Cholesterol Potentiates the Coronary Artery Response to Norepinephrine in Anesthetized and Conscious Dogs

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SUMMARY We investigated the effect of hypercholesterolemia on coronary and cardiac hemodynamic responses to intracoronary norepinephrine (NE) (0.01 to 10.0 μg/min as the bitartrate) in a Gregg cannula autoperfusion system. Coronary blood flow was measured by the radioactive microsphere technique in two groups of open-chest dogs anesthetized with pentobarbital: 10 controls and 8 that were fed a cholesterol-rich diet (CD) which doubled the serum cholesterol level. In the control dogs, NE in doses of 0.01 to 1.0 μg/min had no effect on coronary vascular resistance (CVR) but 10 μg/min caused a significant decrease to 0.58 ± 0.12 of control. In the CD dogs, NE at doses of 1.0 and 10.0 μg/min significantly reduced CVR, to 0.72 ± 0.06 and 0.52 ± 0.11 of control, respectively. There was no consistent effect of NE, at these doses, on myocardial oxygen uptake, left ventricular stroke work index, or maximal positive dP/dt. In a second series of experiments we measured coronary flow with electromagnetic flowmeters in 11 chronically instrumented conscious dogs, 5 controls, and 6 CD. In the control dogs, intravenously administered NE hydrochloride, 0.01 μg/min, reduced CVR to 0.74 ± 0.07 of control, and 1.0 μg/min increased CVR to 1.26 ± 0.09 of control. In the CD animals, these effects were seen at a 10-fold lower NE dose, 0.001 μg/min (0.83 ± 0.11 of control) and 0.1 μg/min (1.32 ± 0.06 of control). The vasodilation was blocked by propranolol, and vasoconstriction by phentolamine. We conclude that NE at low doses activates β-adrenoreceptors to reduce CVR and at higher doses activates α-adrenoreceptors to increase CVR; the vasoconstrictor response is inhibited in pentobarbital anesthetized dogs, and hypercholesterolemia sensitizes coronary vessels to both the dilator and constrictor effects of NE. Circ Res 48: 320-329, 1981

It recently has been shown (Rosendorff and Cranston, 1971; Rosendorff, 1972; Bomzon and Rosendorff, 1975; Bomzon et al., 1975) that in several vascular beds, including the cerebral and renal, norepinephrine (NE) has a dose-dependent effect; small doses of NE dilate and larger doses constrict resistance vessels. The vasodilator effect of the smaller dose is mediated by low-threshold β-adrenoceptors, and the vasoconstrictor effect of the larger doses is the result of both β- and α-receptor activation, with the α-receptor mediated vasoconstriction predominating.

There also is good evidence (Bloom et al. 1975a, 1975b, 1976; Bomzon et al., 1977, 1978) that some steroids, such as cholesterol and β-estradiol, sensitize resistance vessels to NE, both in vitro and in vivo, in the brain and kidney. The effect of sensitization is to lower the dose of NE at which the β-receptor mediated dilation is converted to an α-receptor mediated constriction. We thought that it might be important to assess the effect of hypercholesterolemia on the coronary vascular response to NE; if hypercholesterolemia sensitizes coronary resistance vessels to the constrictor effects of NE, then an important mechanism of coronary spasm may be unmasked.

Two series of experiments were performed, one on anesthetized and one on conscious dogs. In the anesthetized dogs, which were open-chest dogs with the left coronary artery autoperfused via a modified Gregg cannula system, we were able to control the coronary perfusion pressure independently of the aortic pressure, and were able to infuse the NE directly into the coronary artery. The conscious dogs, on the other hand, allowed us to study the coronary and myocardial effects of NE in the absence of pentobarbital anesthesia, which is known to distort coronary hemodynamic patterns (Vatner et al., 1974).

In each group of experiments, in the anesthetized and conscious dog series, the NE dose range chosen was subthreshold for measurable systemic cardiovascular effects. We measured, in each group, the...
effects of these doses on coronary blood flow and vascular resistance as well as on measures of myocardial function, in dogs which had been on a normal diet, and in dogs made hypercholesterolemic by dietary means. The high cholesterol diet was continued for a time sufficient to induce significant hypercholesterolemia, but not long enough to produce any macroscopic or histological change in the coronary arteries. This was done because the purpose of the study was to observe cholesterol-norepinephrine interactions, and not to study the effects of altered coronary artery morphology on coronary NE responses.

If the coronary vascular system behaves in the same way as the cerebral and renal systems, we predicted that we would find a low dose of NE which is vasodilator, an intermediate dose with no effect, and a higher dose which is vasoconstrictor. In the hypercholesterolemic animals this dose-response relationship would be shifted to the left; that is, the dose at which the dilation is converted to a constriction would be lower than in the control dogs fed the normal diet.

