant differences at any point in the course of the experiment in the coronary effluent adenosine or inosine concentrations between the hearts of rats treated with exotoxin A and those of untreated rats. In both groups, adenosine levels in the coronary effluent after 6 min of hypoxic perfusion were slightly but significantly lower than those observed during the first control period, whereas inosine levels were significantly higher. After 6 min of isoproterenol infusion, adenosine and inosine concentrations of the coronary effluent of both groups were increased above the preceding control levels. Infusion of exogenous adenosine obviously resulted in significant increases in effluent levels of adenosine and inosine in both groups, but because of significant uptake and/or degradation of the adenosine during passage of the perfusate through the coronary beds, the adenosine in the effluent was significantly less than the 10 μM that were infused. Again, there was no significant difference between the groups in the metabolism of this exogenous adenosine.

Discussion

The primary findings of this study were that treatment of rats with *P. aeruginosa* exotoxin A resulted in (i) an enhanced automaticity of the isolated heart preparations and (ii) an exaggeration of the functional depression of the isolated hearts induced by hypoxia. The implication of each of these findings is presented separately.

First, the isolated hearts of rats treated with *P. aeruginosa* exotoxin A had significantly higher spontaneous beating rates in comparison to those of hearts from untreated rats. This increased automaticity was evoked by both doses of exotoxin studied, which suggests either that the cardiac pacemakers are quite sensitive to the exotoxin effect or that the purity of the exotoxin was not sufficient to allow a distinction between the two doses. Werdan's studies of the effect of

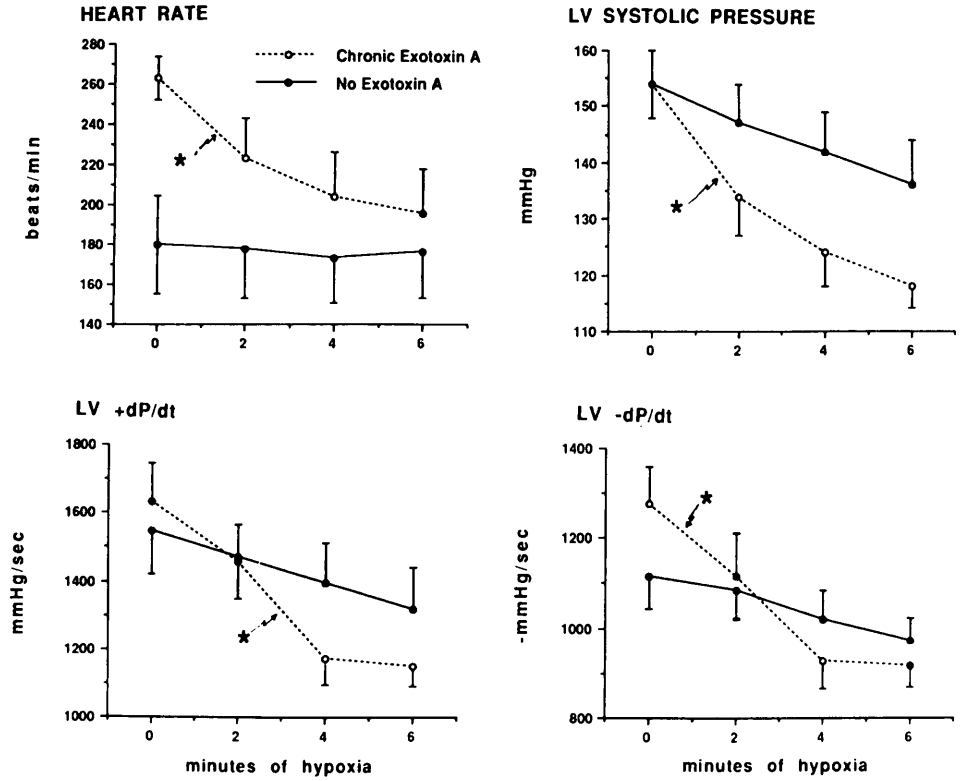

Figure 2. Effect of hypoxia upon characteristics of isolated perfused hearts of rats receiving either no treatment (no Exotoxin A, $n = 11$) or an intraperitoneal injection of *Pseudomonas aeruginosa* exotoxin A (either 7 or 22 μg/kg) 48 hr before the experiment (chronic exotoxin A, $n = 11$). *$P < 0.05$ as compared with response of the untreated group (analysis of variance).

exotoxin A on cultured cardiac myocytes established that a potassium loss begins a few hours after exposure in a dose-dependent manner (13). If such a process also occurs in cardiac pacemaker cells of the intact heart, the depolarization predicted by the potassium loss might cause the observed tachycardia. In addition, such potassium loss might also contribute to a heightened cardiac irritability and an increased susceptibility to arryhythmias or fibrillation, as was suggested by the present study. Since it has been suggested that exotoxin A plays an important role in pathogenesis of Pseudomonas septicemia in humans (8–10), such a heightened potential to develop arrhythmias might also exist in such patients.

