

Catecholamine Tachyphylaxis in Isolated Hearts.* (32600)

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In several species it has been established that repeated injections or continuous infusions of epinephrine or norepinephrine produce progressively diminished blood pressure responses(1,2,3,4). The means by which tolerance or tachyphylaxis to catecholamines occurs has yet to be established. Several alternate hypotheses have been proposed, including saturation of norepinephrine storage sites in adrenergic organs(2) and "receptor saturation"(3). The results of norepinephrine infusions in dogs(5) suggest that the primary locus of the tolerance is on the heart. Increases in serum lactic dehydrogenase have been associated with heart lesions(6). In the above studies the alterations in heart sensitivity to administered catecholamines were not investigated in the absence of reflex influences on the heart.

The purpose of this study was to investigate the effects of *in vivo* and *in vitro* infusions of catecholamines on responses of isolated hearts to catecholamines and to acetylcholine. Once isolated from the animal's body, the heart would no longer be subject to various efferent nervous activity and the levels of circulating catecholamines and metabolic products could easily be controlled.

Methods. *In vivo* infusions. 10 male Dutch rabbits, weighing from 1.2-3.0 kg, were anesthetized with phenobarbital sodium (200 mg/kg) administered intravenously. A femoral artery was cannulated and systemic arterial pressure was measured by a Satham P23AA pressure transducer and recorded on an Offner type RS Dynograph. An external jugular vein was cannulated and the catheter was attached to a calibrated Holter model RD045 infusion pump for the infusion. 5 control animals were infused with saline, 1 ml/min for 40 min. 5 experimental animals received 3 μ g norepinephrine/kg/min for 40 min, at which

time blood pressure was found to be within 30% of preinfusion levels. At the end of the infusion period each animal's heart was excised, the aorta cannulated, and the heart placed in a conventional heart perfusion apparatus. The isolated hearts were perfused at a constant pressure of 35 mm Hg with Locke Ringer solution for isolated hearts warmed to 37°C and aerated by bubbling 95% oxygen—5% carbon dioxide. Contractile force was measured from a Grass FT 0.3 tension transducer and recorded by a Gilson (GME) polygraph. Heart rate was counted from recorder pen deflections at the time of maximal rate response. Inotropic and chronotropic responses of the isolated hearts to 2 dose levels each of epinephrine, norepinephrine, isoproterenol and acetylcholine were determined during 2 perfusion periods: 0-30 min and 30-60 min after excision.

In vitro infusions. 10 male Dutch rabbits were sacrificed by cervical fracture and their hearts prepared as above. Control responses to 2 dose levels of epinephrine, norepinephrine, isoproterenol and acetylcholine were determined. 5 hearts were then infused with Locke Ringer solution containing 3 μ g/ml norepinephrine and 5 hearts were infused with epinephrine, 3 μ g/ml. After the 60-min infusion period, perfusion was continued with Locke Ringer solution containing no catecholamine and the responses to the catecholamines and acetylcholine were again determined.

Drugs and chemicals. Norepinephrine bitartrate (Levophed; Winthrop), epinephrine hydrochloride (Adrenalin; Parke, Davis), isoproterenol hydrochloride (Isuprel; Winthrop) and acetylcholine chloride (Nutritional Biochemicals). All doses were calculated as the free bases and the catecholamines were dissolved in appropriate concentrations in 0.9% sodium chloride.

Statistical analyses were performed by 4 point bioassay(8); Student's *t* test paired comparison(9) and by analysis of variance

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2 × 2 and 4 × 4 factorial randomized complete block design (10). Values of P equal to or less than 0.05 were considered significant.