Methods

Anesthetized Dogs

Eighteen dogs weighing 26-35 kg were anesthetized with sodium pentobarbital (25-30 mg/kg) and given supplemental doses as needed. An endotracheal tube was inserted and the dogs were ventilated with room air by a Harvard respirator; if needed, oxygen was added to maintain a normal arterial oxygen tension. Catheters were placed in a femoral vein for infusing saline and in the right femoral artery for blood sampling.

The pericardium was exposed through a left thoracotomy in the 4th intercostal space and then opened widely. Pressure lines connected to Statham P26Db pressure transducers were placed in the descending aorta through the left femoral artery, the left atrium through its appendage, and the coronary sinus as described by Carlson and Utley (1973). A stiff no. 8 polypropylene pigtail catheter was placed in the left ventricle via the left atrial appendage. The first derivative of left ventricular pressure was measured by active electronic differentiation of the pressure signal and the system was calibrated with triangular wave inputs of known slope. An electromagnetic flow transducer was placed on the circumflex coronary artery near its origin and calibrated by triangular wave inputs of known slope.

After dissecting the fibroareolar tissue covering the origin of the left coronary artery (LCA), we placed a no. 0 silk ligature underneath the origin of the LCA at its junction with the aorta and proximal to all coronary arterial branches. The left common carotid artery was cannulated with a Bardic tube and its flow was diverted through an occlusive roller pump (Sarns Co.) to a modified Gregg cannula (4.25 mm i.d.) via an interposed 1-liter glass bottle in a water bath at 37°C (Fig. 1); the connecting tubing had an internal diameter of 4.5 mm. The tubing in this perfusion system was filled with saline and then the cannula was advanced through an arteriotomy in the left subclavian artery into the root of the aorta. Aortic pressure was recorded through a small tube inside the cannula; the tips of the small inner tube and larger outer cannula were at the same level. Similarity of aortic pressures recorded through this tube and the aortic catheter was verified. The pump was turned on until flows of 100-150 ml/min were reached and then the cannula was advanced into the LCA where it was tied in place. The glass bottle was pressurized with compressed air, its pressure was regulated by a valve to the level of the mean aortic pressure, and then the system was changed from a flow- to a pressure-regulated system. Pressure in the bottle was adjusted until the diastolic pressure in the LCA matched aortic diastolic pressure and the pressurized bottle was used to perfuse the LCA while the pump flow was adjusted to maintain a constant level of arterial blood in the pressure bottle. The coronary perfusion pressure was kept constant throughout the experiment. Adequacy of cannulation was confirmed by finding that, upon occlusion of the perfusion line for 10 seconds, the LCA pressure decreased to 10-15 mm Hg and that, upon reperfusion at constant pressure, there was a hyperemic response.

A catheter attached to the Gregg cannula system
permitted the intracoronary infusion of NE (levar-terenol bitartrate). In each experiment there were four 20-minute infusion periods, consisting of normal saline and three out of the four NE doses used in the study, namely, 0.01, 0.1, 1.0, and 10.0 μg/min.

Doses refer to total mass of l-norepinephrine bitartrate; the amount of norepinephrine base is half the specified dose. In each experiment, the order was saline, then NE from the lowest to the highest dose, to minimize tachyphylaxis.

Transmural blood flow distribution was studied with radioactive microspheres injected 10 minutes after the start of each infusion dose. Four sets of microspheres 9 ± 1 μm in diameter (mean ± SD) and labeled with 125I, 141Ce, 85Sr, or 46Sc (3M Company) were prepared as previously described by Heymann et al. (1977). For each myocardial blood flow measurement we injected 3 × 10⁵ to 1 × 10⁶ microspheres into the LCA perfusion line by flushing them in with 12 ml of arterial blood taken from the femoral artery. The microsphere injections produced no changes in heart rate, systemic or coronary arterial pressures, or coronary blood flow; coronary arterial pressure was stable because the pressure bottle acted as a buffer. Starting immediately before the microsphere injection, blood was withdrawn from a side arm on the cannula at a rate of 10-15 ml/min by a Holter pump to fill successively four collecting vials for 30 seconds each. The microsphere injecting and withdrawal sites were situated on the tubing connecting the pressure bottle to the cannula, were 40 cm apart, and were separated by two mixing chambers. At the same time we injected microspheres, with a different isotope, into the right atrium, and collected reference samples from the pulmonary artery for cardiac output measurement.

