Uptake and Subcellular Distribution of Norepinephrine in the Canine Heart Following Experimental Myocardial Infarction[1] (35084)

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Increasing evidence suggests that the activity of the sympathetic nervous system is altered following myocardial infarction. Elevated plasma norepinephrine (NE) levels and increased urinary excretion of catecholamines have been demonstrated in human subjects as well as in experimental animals (1–3). In previous observations from this laboratory it could be shown that following experimental myocardial infarction in dogs, the non-infarcted heart muscle shows a marked decline in norepinephrine levels in the postinfarction period, reaching the lowest levels in the second week after infarction (4).

The purpose of this study was to examine if changes in myocardial NE-uptake might be responsible for the decline in myocardial NE-stores. Uptake and metabolism of tracer doses of *dl*-norepinephrine-7-^{14}C were determined as well as the subcellular distribution of endogenous NE and the uptake of the labeled amine into the subcellular fractions of non-infarcted heart muscle. Determinations were carried out 10 days after infarction when the lowest endogenous NE-levels were observed, as well as 6 weeks after the infarct, when endogenous NE-levels had returned to normal.

Methods. Experiments were performed on 18 mongrel dogs, weighing from 16 to 24 kg. Myocardial infarction was produced by ligation of several branches of the anterior descending and circumflex coronary arteries to obtain an anterolateral infarction of approximately uniform size as described previously (5). The animals were divided into three groups of six each; (i) a control group, (ii) animals studied 10 days after infarction, and (iii) animals studied 6 weeks after infarction. The animals were anesthetized with a combination of Sublimaze (0.04 mg/kg of body wt), inaprine (2 mg/kg) and sodium pentobarbital (6–8 mg/kg) to avoid depression of the respiratory activity. *Dl*-Norepinephrine-7-^{14}C, (0.27 mCi/mg) 1 μCi/kg of body weight was injected intravenously, and 1 hr later, the animals were sacrificed with an intravenous injection of saturated potassium chloride solution. The heart was immediately removed, dissected on crushed ice and analyzed for endogenous and radioactive NE content. The endogenous myocardial norepinephrine content was determined in non-infarcted left and right ventricular myocardium. The determination was done in 2-g tissue samples, which were grossly freed from blood, fat, and connective tissue, according to the method of Anton and Sayre (6) as modified by De Champlain *et al.* (8) as reported previously (4).

Duplicate recoveries were run with each assay, and the final values for endogenous as well as radioactive NE were corrected for these recoveries. The average recovery was 81 $\pm$ 6.4% SD. All determinations for left ventricular tissue were done in duplicate, and the standard deviation for these duplicates was $\pm$5.1%. The radioactivity of 1.0-ml aliquots of the neutralized perchloric acid extract and the acetic acid eluate was determined in a liquid scintillation spectrometer

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after addition of 15 ml of dioxane–phosphor scintillation mixture. In this system, the counting efficiency for ^{14}C was 82%, and external radioactive standards were used to correct for individual variations. Sufficient counts were obtained to maintain a standard deviation of counting below 1%. An estimate of the total NE-^{14}C-metabolites was obtained by calculating the difference between the radioactivity of the perchloric acid extract and the radioactivity of the acetic acid eluate from the alumina.

Studies of the subcellular distribution of norepinephrine were done by differential centrifugation of a homogenate of non-infarcted left ventricular muscle according to the method of Campos and Shideman (7). The tissue was homogenized in ice-cold 0.075 *M* potassium phosphate buffer, pH 7.5, in a glass homogenizer. The homogenate was centrifuged at 2000*g* for 5 min at 4°. The supernatant was then transferred into a polyethylene tube and centrifuged at 100,000*g* for 1 hr at 4°. The pellets were resuspended and rehomogenized in 0.4 *M* perchloric acid, and the supernatant was acidified by addition of 1.2 *M* perchloric acid to obtain a final concentration of 0.4 *M* perchloric acid. All fractions were centrifuged again at 18,500*g* for 15 min and the norepinephrine content was measured as described above. The radioactivity of 1.0-ml aliquots of the acetic acid eluate from the alumina columns was determined as described above.