Results. *In vivo* infusions. The isolated hearts taken from rabbits which had been infused with norepinephrine were found to be much less reactive to epinephrine, norepinephrine and isoproterenol than the hearts removed from the saline-infused animals (positive inotropic actions to the catecholamines). As can be seen in Table I, the hearts from norepinephrine-infused animals were only 1/8 to 1/6 as reactive to the catecholamines (as determined by the relative potency calculations) as hearts from animals infused with saline. Reactivity of neither group of hearts was significantly altered during the 2nd perfusion period (30-60 min after excision). No relative potency calculation could be performed on the responses to isoproterenol during the second period, since analysis of variance provided evidence that the regression was not significant. There was no systematic decay of positive inotropic responses after 30 min of perfusion of hearts from saline-infused rabbits or improvement in reactivity of hearts from animals infused with norepinephrine.

Comparison of mean pre-stimulus contractile force (average of 8 pre-drug control periods for each heart during each perfusion period) developed spontaneously by the hearts revealed that in the control (saline-infused) group there was a significant decrease in mean contractile force in the 2nd perfusion period (Table II), and a slight, nonsignificant increase in the norepinephrine-infused group. Similarly, there was a significant decrease in mean spontaneous heart rate in the 2nd infusion period in the control hearts but, due to more variability, the decrease in spontaneous heart rate in the hearts from norepinephrine-infused animals was not significant between the 2 perfusion periods. Comparison of the 2 groups of hearts provided evidence that the difference in pre-drug contractile force, but not heart rate, between the saline-infused and the norepinephrine-infused animals was statistically significant (Table II).

The action of acetylcholine on the isolated hearts was not altered by infusing the ani-

TABLE I. Effects of Prior Infusion *in vivo* with Saline or Norepinephrine on Positive Inotropic Responses of Isolated Rabbit Hearts to Catecholamines During Two *in vitro* Perfusion Periods (0-30 Min and 30-60 Min After Excision). Parallel line bioassay.

Stimulus	No. of hearts	0-30 min after excision						30-60 min after excision					
		Saline-infused			Norepinephrine-infused			Saline-infused			Norepinephrine-infused		
		Dose of stimulus (μg)	Mean ↑ CF (g)	Relative potency with 95% limits	Dose of stimulus (μg)	Mean ↑ CF (g)	Relative potency with 95% limits	Dose of stimulus (μg)	Mean ↑ CF (g)	Relative potency with 95% limits	Dose of stimulus (μg)	Mean ↑ CF (g)	Relative potency with 95% limits
Epinephrine	5	0.4	4.6	0.12 (0.03-0.20)	2.5	4.6	0.12 (0.03-0.20)	0.4	3.6	0.20 (0.10-0.44)	2.5	3.8	0.20 (0.10-0.44)
		0.8	6.2		5.0	5.2		5.0	6.4				
Norepinephrine	5	0.4	4.2	0.15 (0.14-0.22)	2.5	4.6	0.15 (0.14-0.22)	0.4	4.2	0.16 (0.09-0.29)	2.5	4.6	0.16 (0.09-0.29)
		0.8	6.4		5.0	5.8		5.0	5.8				
Isoproterenol	5	0.2	7.8	0.17 (0.02-0.38)	1.0	7.2	0.17 (0.02-0.38)	0.2	9.2	Regression not significant	1.0	6.6	Regression not significant
		0.4	8.6		2.0	8.6		2.0	7.0				

TABLE II. Comparisons of Mean Pre-Stimulus Values for Spontaneous Heart Rate (HR) and Contractile Force (CF) in Hearts Taken from Rabbits Infused *in vivo* with Saline or Norepinephrine and Effects of Duration of *in vitro* Perfusion.

Infused <i>in vivo</i> with	0-30 min <i>in vitro</i>	30-60 min <i>in vitro</i>	Difference ± S.E.	No. of hearts	Change P*	Saline vs norepinephrine P†
	Mean CF (g)	Mean CF (g)				
Saline	6.6	5.8	-0.8 ± 0.3	5	<0.05 } NS }	<0.05
Norepinephrine	3.0	3.4	+0.4 ± 0.4	5		
	Mean HR (/min)	Mean HR (/min)				
Saline	181	165	-16 ± 5	5	<0.05 } NS }	NS
Norepinephrine	202	166	-36 ± 24	5		

* Level of significance, Student's t test, paired comparison. NS = not statistically significant.