Second, the exotoxin A-induced exaggeration of the hypoxia-dependent functional depression of the isolated hearts included enhanced suppression of heart rate, left ventricular systolic pressure, and rates of left ventricular contraction and relaxation. The reasons for these differences are not clear, but could reflect either exotoxin-induced alterations in processes associated with energy production under hypoxic conditions or exotoxin-induced changes in excitation-contraction coupling processes. We know of no specific studies that address these possibilities. Since both doses of exotoxin A evoked similar exaggeration of the hypoxia-dependent suppression, it is likely that whatever mechanism is involved is evoked fully by a low dose. Alternatively, the purity of the exotoxin may not have been sufficient to allow a distinction between the two doses. In any case, the data suggest that chronic exposure of hearts to exotoxin A enhances their vulnerability to the depressant effects of hypoxic insults.

The absence of any significant difference in the adenosine and inosine concentrations in the coronary effluent between the hearts of exotoxin A-treated rats and the untreated rats during the various experimental maneuvers suggest that purine metabolism is not significantly altered by this exotoxin treatment and that similar tissue adenosine levels were likely to have been achieved by infusion of exogenous adenosine.

We had expected that chronic exposure of rats to exotoxin A might enhance the responses of the isolated hearts to isoproterenol and decrease the negative chronotropic responses of the hearts to exogenous adenosine. These expectations were based upon the study by Reithmann *et al.* (14) showing that an increase in the α-subunit of the G_i protein, induced in cardiac myocytes by chronic noradrenaline exposure, was completely abolished when the cells were additionally exposed to *P. aeruginosa* exotoxin A. These findings suggested that exotoxin A could lead to a partial attenuation of catecholamine-induced desensitization and possibly to an increased catecholamine cardiotoxicity. If chronic ex-