Before injecting the microspheres we measured arterial blood gases and pH, and corrected them to normal if necessary. After the microsphere injection, a blood sample was withdrawn from the cannula for the measurement of arterial oxygen and carbon dioxide tensions, pH, and hematocrit; hemoglobin and percentage oxygen saturation were measured with a hemoxymeter (Radiometer OSM 2) and the oxygen content was calculated assuming an oxygen capacity of 1.36 ml/g of hemoglobin. Blood samples from the coronary sinus and the cannula were collected simultaneously for oxygen content measurements.

At the end of each experiment the heart was arrested by injecting a concentrated solution of potassium chloride, removed, weighed, and fixed in 10% formalin for 1 week. Fat, epicardial vessels, and heart valves were discarded; then the atria and right ventricular free wall were cut off and discarded. The left ventricle was divided into four layers of about equal thickness from endocardial to epicardial surfaces or from the left to right septal surfaces. The blood samples and muscle were placed in preweighed vials, weighed, and then counted in a well scintillation counter with a NaI (TI) crystal (Searle Analytic, Inc.) connected to a 512 channel pulse height analyzer. The total activity of each nuclide was calculated by the stripping method of Heymann et al. (1977). From these counts the flow in the cannula was computed as the quotient of flow and activity (in counts/min) in the reference sample taken from the cannula, multiplied by the total amount of radioactivity injected into the heart (total counts/min in the vial before injection minus residual counts in the vial). The total and regional flows in the heart were calculated as flow in the reference sample from the cannula, multiplied by the quotient of counts/min in the heart or region and the counts/min in the reference sample (Domenech et al., 1969). Cardiac output was calculated as the total counts injected multiplied by the quotient of flow and activity (cpm) in the reference sample taken from the pulmonary artery.

The following indices of myocardial performance were calculated:

1. RPP = LVP_syst · HR, where RPP = the rate pressure product (mm Hg/min), LVP_syst = left ventricular systolic pressure (mm Hg) and HR = heart rate/min.

2. MVO_o = \( \dot{Q}_{LV} \) (A_o - CSo), where MVO_o = the myocardial oxygen consumption (ml/100 g per min), \( \dot{Q}_{LV} \) = total left ventricular blood flow (liters/100 g per min), A_o = oxygen content of coronary artery blood (ml/liter), and CSo = oxygen content of coronary sinus blood (ml/liter).

3. \( \text{SPTI} = \text{HR} (\text{P}_{LV_{t_0}} - \text{P}_{LA_{t_0}}) \) where SPTI = the systolic pressure-time index (mm Hg sec/min), \( \text{HR} \) = heart rate per minute, \( \text{P}_{LV} \) = the mean left ventricular pressure, measured by electrical damping (mm Hg), \( \text{t}_0 \) = duration of the cardiac cycle (sec), \( \text{P}_{LA} \) = mean left atrial pressure (mm Hg) and \( \text{t}_0 \) = duration of diastole (sec).

4. LVSW = \( \text{SI} \cdot \text{P}_{LV_{syst}} \cdot 0.0136 \), where LVSW = left ventricular stroke work (gram-meters/kg), SI = stroke index (ml/kg), \( \text{P}_{LV_{syst}} \) = mean left ventricular systolic pressure (mm Hg), and 0.0136 = a conversion factor from mm Hg × ml to gram-meters.

5. \( \text{dP/dt}_{\text{max}} \) = the maximum positive value of the first differential of left ventricular pressure by time (mm Hg/sec).

In addition, we calculated coronary vascular resistance as diastolic coronary arterial minus mean left atrial pressures (mm Hg) divided by total left ventricular flow (ml/100 g per min).

Ten of the dogs were fed a normal diet, including one can of Calcan dog food per day, while the remaining eight dogs were fed the normal diet to which was added each day 100 ml of coconut oil (PVO International) and 20 g of cholesterol (ICN Pharmaceuticals). Non-hydrogenated coconut oil consists of 90% saturated fats, 8% mono- and 2%
polyunsaturated fats (Hornstra, 1975). The high cholesterol diet was continued for 27–36 days before the experiments were performed; this period was found to be sufficient for a significant increase in plasma cholesterol concentration but was not long enough for any morphological changes to develop. Serum cholesterol and triglyceride concentrations were estimated routinely by automated methods (Rush et al., 1970). Lipids in ultracentrifugally separated lipoprotein fractions were measured by standard techniques (Myers et al., 1976).