Results. Endogenous norepinephrine levels. The endogenous level of norepinephrine fell from a control value of 0.99 ± 0.02 to 0.35 ± 0.01 μg of norepinephrine/g of tissue in the noninfarcted portion of the left ventricle ($p<0.001$) 10 days after infarction. In the right ventricle, the finding was similar; the NE-level fell from a control value of 0.97 ± 0.03 to 0.34 ± 0.02 μg/g of tissue (Table I). The third group, sacrificed 6 weeks after infarction, had essentially normal NE-levels; 0.92 ± 0.05 μg in the left and 0.97 ± 0.03 μg of NE/g of tissue in the right ventricle.

Subcellular distribution of norepinephrine. The concentration of norepinephrine in homogenates of heart muscle prepared in phosphate buffer did not differ significantly from those determined in homogenates prepared in 0.4 *N* perchloric acid.

Normal left ventricular muscle contained 0.95 ± 0.04 μg of NE/g of tissue. Ten days after infarction, the non-infarcted left ventricular muscle contained 0.36 ± 0.03 μg, and 6 weeks after infarction, 0.93 ± 0.07 μg of NE/g of tissue.

The subcellular distribution is illustrated in Fig. 1. In normal left ventricular muscle, the particle bound fraction contained 58.9% and the soluble fraction contained 13.7% of the total norepinephrine content. 10 days after infarction, the relative distribution remained similar despite marked changes in total content. The particulate fraction contained 61.1% and the soluble fraction 13.8% of the total norepinephrine. 6 weeks after infarction, with norepinephrine levels close to the control, the subcellular distribution was unchanged.

Uptake and metabolism of dl-norepinephrine-7-^{14}C. The term "uptake" here refers

TABLE I. Uptake, Metabolism, and Endogenous Levels of Norepinephrine.

Ventricle	Test	Control $N=6$	10 Days after infarction $N=6$	6 Weeks after infarction $N=6$
Left	Endogenous norepinephrine (μg/g of tissue)	0.99 ± 0.02	0.35 ± 0.01[a]	0.92 ± 0.05
	^{14}C-NE (1×10^{-12} moles/g of tissue)	118.0 ± 5.26	115.3 ± 7.03	130.6 ± 10.9
	^{14}C-Metabolites (1×10^{-12} moles/g of tissue)	14.2 ± 1.54	14.3 ± 3.25	11.8 ± 0.88
Right	Endogenous norepinephrine (μg/g of tissue)	0.97 ± 0.03	0.34 ± 0.02[a]	0.97 ± 0.03
	^{14}C-NE (1×10^{-12} moles/g of tissue)	116.4 ± 7.62	100.4 ± 7.03	112.1 ± 10.75
	^{14}C-Metabolites (1×10^{-12} moles/g of tissue)	15.1 ± 2.13	13.9 ± 1.18	19.5 ± 5.49

[a] $p<0.001$.

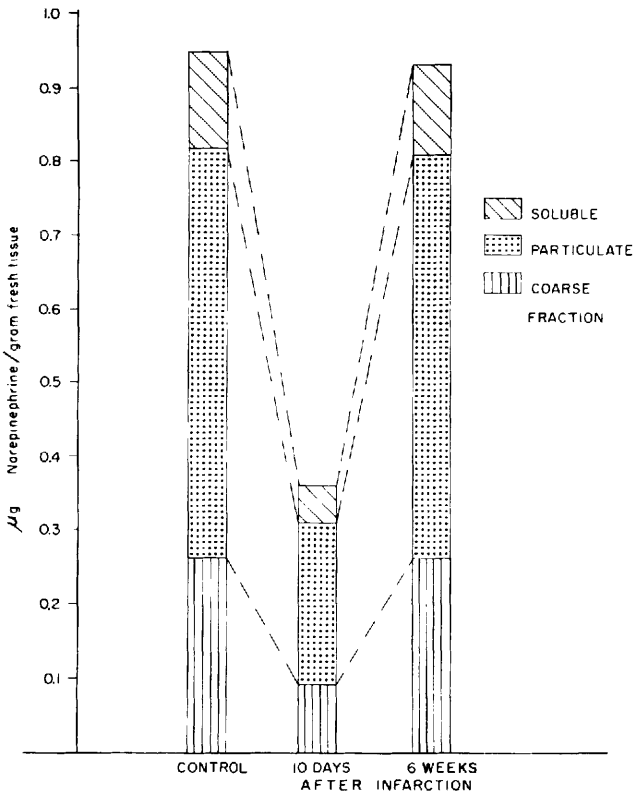

FIG. 1. Subcellular distribution of norepinephrine in non-infarcted heart muscle following myocardial infarction.