† Level of significance, analysis of variance, 2 × 2 factorial randomized complete block design. NS = not statistically significant.

mals with norepinephrine (Table III). There was no significant difference in the negative chronotropic or inotropic effects of acetylcholine in the 2 groups of hearts or between perfusion periods. Comparison of the effects of catecholamines on heart rate responses showed that there was no significant alteration of rate responses in either group of hearts

(saline- or norepinephrine-infused) between the 2 periods of perfusion (Table IV). There was a significant increase in the rate responses to epinephrine, but not to norepinephrine or isoproterenol, in the hearts from animals infused with norepinephrine as compared to the saline-infused controls. In these experiments the catecholamines often produced slight

TABLE III. Effects of Acetylcholine (ACh) on Isolated Rabbit Hearts Infused *in vivo* with Norepinephrine or *in vitro* with Either Epinephrine or Norepinephrine on Heart Rate (HR) and Contractile Force (CF).

Infusion	Period	Dose of ACh (μg)	↓CF (g)	Period	Dose of ACh (μg)	↓CF (g)	No. of hearts	Change P*	Saline vs norepinephrine P†
Saline <i>in vivo</i>	0-30 min	2.0	1.2	30-60 min	2.0	1.2	5 } 5 }	NS	} NS
		4.0	3.2		4.0	3.2			
Norepinephrine <i>in vivo</i>	0-30 min	2.0	1.2	30-60 min	2.0	1.2	5 } 5 }	NS	
		4.0	1.4		4.0	2.8			
Norepinephrine <i>in vitro</i>	Control	2.0	5.0	After infusion	2.0	2.2	5 } 5 }	<0.005	
		4.0	5.6		4.0	3.0			
Epinephrine <i>in vitro</i>	Control	2.0	1.6	After infusion	2.0	1.2	5 } 5 }	0.05	
		4.0	3.2		4.0	1.2			
			↓HR (/min)			↓HR (/min)			
Saline <i>in vivo</i>	0-30 min	2.0	85	30-60 min	2.0	81	5 } 5 }	NS	} NS
		4.0	115		4.0	111			
Norepinephrine <i>in vivo</i>	0-30 min	2.0	119	30-60 min	2.0	96	5 } 5 }	NS	
		4.0	168		4.0	136			
Norepinephrine <i>in vitro</i>	Control	2.0	84	After infusion	2.0	129	5 } 5 }	<0.05	
		4.0	118		4.0	171			
Epinephrine <i>in vitro</i>		2.0	130	After infusion	2.0	108	5 } 5 }	NS	
		4.0	176		4.0	172			

* Level of significance, analysis of variance, 2 × 2 factorial randomized complete block design. NS = not statistically significant.

† Level of significance, analysis of variance, 4 × 4 factorial randomized complete block design. NS = not statistically significant.

TABLE IV. Effects of Epinephrine, Norepinephrine and Isoproterenol on Changes in Heart Rate (HR) in Isolated Rabbit Hearts Infused *in vivo* with Norepinephrine or *in vitro* with Epinephrine or Norepinephrine.