Conscious Dogs

In a second series of experiments, the effect of cholesterol feeding on the coronary vascular response to NE was measured in 11 conscious dogs, 5 controls, and 6 on the same high cholesterol diet as described above, for 26–32 days. Dogs weighing 23–28 kg were anesthetized with sodium pentobarbital, 25–30 mg/kg, and given supplemental doses as needed. They were ventilated by a Harvard respirator via an endotracheal tube, and the heart was exposed through a left thoracotomy. A 2.5- or 3-mm electromagnetic flow transducer (Biotronex) was placed around the circumflex branch of the left coronary artery and secured by the technique described by Alexander et al. (1969). A 3-mm internal diameter hydraulic snare was placed just distal to the flow transducer, for zero-flow calibration of the baseline. The transducer was calibrated in an in vitro dog blood perfusion system before implantation. A saline-filled catheter was inserted in the descending thoracic aorta through its wall and sutured in place. Lead wires and catheters were brought out through the dorsal chest wall between the scapulae, and taped to the skin. The pericardium was reapproximated with no. 00 silk, and the chest wall closed in the conventional way. The animals were given procaine penicillin, 2 million U, and streptomycin, 0.5 g, intramuscularly daily for 3–4 days postoperatively. Two to three weeks later the cholesterol diet was commenced in the six test animals; all experiments were performed approximately 6–7 weeks after operation.

During the experiments the dogs were unanesthetized and unsedated, restrained only by a canvas cradle to which they had become accustomed during twice-weekly training sessions between the operation and the experiment. A 21 (0.8 mm) Butterfly (luer) needle was inserted into a superficial vein in the foreleg. The experiments consisted of the intravenous infusion, over a period of 20 minutes each as the hydrochloride of saline, then NE (as the hydrochloride), 0.0001, 0.001, 0.01, 0.1, and 1.0 μg/min, in that order to minimize tachyphylaxis. During the last 5 minutes of each period, five recordings, a minute apart, were taken of phasic and mean coronary blood flow and phasic and mean aortic pressure. a- and β-adrenergic blockade was achieved by the technique described by Sommer et al. (1970). Serum cholesterol and triglyceride concentrations for the dogs were measured at the commencement of the propranolol infusion, a 20-minute saline infusion was followed by a 20-minute infusion of NE, 0.01 μg/min. The experiment then was repeated with combined β- and α-blockade, with propranolol, 0.5 mg/min, and phentolamine, 1.0 mg/min, respectively. The experiment was repeated on another day with NE, 1.0 μg/min, with α-blockade, and with combined α- and β-blockade, with the same doses of phentolamine and propranolol. These experiments were done on the conscious control (five experiments) and cholesterol-fed (six experiments) dogs.

Results

Effects of Cholesterol on NE Responses in Anesthetized Dogs

In the dogs fed the high cholesterol, high saturated fat diet, the serum total cholesterol increased from a mean of 160 ± 8 (SEM) mg/dl before the diet was commenced, to a mean of 257 ± 18 mg/dl at the time of the experiment. The serum triglyceride concentration increased from 42 ± 6 mg/dl to 214 ± 269 (1,376 ± 200) mm Hg/sec. There were no evidence that aortic dP/dt is exactly equivalent to left ventricular dP/dt, but this has been used as a rough index of left ventricular function (Rushmer, 1964; Prokop et al., 1970).

In a further series of experiments on these chronically instrumented conscious animals, we measured the effects of β- and α-adrenergic receptor blockade on the coronary vascular resistance responses to NE. Beta-blockade was produced by the intravenous infusion of propranolol, 0.5 mg/min, throughout the experiment. Thirty minutes after the commencement of the propranolol infusion, a 20-minute saline infusion was followed by a 20-minute infusion of NE, 0.01 μg/min. The experiment then was repeated with combined β- and α-blockade, with propranolol, 0.5 mg/min, and phentolamine, 1.0 mg/min, respectively. The experiment was repeated on another day with NE, 1.0 μg/min, with α-blockade, and with combined α- and β-blockade, with the same doses of phentolamine and propranolol. These experiments were done on the conscious control (five experiments) and cholesterol-fed (six experiments) dogs.
FIGURE 2 Dose response relations for NE in the control (left) and cholesterol-fed (right) anesthetized dogs. CVRNE = coronary vascular resistance during the NE infusion, calculated by dividing coronary perfusion pressure (diastolic coronary artery minus mean right atrial pressures) by total left ventricular flow. CVRSAL = coronary vascular resistance during saline infusion (control). CVRNE/CVRSAL is the ratio of the two resistances: a value greater than 1 represents an increase in CVR during NE infusion (vasoconstriction), and a value less than 1 is a decrease in CVR during NE infusion (vasodilation). T = total LV resistance, I = inner (subendocardial) LV resistance and O = outer (subepicardial) LV resistance. Bars represent one standard error on either side of the mean. Note that there is significant vasodilation at the 10.0 μg/min dose in the control dogs but, in the cholesterol-fed dogs, significant coronary vasodilation is seen at both the 1.0 and the 10.0 μg/min doses, i.e., there has been a ten-fold potentiation of the vasodilator effect of NE in the cholesterol-fed dogs. No vasoconstrictor effects were seen at these doses. Coincident with this observation is the significant vasodilation at the 10.0 μg/min dose in the control dogs but, in the cholesterol-fed dogs, significant coronary vasodilation is seen at both the 1.0 and the 10.0 μg/min doses, i.e., there has been a ten-fold potentiation of the vasodilator effect of NE in the cholesterol-fed dogs. No vasoconstrictor effects were seen at these doses. ♦ = P < 0.05; ○ = P < 0.01; ▼ = P < 0.005.