to the uptake and accumulation of *dl*-norepinephrine-7-^{14}C in heart muscle during 1 hr after the injection of 1 μCi of NE-^{14}C/kg of body weight. One hr after injection, the normal heart contains 118 $\pm$ 5.20 $\times$ 10^{-12} moles/g of tissue of *dl*-norepinephrine-7-^{14}C. The amount of radioactive metabolites present at this time is 14.2 $\pm$ 1.54 $\times$ 10^{-12} moles/g of tissue, roughly 12% of the intact norepinephrine taken up. Despite the marked decrease in endogenous norepinephrine content 10 days after infarction, the heart takes up approximately the same amount of NE-^{14}C, 115.3 $\pm$ 7.03 $\times$ 10^{-12} moles/g of tissue.

Findings in the right ventricle were similar to those in the left ventricle. Despite the marked decline in the norepinephrine content, the uptake and degradation of injected material was essentially unchanged from the control. Furthermore, no difference was noted between right and left ventricle in the ability to take up and metabolize the injected *dl*-norepinephrine-7-^{14}C (Fig. 2). Six weeks after infarction, the norepinephrine content reached control values again. Uptake and the rate of degradation were nearly identical to values observed in control animals or in animals studied 10 days after infarction.

Uptake of dl-norepinephrine-7-^{14}C into subcellular fractions. Figure 3 depicts the amount of norepinephrine-^{14}C taken up 1 hr after injection of 1 μCi/kg of weight into the subcellular fractions. The amount taken up corresponds closely to the endogenous level of each fraction. In control animals, 59% of the endogenous norepinephrine content and 60.2% of the injected NE-^{14}C is found in the particulate fraction; the correlation between uptake and endogenous levels is equally close in the coarse and soluble fractions. Ten days after infarction, the uptake into the subcellular fractions in absolute terms is equal to

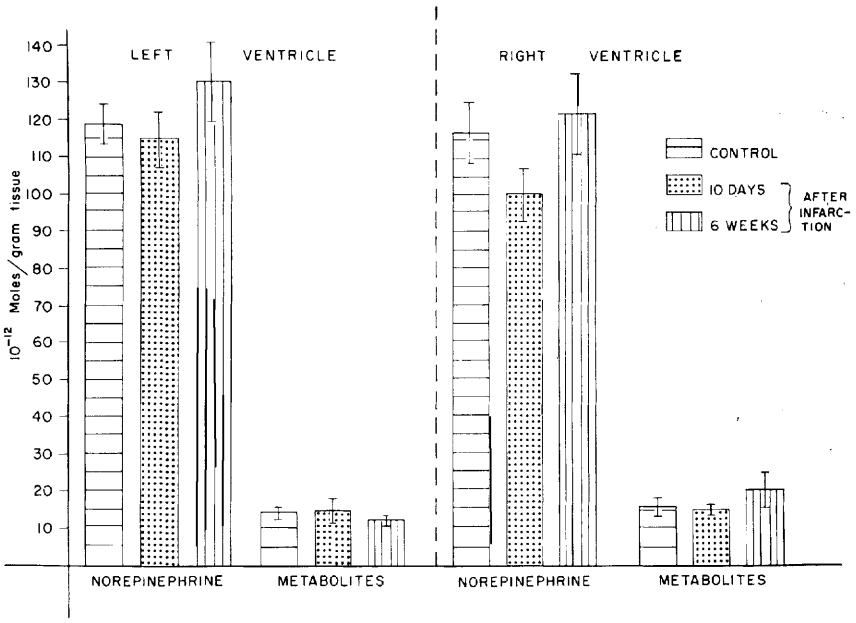

FIG. 2. The uptake and metabolism of *dl*-norepinephrine-7-^{14}C in non-infarcted heart muscle following myocardial infarction. The labeled amine and its metabolites were measured 1 hr after intravenous administration. Vertical bars represent ±SE.

control animals, despite the above described changes in pool size. Six weeks after infarction, the uptake in absolute terms as well as the relative distribution is again very close to control values, excluding a shift in intracellular pools or a change in the uptake mechanism as functional cause for the restoration of the norepinephrine stores.