Stimulus	Infusion	Period	Stimulus dose (μg)	Mean Δ HR (/min)	Period	Stimulus dose (μg)	Mean Δ HR (/min)	No. of hearts	Change P*	Saline vs norepinephrine P†
Epinephrine	Saline <i>in vivo</i>	0-30 min	0.4	- 4	30-60 min	0.4	- 4	5	NS	<0.05
		0-30 min	0.8	+ 4		0.8	- 2			
Norepinephrine	<i>in vivo</i>	0-30 min	2.5	+ 34	30-60 min	2.5	+ 14	5	NS	
		0-30 min	5.0	+ 32		5.0	+ 26			
Norepinephrine	Saline <i>in vivo</i>	0-30 min	0.4	+ 4	30-60 min	0.4	+ 2	5	NS	
		0-30 min	0.8	- 2		0.8	+ 2			
Isoproterenol	Norepinephrine <i>in vivo</i>	0-30 min	2.5	+ 4	30-60 min	2.5	+ 18	5	NS	
		0-30 min	5.0	+ 12		5.0	+ 16			
Epinephrine	Saline <i>in vivo</i>	0-30 min	0.2	+ 12	30-60 min	0.2	+ 44	5	NS	
		0-30 min	0.4	+ 38		0.4	+ 38			
Norepinephrine	<i>in vivo</i>	0-30 min	1.0	+ 34	30-60 min	1.0	+ 32	5	NS	
		0-30 min	2.0	+ 32		2.0	+ 58			
Epinephrine	Norepinephrine <i>in vitro</i>	Control	0.4	0	After-in-fusion	5.0	+ 60	5	NS	
		"	0.8	- 20		10.0	+ 110			
Isoproterenol	"	"	0.2	- 10	"	2.5	- 30	5	NS	
		"	0.4	- 10		5.0	+ 120			
Epinephrine	<i>in vitro</i>	"	0.2	+ 150	"	1.0	+ 210	5	NS	
		"	0.4	+ 180		2.0	+ 260			
Norepinephrine	"	"	0.2	- 2	"	5.0	+ 8	5	NS	
		"	0.4	+ 20		10.0	+ 46			
Isoproterenol	"	"	0.2	- 4	"	5.0	+ 14	5	<0.05	
		"	0.4	- 2		10.0	+ 30			
Epinephrine	"	"	0.2	+ 56	"	1.0	+ 20	5	NS	
		"	0.4	+ 50		2.0	+ 70			

* Level of significance, analysis of variance 2×2 factorial randomized complete block design. NS = not significant.
 † Level of significance, analysis of variance 4×4 factorial randomized complete block design. NS = not significant.

TABLE V. Effects of Infusion *in vitro* with Either Epinephrine or Norepinephrine on Contractile Force (CF) Responses to Epinephrine, Norepinephrine and Isoproterenol. Parallel line bioassay.

Stimulus	No. of hearts	Control period		Infused with	After infusion		Relative potency with 95% limits
		Dose of stimulus (μg)	$\uparrow\text{CF}$ (g)		Dose of stimulus (μg)	$\uparrow\text{CF}$ (g)	
Epinephrine	5	0.4	3.8	Norepinephrine, 3 $\mu\text{g}/\text{min}$	5.0	2.6	0.052 (0.0001-0.094)
		0.8	6.0		10.0	4.6	
Norepinephrine	5	0.2	4.0	"	2.5	4.2	0.064 (0.007 -0.13)
		0.4	8.4		5.0	6.2	
Isoproterenol	5	0.2	6.2	"	1.0	3.8	0.10 (0.002 -0.24)
		0.4	10.0		2.0	6.2	
Epinephrine	5	0.2	1.8	Epinephrine, 3 $\mu\text{g}/\text{min}$	5.0	2.6	0.045 (0.024 -0.11)
		0.4	5.2		10.0	5.4	
Norepinephrine	5	0.2	3.6	"	5.0	3.8	0.033 (0.014 -0.055)
		0.4	5.6		10.0	4.6	
Isoproterenol	5	0.2	7.2	"	1.0	3.4	0.068 (0.015 -0.11)
		0.4	9.8		2.0	5.8	

(nonsignificant) slowing of the hearts during the height of the positive chronotropic responses. This was most often observed in the control (saline-infused) group of hearts and may have been a consequence of the doses of catecholamines employed. Larger doses of catecholamines in non-pretreated hearts usually produce positive chronotropic effects during the maximum inotropic response.