Figure 3 Effect of NE, in control and cholesterol-fed anesthetized dogs, on aspects of myocardial function. The values shown are the mean (and standard error of the mean) differences between the NE and saline infusion values. For example, Δ RPP is the mean difference between the rate-pressure product during a NE infusion (RPPNE) and that during the saline infusion (RPPs). Other symbols are defined in the text. N = number of experiments. The only value which significantly differed from O was the mean dP/dtmax during the 10.0 μg/min NE dose in the cholesterol-fed dogs.
tillity that would be expected to cause autoregulatory decreases of coronary blood flow at a constant perfusion. At the highest dose infused, \( \text{MVO}_2 \) increased about 20-25% in each group; this change is not enough to explain a 40-50% fall in coronary vascular resistance.

**Effect of Cholesterol on NE Responses in Conscious Dogs**

A second series of experiments was carried out on conscious dogs. In the CD dogs the total serum cholesterol increased from 131 ± 10 (SEM) mg/dl to 206 ± 8 mg/dl by the time of the experiment. The serum triglyceride concentration increased from 41 ± 3 mg/dl to 158 ± 7 mg/dl during this period. Values of serum total cholesterol and triglyceride concentrations for the control dogs at the times of the experiments were 145 ± 14 and 48 ± 7 mg/dl, respectively.

In Figure 4, the results are expressed as a ratio of the coronary vascular resistance during NE infusion to that during the saline infusion at the five different doses of NE for both the control and the cholesterol-fed dogs. A different pattern from that seen in the anesthetized dogs was observed.

In control dogs, there was a significant decrease in the coronary resistance with the 0.01 \( \mu \text{g/min} \) dose of NE. The highest dose, 1 \( \mu \text{g/min} \), was constrictor, the increase in resistance being significantly elevated over the saline control. The intermediate dose, 0.1 \( \mu \text{g/min} \), had no effect on coronary flow.

In the cholesterol fed dogs, however, the vasodilator dose (0.001 \( \mu \text{g/min} \)) was 10-fold lower than in the control dogs, and significant (\( P < 0.01 \)) vasoconstriction was produced by the 0.1 and 1.0 \( \mu \text{g/min} \) doses. This represents a one order of magnitude shift of the dose response curve to the left, i.e., a 10-fold increase in the sensitivity of coronary resistance vessels with respect to the vasoconstrictor response to NE. The data suggest that a similar shift occurs for the lower dose vasodilator effects of NE, but in only the control was the decrease in coronary vascular resistance statistically significant. There was no change in mean arterial blood pressure in either group of dogs at all five doses, and, as in the anesthetized dogs, there was no significant change in the rate pressure product (calculated here as the product of the aortic systolic blood pressure and the heart rate) or in the maximal positive aortic dP/dt. Therefore the coronary vascular effects of the NE, at the doses used, are unlikely to be secondary to any autoregulatory response to altered systemic arterial blood pressure or myocardial work.

In the series of experiments on the conscious dogs with adrenergic blocking agents (Fig. 5), the vasodilator effect of the NE (0.01 and 0.001 \( \mu \text{g/min} \) in the control and cholesterol-fed animals, respectively) was attenuated by \( \beta \)-blockade with propranolol, 0.5 mg/min. The further addition of the \( \alpha \)-blocker, phentolamine, had no significant effect. On the other hand, in both the control and cholesterol-fed dogs, the vasoconstrictor effect of the larger NE dose (1.0 \( \mu \text{g/min} \)) was abolished, even reversed, by phentolamine. These results are consistent with the interpretation that \( \alpha \)-blockade has abolished the \( \alpha \)-receptor mediated vasoconstriction of the higher NE dose, and unmasked the \( \beta \)-receptor mediated dilator response. This is confirmed by the reduction of the vasodilation by the addition of propranolol.