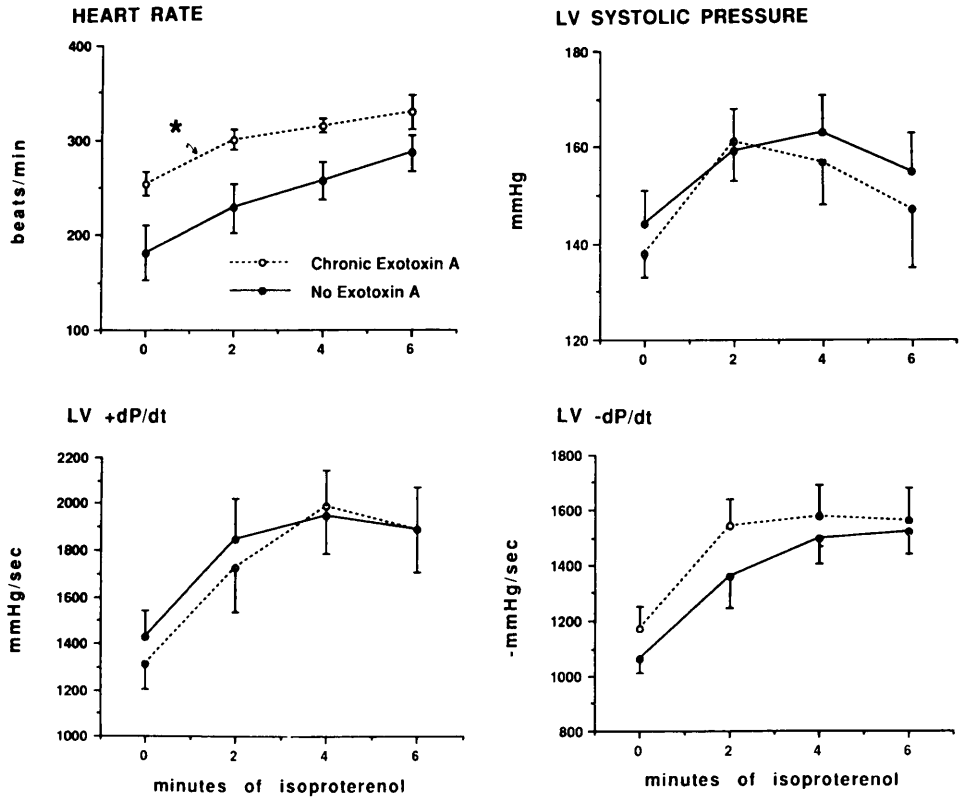

Figure 3. Effect of infusion of isoproterenol (10 n*M*) upon characteristics of isolated perfused hearts of rats receiving either no treatment (no exotoxin A, $n = 11$) or an intraperitoneal injection of *Pseudomonas aeruginosa* exotoxin A (either 7 or 22 μg/kg) 48 hr before the experiment (chronic exotoxin A, $n = 11$). $^*P < 0.05$ as compared with the untreated group (analysis of variance).

Table II. Effect of Adenosine Infusion on Beating Rates of the Isolated Perfused Hearts Untreated or Treated with *Pseudomonas aeruginosa* Exotoxin A 48 hr before Experiment

	No exotoxin A ($n = 11$)	Exotoxin A treated ($n = 11$)
Heart rate		
Initial value (bpm)	184 ± 26	217 ± 18
+Adenosine (10 μ*M*, 6 min) (bpm)	105 ± 17[a]	153 ± 26[a]
Percentage of initial value	63 ± 7	72 ± 11

[a] $P < 0.05$ as compared with initial value (paired *t* test).

otoxin A exposure could lead to a specific suppression of this inhibitory G protein production in the absence of added noradrenaline, we predicted that the response of the exotoxin-treated preparations to acute infusions of relatively low concentrations of isoproterenol might be enhanced. However, in our study, we did not find any significant changes in response to acute treatment with isoproterenol in the exotoxin-treated preparations. Furthermore, since the negative chronotropic effect of exogenous adenosine is governed by G_i protein-mediated signal transduction mechanisms (both cAMP and K channel-dependent processes) (15), we predicted that, if chronic exotoxin A exposure reduced the *specific* production of the α-subunit of the G_i protein, the negative chronotropic responses to exogenous adenosine would be depressed. However, we did not find any significant exotoxin A-induced changes in the negative chronotropic response to exogenous adenosine. Taken together, these findings suggest that although chronic exposure to exotoxin A may prevent an *increase* in the myocyte α-subunit of the G_i protein (14), it does not cause an actual *decrease* in the G_i protein that can be assessed functionally. Alternatively, the data might also be explained if chronic exposure to exotoxin A resulted in a simultaneous alteration of the stimulatory G_s protein, which would then counteract the suppression of the G_i protein.

One possible explanation for the lack of expected effects of exotoxin A treatment on responses of isolated hearts to isoproterenol and adenosine might be that the heart was not affected by the intraperitoneal injection of the exotoxin A. Studies by Iglewski *et al.* (12), however, indicate that the heart is indeed a target organ for the effects of exotoxin A. In their studies, exotoxin A injected intraperitonally into mice decreased the amount of elongation factor 2 in all organs except the brain. The decrease in the heart was approximately 50% of that achieved in the liver.

A second possible explanation for the lack of ex-

Table III. Adenosine and Inosine Concentrations in Coronary Effluent of Isolated Perfused Rat Hearts[a]

	Adenosine (n*M*)		Inosine (n*M*)	
	Untreated ($n = 11$)	Exotoxin A treated ($n = 11$)	Untreated ($n = 11$)	Exotoxin A treated ($n = 11$)
Control period 1	65 ± 15	82 ± 32	67 ± 13	119 ± 30
+ 6 min hypoxia (30% O_2)	29 ± 7[b]	59 ± 23[b]	98 ± 25[b]	185 ± 57[b]
Control period 2	7 ± 4	45 ± 17	17 ± 3	67 ± 23
+ 6 min isoproterenol (10 n*M*)	49 ± 16[b]	117 ± 27[b]	98 ± 31[b]	336 ± 110[b]
Control period 3	22 ± 9	11 ± 5	10 ± 4	15 ± 6
+ 6 min exogenous adenosine (10 μM)	4753 ± 327[b]	4024 ± 384[b]	2006 ± 107[b]	2302 ± 249[b]

[a] Rats were either untreated or injected with *Pseudomonas aeruginosa* exotoxin A 48 hr before the experiment.
[b] $P < 0.05$ as compared with the preceding control value within the group (paired t test).

pected effects on isoproterenol and adenosine responses might be that the concentrations of exotoxin A used were too low to be effective. The concentrations (7 μg/kg or 22 μg/kg) used in this study were well above the LD_{50} determined in preliminary studies (J. Patzer, unpublished data) for mice weighing 18–20 g (5 μg/kg), but was substantially below that determined in this study for rats weighing 400–500 g (33 μg/kg). Thus, the relative levels chosen for these experiments may have been too low to evoke full-blown expression of all the toxic effects in the rat.