Examination of the hearts from the animals infused with norepinephrine failed to reveal any gross pathological changes, but no detailed pathological studies were performed. Heart weight (measured before perfusion *in vitro*) was not altered by prior norepinephrine infusion (hearts from saline-infused animals weighed 3.1 ± 0.4 g/kg, from norepinephrine-infused animals weighed 3.3 ± 0.2 g/kg) and the hearts could not be said to be edematous.

In vitro infusions. Since the effects of *in vivo* infusion of norepinephrine on the subsequently isolated hearts could have been due to a number of non-cardiac effects of norepinephrine (reflex effects, general metabolic actions, alteration of blood electrolytes, peripheral release of cardiac depressant substances, etc.), hearts were infused *in vitro* with norepinephrine to provide a comparison of the effects of infusion *in vivo* and *in vitro*. A 2nd group of hearts was infused *in vitro* with epinephrine to compare the effects on the hearts of infusion of epinephrine *vs* nor-

epinephrine. As can be seen in Table III, infusion of hearts *in vitro* with norepinephrine and with epinephrine significantly decreased the absolute negative inotropic effects of acetylcholine, but there was little or no alteration in the percent changes induced. Infusion with norepinephrine, but not with epinephrine, significantly increased the absolute and percent negative chronotropic effects of acetylcholine. Perfusion of hearts with norepinephrine *in vitro* did not alter the rate responses to epinephrine, norepinephrine or isoproterenol (Table IV). Infusion with epinephrine resulted in a significant enhancement of the rate responses to norepinephrine, but not to epinephrine or isoproterenol (Table IV).

Infusion of either norepinephrine or epinephrine *in vitro* drastically reduced reactivity (positive inotropic responses) to subsequently administered catecholamines (Table V). After the *in vitro* infusion of catecholamines the hearts were only 1/30th to 1/10th as reactive to epinephrine, norepinephrine or isoproterenol as during the pre-infusion control test period. Comparison of mean pre-drug contractile force before and after catecholamine infusion demonstrated that infusion of either norepinephrine or epinephrine significantly reduced pre-stimulus contractile force (Table VI). Mean pre-drug spontaneous heart rate was similarly decreased after the catecholamine infusion (Table VI).

Discussion. It is obvious from these data

TABLE VI. Comparisons of Mean Pre-Stimulus Values for Spontaneous Heart Rate (HR) and Contractile Force (CF) in Isolated Rabbit Hearts Perfused *in vitro* with Either Epinephrine or Norepinephrine.

Mean CF (g) pre-infusion control	Infusion with	Mean CF (g) after infusion	Difference ± S.E.	No. of hearts	P*
10.7	Norepinephrine, 3 µg/min	5.2	-5.5 ± 1.0	5	<0.01
6.3	Epinephrine, 3 µg/min	3.5	-2.8 ± 0.8	5	<0.05
Mean HR (/min) pre-infusion control		Mean HR (/min) after infusion			
184	Norepinephrine, 3 µg/min	168	-16 ± 7	5	<0.05
205	Epinephrine, 3 µg/min	162	-43 ± 11	5	<0.01

* Level of significance, analysis of variance 2 × 2 factorial randomized complete block design.

that the infusion of norepinephrine *in vivo* in rabbits results in altered reactivity of subsequently isolated hearts and that this phenomenon can also be produced by *in vitro* infusion of either norepinephrine or epinephrine. It has previously been suggested that tachyphylaxis to catecholamines could result from saturation of either norepinephrine storage sites(2) or catecholamine receptors(3). If either of these ideas were true, one would expect desaturation of either site after the hearts were removed and perfused with an artificial solution containing no catecholamine. The "receptor saturation" theory, previously very attractive, holds that the speed with which the adrenergic receptors become saturated is a function of the plasma concentration of catecholamine molecules(4); conversely the recovery rate should be inversely related to plasma catecholamine concentration. Since no improvement in reactivity to catecholamines or spontaneous activity occurred even 30-60 min after isolation of the heart, these theories become doubtful. Similarly, since both control and experimental hearts were perfused *in vitro* with artificial solutions of exactly the same composition, accumulation of metabolic products cannot be blamed for altered reactivity of hearts from animals infused with norepinephrine.