**Discussion**

The coronary vessels of the dog have a rich monaminergic innervation, as demonstrated by the histochemical fluorescence method (Schenk and El-Badawi, 1968; Dolezel et al., 1978). The distribution of monoaminergic terminals in the arterial wall depends upon the diameter of the artery. In large arteries, the terminals are regularly distributed around the entire circumference, the fibers being situated between the elastic lamellae of the adven-
CHEST DOGS, AUTOPOURED VIA A MODIFIED GREGG CANNULA SYSTEM, INFUSIONS OF NOREPINEPHRINE AT Doses Too Low To Have Any Measurable Myocardial Effect Cause A Dose-Related Coronary Vasodilation In Both The Inner And Outer Layers Of The Myocardium. This Finding Is In Accordance With Those Studies That Have Shown Dilation Of Coronary Vessels In Response To Either Sympathetic Nerve Stimulation Or Catecholamine Injection Or Infusion, Independent Of An Increase In Myocardial Metabolism (Klocke et al., 1965; Mark et al., 1972; McRaven et al., 1971). The Coronary Vascular Receptors For This Effect Are Of The $\beta$-2 Type, Since Selective $\beta$-1 Blockade Does Not Affect The Coronary Dilator Response To Isoproterenol (Adam et al., 1970; Gross and Feigl, 1975; McRaven et al., 1971). Our Finding That Propranolol Blocks NE Dilation Is Consistent With These Reports.

We Have Found Also That The Effect Of Norepinephrine On The Coronary Vascular Bed In Conscious Animals Is Dose-Dependent; A Smaller Dose Causes Vasodilation And Larger Doses Cause Vasoconstriction. Both Effects Are Independent Of Any Change In Our Measured Indices Of Myocardial Metabolism, And Are Not Due To Autoregulatory Responses To Changes In Systemic Arterial Blood Pressure. Vatner et al. (1974) Also Have Shown A Sustained Increase In Mean Coronary Vascular Resistance Induced By Norepinephrine In Conscious But Not Anesthetized Animals; The Change Was Abolished By $\alpha$-Receptor Blockade. This Constriction Therefore Undoubtedly Is Produced By A Direct Effect On $\alpha$-Receptors In The Coronary Vascular Bed.

Other Studies Have Demonstrated The Capacity For The Development Of Neurally Mediated Coronary Vasoconstriction. Examples Include The Increase In Coronary Vascular Resistance Following Severe Baroreceptor Hypotension, When The Reflex Tachycardia And Augmentation Of Myocardial Activity Are Blocked With Propranolol (Szentivanyi and Juhasz-Nagy, 1963). This Increase In Coronary Vascular Resistance Can Be Prevented By Interruption Of The Cardiac Efferent Adrenergic Nerves (Brachfeld et al., 1960; Hackett et al., 1972).

It Is Likely That Neurally Mediated Effects On Coronary Blood Flow May Be Present Even During Profound Metabolically Induced Coronary Vasodilation. For Example, During Reactive Hyperemia, When The Coronary Vasodilation Occurs As A Result Of Powerful Metabolic Stimulation, Stellate Ganglionectomy And The Administration Of Phentolamine Cause A Substantial Reduction In The Coronary Vascular Resistance. The Reduction May Be Due To Some $\alpha$-Receptor "Tone" Even In The Presence Of A Powerful Vasodilating Influence (Schwartz and Stone, 1977). It Is Likely Therefore That Sympathetic Vasoconstriction Competes With Metabolic Vasodilation In The Coronary Circulation (Mohrman and Feigl, 1978). Also, Since We Have Shown In This Study That Dilation Or Constriction May Be Produced By Norepinephrine, Depending Upon The Dose, It Is Possible That The Degree Of Metabolic Dilation Produced Under Phys-
ior physiological conditions may be influenced considerably by the intensity of sympathetic efferent stimulation, or by alterations in the local modulation of adrenergic transmitter release or of the norepinephrine-adrenoreceptor interaction.

The pattern of vascular reactivity to NE in the coronary circulation, as shown in this study, is identical to that previously described for the brain (Rosendorff, 1972). In both the cerebral and coronary circulations, NE has dose-dependent effects on blood flow; small doses dilate resistance vessels, larger doses are vasoconstrictor. The vasodilator effects of the smaller doses are attenuated by β-adrenoreceptor blockers; the vasoconstrictor effect of the larger dose is reversed by α-blockers. This suggests that the β-receptors have a lower activation threshold, but that with larger doses of NE, both β- and α-receptors are stimulated, the net result being an α-receptor mediated vasoconstriction. The only qualitative difference between the anesthetized and the conscious animals is the absence of a vasoconstrictor component in the anesthetized dogs. This type of analysis predicts that higher doses of norepinephrine in anesthetized animals would have a vasoconstrictor effect, but unfortunately higher doses also produced a very significant myocardial stimulation with a further metabolic vasodilation. However, even after β-1 blockade of the myocardial effects of the higher doses of norepinephrine, Rosendorff and Lewis (1977) were unable to show vasoconstrictor effects at those doses. The absence of a vasoconstrictor effect of NE in pentobarbital-anesthetized animals, in this and other studies (Rosendorff and Lewis, 1977; Vatner et al., 1974), therefore probably is due to the direct effects of the anesthetic agent on coronary vessels. Pentobarbital and many other general anesthetic agents have a variety of effects on vascular smooth muscle including a direct dilator action (Hershey et al., 1953), inhibition of the development of spontaneous mechanical activity, dose-dependent attenuation of vasopressor-induced contractions, non-competitive displacement of the dose-response curves of vasoactive substances, and the attenuation of the calcium-induced contractions of potassium-depolarized vascular smooth muscle strips (Altura and Altura, 1975a, 1975b). For all of these reasons, it seems that pentobarbital-anesthetized animals are capable of producing only the vasodilator responses to norepinephrine, but conscious animals show the full pattern of low-dose β-adrenoreceptor mediated vasodilation, and a high-dose α-adrenoreceptor mediated constriction.