Since in this study both the low and high doses of exotoxin A increased automaticity and enhanced the depression evoked by hypoxia to a similar extent, it would appear that these particular alterations are both evoked by low levels of exotoxin A which might be more nearly equivalent to the relative levels found in nonfatal Pseudomonas septicemia in patients. On the basis of the current findings, one might expect such patients to have an enhanced cardiac excitability and an increased cardiac vulnerability to the hypoxic insults that might accompany a Pseudomonas-induced pneumonia.

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1. Carney SA, Dyster RE, Jones RJ. The invasion of burned skin by *Pseudomonas aeruginosa*. Br J Dermatol **88**:539–545, 1973.
2. Hoiby N. Microbiology of lung infections in cystic fibrosis patients. Acta Paediatr Scand [Suppl] **301**:33–54, 1982.
3. Wood RE. *Pseudomonas*: The compromised host. Hosp Pract **11**:91–100, 1976.
4. Liu PV. Extracellular toxins of *Pseudomonas aeruginosa*. J Infect Dis **130**:S94–S99, 1974.
5. Cross AS, Sadoff JC, Iglewski BH, Sokol PA. Evidence for the role of toxin A in the pathogenesis of infection with *Pseudomonas aeruginosa* in humans. J Infect Dis **142**:538–546, 1980.
6. Nicas TI, Iglewski BH. The contribution of exoproducts to virulence of *Pseudomonas aeruginosa*. Can J Microbiol **31**:387–392, 1985.
7. Vasil ML, Chamberlain C, Grant CCR. Molecular studies of *Pseudomonas aeruginosa* exotoxin A gene. Infect Immun **52**:538–548, 1986.
8. Pollack M, Callahan LT, Taylor N. Neutralizing antibodies to *Pseudomonas aeruginosa* exotoxin A in human sera: Evidence for in vivo production during infections. Infect Immun **14**:942–947, 1976.
9. Pollack M, Young LS. Protective activity of antibodies to exotoxin A and lipopolysaccharide at the onset of *Pseudomonas aeruginosa* septicemia in man. J Clin Invest **63**:276–286, 1979.
10. Jagger KS, Robinson DL, Franz MN, Warren RL. Detection by enzyme-linked immunosorbent assay of antibodies for *Pseudomonas aeruginosa* proteases and exotoxin A in sera from cystic fibrosis patients. J Clin Microbiol **15**:1054–1058, 1982.
11. Iglewski BH, Kabat D. NAD-dependent inhibition of protein synthesis by *Pseudomonas aeruginosa* toxin. Proc Natl Acad Sci USA **72**:2284–2288, 1975.
12. Iglewski BH, Liu PV, Kabat D. Mechanism of action of *Pseudomonas aeruginosa* exotoxin A: Adenosine diphosphate-ribosylation of mammalian elongation factor 2 in vitro and in vivo. Infect Immun **5**:138–144, 1977.
13. Werdan K, Melnitski SM, Pilz G, Kapsner T. The cultured rat heart cell: A model to study direct cardiotoxic effects of *pseudomonas* endo- and exotoxins. Prog Clin Biol Res **308**:247–251, 1989.
14. Reithmann C, Gierschik P, Muller U, Werdan K, Jakobs KH. *Pseudomonas* exotoxin A prevents β-adrenoceptor-induced up-regulation of G_i protein α-subunits and adenylyl cyclase desensitization in rat heart muscle cells. Mol Pharmacol **37**:631–638, 1990.
15. Olsson RA, Pearson JD. Cardiovascular purinoceptors. Physiol Rev **70**:761–844, 1990.
16. Heller LJ, Mohrman DE. Estimates of interstitial adenosine from surface exudates of isolated rat hearts. J Mol Cell Cardiol **20**:509–523, 1988.
17. Mohrman DE, Heller LJ: Transcapillary adenosine transport in isolated guinea pig and rat hearts. Am J Physiol **259**:H772–783, 1990.