Infusions of isolated hearts *in vitro* with either norepinephrine or epinephrine produced results qualitatively comparable to those after *in vivo* infusions of norepinephrine. These findings indicate that the infused norepinephrine acted directly on the heart to produce those changes reflected as decreased reactiv-

ity to subsequently administered catecholamines and altered spontaneous inotropic and chronotropic activity. Non-cardiac influences cannot now be implicated as playing causative roles in the development of the altered cardiac performance observed after norepinephrine infusion. Since little alteration of responses to acetylcholine were noted, the cardiac parasympathetic system can be eliminated as the primary factor resulting in altered responsiveness to exogenous catecholamines.

It would therefore appear likely that the infusion of norepinephrine produces either metabolic or pathologic changes in the heart which persist for some time after removal of the norepinephrine. These findings would be most closely related to those in the dog heart by Schenk *et al*(7). The method of producing tachyphylaxis to catecholamines *in vitro* should be a useful means of studying these phenomena.

Summary. Hearts isolated from rabbits infused *in vivo* with norepinephrine were found to be 5-8 times less reactive (positive inotropic effects) to epinephrine, norepinephrine and isoproterenol than were hearts removed from animals similarly infused with saline. Mean non-stimulated contractile force, but not mean spontaneous heart rate, was found to be significantly depressed in hearts from the norepinephrine-infused animals. Negative inotropic and chronotropic responses to acetylcholine were unaltered. Performance of hearts from norepinephrine-infused animals did not improve with time up to 60 min after isolation. Hearts from non-infused animals were infused *in vitro* with epi-

nephrine or norepinephrine and showed alterations in reactivity similar to those observed with hearts from animals infused *in vivo* with norepinephrine. The results suggest that the infused catecholamines interact directly with mammalian hearts to produce persistent metabolic or pathologic alterations in reactivity.

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The Cell Cycle of Leukemia L1210 Cells *in vivo* and *in vitro*.* (32601)

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Leukemia L1210 has been and is being used extensively for evaluation of candidate anticancer agents. Ascitic cells of this neoplasm can be cultured *in vitro*, and it has been demonstrated that when cultured cells are implanted into mice, a disease indistinguishable from Leukemia L1210 results(1). In view of the current interest in the relationship between the phases of the cell cycle and the susceptibility of cells to various drugs and other agents, it seemed desirable to determine the length of the cell cycle and of the different phases of the cycle for these cells. It also seemed worthwhile to compare the cycles of cells proliferating in the mouse, of cells proliferating *in vitro*, and of cells proliferating in the mouse following implantation of cultured cells. If the cycle of cells in culture is similar to that of cells in mice, then the results of experiments with cultured

cells would have increased importance.

Methods and materials. For *in vivo* experiments mice (BDF₁) were inoculated intraperitoneally with 10⁵ L1210 cells, and on the 6th day thereafter they were given thymidine-(methyl-³H) (specific activity, 6.7 c per millimole) intraperitoneally at a dosage of 1 μ c per gram of body weight. Groups of 3 to 5 animals were killed by asphyxiation with carbon dioxide at various times following the injection of the radioactive substrate, and the ascites cells were taken in syringes containing small quantities of heparin and pooled. The cells were separated by centrifugation in a clinical centrifuge and suspended in 4 ml of cold 0.1 M citric acid. The tube containing the suspension was placed in a water bath at 37°C for 1/2 minute, and the cells were again collected by centrifugation. (In experiments in which the initial number of cells per unit volume was large, only a portion of the suspension of the cells in citric acid was used for the remainder of the procedure.) The cells were suspended in 0.4 ml of ethanol-

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