It is of interest to note that the vasodilator doses were much lower in the conscious than in the anesthetized dogs. We infused NE into the coronary arteries in the anesthetized dogs and intravenously in the conscious dogs; concentrations of NE reaching the coronary vessels therefore must have been quite different. For intracoronary infusion, 1 μg/min would have been diluted in about 100 ml/min of coronary flow, thus giving a concentration of 10 μg/liter of total agent or 5 μg/liter of norepinephrine base. For the intravenous infusion, 1 μg/min of NE was diluted in the cardiac output of, say, 2000 ml/min to give a concentration reaching the coronary arteries of about 0.5 μg/liter. The body load of NE was the same for each, but the concentration of NE reaching the coronary arteries was quite different; we assume that, in this study, the effects depend upon the concentration of NE at coronary vascular adrenergic receptors, so that the sensitivity to NE was enhanced even more in conscious dogs than the actual data show.

Perhaps the most interesting finding in this study is the leftward shift of all the NE dose-response curves in the hypercholesterolemic dogs; cholesterol sensitizes coronary vessels to NE. This also has been shown in the kidney, both in vivo and in vitro, in the brain and in skeletal muscle. Cholesterol-rich plasma, from patients with obstructive jaundice (Bloom et al., 1975a) or Type IIa hyperlipoproteinemia or prepared by adding β-lipoprotein concentrate to normal plasma (Bloom et al., 1975b), causes a leftward shift of the dose-response curve of the effect of NE on the vascular resistance of an isolated, perfused rabbit kidney preparation. Similar effects are seen in the baboon kidney in vivo (Bloom et al., 1976; Bonszon et al., 1978), and here the NE potentiation is due to sensitization of α-adrenoreceptors (Bonszon et al., 1977). The present study has shown cholesterol sensitization of both β-adrenoreceptors (in anesthetized and conscious dogs) and α-adrenoreceptors (conscious dogs only) in coronary vessels.

Recently, Yokoyama and Henry (1979) have shown that cholesterol enhances the constrictor effects of calcium chloride on isolated coronary arteries, probably by a nonadrenergic mechanism. Therefore cholesterol sensitization of coronary artery responses is not specific for NE. It is probably not specific for cholesterol either. For example, a depot estrogen and progesterone preparation sensitizes cerebral resistance vessels to the vasoconstrictor effects of 5-hydroxytryptamine (Mendelow et al., 1977); β-estradiol does the same thing, but dexamethazone does not. Therein lies the clue to a possible mechanism of action of cholesterol in potentiating NE effects, because β-estradiol is a very potent inhibitor of the tissue (non-neuronal) uptake (“uptake-2”) of NE released from sympathetic nerve terminals, whereas dexamethazone is not (Salt, 1972). Inhibition of this uptake mechanism could result in an increase in the concentration of free NE available to interact with adrenoreceptors, and this increase will shift the dose-response curve to the left. This idea is supported by the observation that the specific uptake-2 blocker, a haloalkylamine coded GD 131, has very similar effects to cholesterol and β-estradiol on vascular sensitivity to NE (McCalden et al., 1977). In addition to uptake-2 inhibition, there are other possible mechanisms of
steroid-sensitization of vascular smooth muscle to NE. The interaction between NE and α- and β-receptors can be affected by a modulation in the rate of release of the neurotransmitter from nerve terminals, the rate of its uptake back into nerve terminals (uptake-1) or into vascular smooth muscle (uptake-2), the efficiency of other inactivation mechanisms including the enzymes monoamine oxidase and catechol-α-methyl transferase, the drug receptor affinity, the number or at least the relative ratio of α- and β-receptors, and the transduction process whereby adrenergoreceptor activation is translated into a measurable physiological or pharmacological effect. We do not yet have the answer to which of these mechanisms may be acting in cholesterol-induced coronary sensitization to norepinephrine. However, recent work from the laboratory of one of us (C.R.) suggests that there may be a change, in cholesterol-fed animals, in the binding characteristics of the α-adrenoreceptors in vascular smooth muscle cells, and that this is a cholesterol-induced increase in the number of membrane-bound α-binding sites with no change in α-ligand receptor affinity.