Discussion. Ligation of several neighboring branches of the left descending and cir-

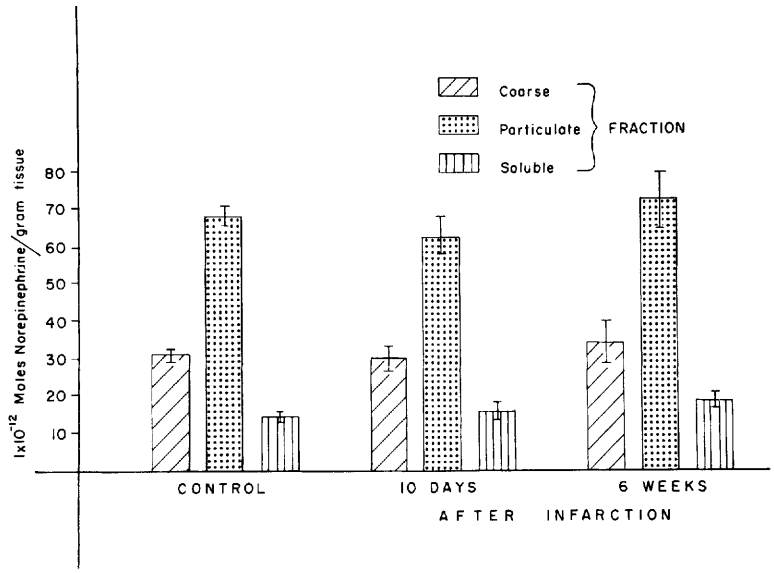

FIG. 3. The uptake and accumulation of *dl*-norepinephrine-7-^{14}C into subcellular fractions of non-infarcted heart muscle following myocardial infarction. Norepinephrine-^{14}C was measured 1 hr after intravenous administration. Vertical bars represent ±SE.

cumflex coronary arteries results in ischemic necrosis of the affected myocardium, an event that very closely resembles the clinical picture of myocardial infarction (5). In previous observations in this laboratory, it was shown that following such an experimental infarction, the entire heart loses a large portion of its norepinephrine stores. The infarcted tissue has lost its NE-content entirely after 48 hrs (4). A rapid decline in the norepinephrine content of the functioning myocardium can be noted. The lowest levels are reached by the tenth day and they persist until the end of the second week. Subsequently the tissue levels gradually rise and reach control values again six weeks after infarction. The decrease in NE-stores did not correlate with changes in energy rich phosphate levels or with changes in myocardial function (4). Myocardial ischemia could be ruled out as a factor causing the decline in norepinephrine stores in non-infarcted tissue, because tissue levels of lactate showed only minor, insignificant variations following infarction. No detectable amount of edema or fibrosis developed in non-infarcted muscle and possible myocardial hypertrophy ensuing after infarction could not account for the observed decline in NE-stores.

The uptake and accumulation of tracer doses of the labeled norepinephrine in non-infarcted heart muscle did not show a detectable alteration following myocardial infarction. The subcellular distribution (Fig. 1) of endogenous and exogenous NE was nearly identical in all three groups; approximately 60% of the total amount of norepinephrine was found in the particulate fraction, and this value remained quite constant after infarction, despite the marked variations in total NE-content.

It appears that the decline in cardiac norepinephrine stores following infarction is not due to a decreased ability of the sympathetic nerve ending to take up and accumulate circulating norepinephrine, or to an increased rate of degradation. The subcellular distribution of NE and the uptake of exogenous NE into these fractions appears to be unaffected, despite significant reduction in endogenous levels.

These observations indicate that the function of the sympathetic nerve ending is not impaired following infarction. An impairment in myocardial NE-synthesis may contribute to the depletion of the NE-stores, but the increase in the activity of the sympathetic nervous system following myocardial infarction and the increase in NE-release is probably the major factor responsible for the gradual exhaustion of the myocardial NE-stores. Following completion of compensatory processes and restoration of myocardial function, the activity of the sympathetic nervous system returns to normal and subsequently the NE-stores are restored.

Summary. Following surgical myocardial infarction in dogs, a marked decline in cardiac norepinephrine stores has been observed. In an attempt to elucidate the mechanism of the NE-depletion, the uptake, degradation, and subcellular distribution of norepinephrine in the canine myocardium were determined after myocardial infarction. The subcellular distribution, uptake and accumulation of tracer doses of *dl*-norepinephrine-7-^{14}C were unchanged and the rate of degradation was not significantly increased. It appears thus, that the decline in cardiac norepinephrine stores following infarction is neither due to a decreased ability of the sympathetic nerve ending to take up and accumulate NE, nor to an increased rate of degradation.

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