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AGE AND MYOCARDIAL Na⁺-K⁺-ATPase Activity and Digitalis Intoxication in the Dog and Guinea Pig

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SUMMARY Infants and young animals tolerate higher doses of digitalis glycosides, relative to body weight, than adults. One possible explanation for this could be an age-dependent difference in the myocardial digitalis receptor, the Na⁺-K⁺-ATPase. Two functions of this enzyme were studied in adult, 1- and 6-week-old dogs and guinea pigs: in vitro myocardial uptake of rubidium (⁶⁷⁸Rb) and binding of ouabain. In guinea pigs, rubidium uptake (pmol Rb/mg LV per 15 min) was: 1 week old: 100.9 ± 7.1 (mean ± SE); 6 week: 79.8 ± 6.7; adult: 55.2 ± 7.9; (1 week: 6 week: P < 0.025; 1 week: adult, P < 0.001; 6 week: adult, P < 0.025). Similarly in dogs, rubidium uptake was significantly greater at 1 week than at 6 weeks (208 ± 13 vs. 144 ± 9; P < 0.001) and the latter greater than in adults (111 ± 4) (P < 0.005). Other groups of anesthetized adult and 6-week-old dogs were given digoxin, 0.3 mg/kg, iv. The young dogs took significantly longer to become cardiotoxic (17.3 ± 3.4 min vs. 9.3 ± 1.4 min; P < 0.001). The myocardial uptake and the latter greater than in adults (111 ± 4) (P < 0.005). Other groups of anesthetized adult and 6-week-old dogs were given digoxin, 0.3 mg/kg, iv. The young dogs took significantly longer to become cardiotoxic (17.3 ± 3.4 min vs. 9.3 ± 1.4 min; P < 0.001). Similarly, in dogs, rubidium uptake was significantly greater at 1 week than at 6 weeks (208 ± 13 vs. 144 ± 9; P < 0.001) and the latter greater than in adults (111 ± 4) (P < 0.005). Other groups of anesthetized adult and 6-week-old dogs were given digoxin, 0.3 mg/kg, iv. The young dogs took significantly longer to become cardiotoxic (17.3 ± 3.4 min vs. 9.3 ± 1.4 min; P < 0.001), while their myocardial digoxin uptake was at least as great. Rubidium uptake showed an average decrease of 58% after digoxin but residual uptakes were not different in the two groups. Data for ouabain binding showed similar differences between the various groups of dogs studied. Increased myocardial Na⁺-K⁺-ATPase activity, reflected in greater active cation transport and specific enzyme binding, has been demonstrated in young animals and may be partly responsible for their greater tolerance to digitalis glycosides. Circ Res 48: 329-333, 1981

INFANTS tolerate higher doses of digitalis relative to body weight or surface area than adults (Sapin et al., 1956; Neill, 1965; Wettrell and Andersson, 1977), and higher serum levels of digoxin can be maintained in infants and children without the development of cardiac arrhythmias (Rogers et al., 1972; Hayes et al., 1973). Young animals also are relatively tolerant of digitalis, and there is some evidence that the myocardial effects of the cardiac glycosides are age dependent (Wollenberger et al., 1953; Boerth, 1975; Rosen et al., 1975; Berman et al., 1977). The explanation for this remains uncertain, but one possibility would be an age-dependent difference in membrane Na⁺-K⁺-ATPase, which is considered to be the digitalis receptor (Matsui and Schwartz, 1968; Schwartz et al., 1971; Dahl and Hoken, 1974; Wallick et al., 1979).

In this study we examined myocardial Na⁺-K⁺-ATPase activity in dogs and guinea pigs of various ages, measuring the function of the enzyme in vitro in terms of active rubidium uptake (Hougen and Smith, 1978; Hougen et al., 1979) and specific ouabain binding (Schwartz et al., 1971). In other dogs, myocardial enzyme activity and digoxin content were measured after administration of a toxic dose of digoxin.

Methods

To determine the influence of age on myocardial Na⁺-K⁺-ATPase activity (protocol A) and susceptibility to digitalis cardiotoxicity (protocol B), myocardial-specific ouabain binding and active rubidium uptake were measured in the following groups of animals.
Cholesterol potentiates the coronary artery response to norepinephrine in anesthetized and conscious dogs.
C Rosendorff, J I Hoffman, E D Verrier, J Rouleau and L E Boerboom

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