Myocardial Extraction and Production of Catechol Amines

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The classic experiments of Loewi on the heart of the frog were the first to demonstrate that a sympathetic transmitter is released from cardiac tissue on cardiac sympathetic nerve stimulation. By observing the effect on the nictitating membrane of the cat, Cannon and Rosenblueth demonstrated that an adrenergic material is also released from mammalian myocardium during cardiac sympathetic nerve stimulation, and it was subsequently shown that this effect is blocked by dibenamine. Cannon and Lissak showed that cat ventricle contained a sympathomimetic which they believed to be epinephrine. Von Euler and, later, Goodall identified and quantified norepinephrine in bovine myocardium. Raab and Humphreys and Goodall and Kirshner, the latter using von Euler's method, showed that chronic cardiac sympathectomy decreased the content of norepinephrine in canine heart muscle. Outschoorn and Vogt, using a bioassay technique, demonstrated that cardiac sympathetic nerve stimulation in the dog is accompanied by an increase in the quantity of norepinephrine in coronary sinus blood.

The availability of an accurate and specific chemical technique for the isolation and identification of catechol amines in blood and the dichloro analogue of isoproterenol, which blocks cardiac adrenergic responses, has made it possible to examine some of the factors influencing the arteriovenous difference of catechol amines across the functioning myocardium and to attempt to relate the hemodynamic consequences of direct cardiac sympathetic nerve stimulation to the myocardial production and extraction of catechol amines.

Methods

A vagotomized canine preparation under pentobarbital anesthesia (30 mg./Kg.) was employed in which the total vena caval return was diverted into a reservoir and then pumped at a predetermined constant flow into the main pulmonary artery, via the branch to the right upper lobe, so as to bypass the right heart (fig. 1). The main pulmonary artery was ligated immediately distal to the pulmonary valve, so that the right ventricle ejected the entire coronary venous outflow via a cannula into a recording rotameter circuit, from which it was returned to the venous reservoir. In some preparations, a shunt was established from the femoral arteries to the vena caval reservoir to make possible the regulation of arterial pressure and, thus, coronary blood flow without a change in cardiac output. Except where indicated, the heart was paced by bipolar electrodes attached to the left atrium so as to maintain a constant stroke volume. Mean left atrial (LA) or left ventricular end-diastolic (LVED) and aortic (AP) pressures were continuously recorded. Left ventricular end-diastolic pressure was obtained through a rigid metal cannula inserted into the ventricle through the apical dimple. The cannula was connected directly to a pressure transducer. The gain of the recording system was adjusted so that full-scale deflection of the galvanometer was from 0 to 50 cm. H₂O and a paper speed of 100 mm. per second was used for high resolution tracings. Sanborn differential manometers and direct-writing recording equipment were used throughout. The tip of the cannula was used as the zero reference point for all intracardiac pressures.

Coronary venous blood samples were taken from the rotameter circuit at the point at which it emptied into the venous reservoir. Arterial samples were drawn from the femoral artery. All blood used in the circuit was obtained fresh from heparinized donors on the morning of the experiment.

Direct stimulation of the cardiac sympathetic efferent nerves was accomplished by bilateral stimulation of the stellate ganglia to which all rami had previously been sectioned. This will be referred to as a sympathectomized heart. Unipolar stimulation of the ganglia was accomplished by means of a model S₄ Grass impulse generator. Stimulation...
tion strengths of from 3 to 7 volts at frequencies ranging from 3 to 9 per second with a duration of 1.5 msec. were used.

The analyses of arterial and coronary venous plasma for catechol amines were carried out by the ethylenediamine condensation method of Weil-Malherbe and Bone (EDA)\textsuperscript{15,16} as well as by a modification of the trihydroxyindole (THI) technique of Lund\textsuperscript{10,17} developed by Croout.\textsuperscript{11} When comparison was made between the two methods, it was done with the same eluate divided into two parts. Control series of duplicate samples analyzed by the THI method showed a 94.6 per cent recovery with a standard deviation of ± 6.7 per cent for total catechol amines. Similar series analyzed by the EDA method showed a 67.2 per cent recovery with a standard deviation of ± 8.5 per cent for norepinephrine and a 90.2 per cent recovery with a standard deviation of ± 2.4 per cent for epinephrine.

In some experiments, dichloroisoproterenol\textsuperscript{*} was injected intravenously. In vitro experiments demonstrated that neither dichloroisoproterenol nor heparin, in the concentrations employed, interfered with the catechol amine analysis. 1-Norepinephrine (Levophed) was infused in selected experiments and concentrations expressed as µg. of the base.

**Results**

The results represent the data from 46 successful experiments. A representative example of the cardiodynamic response to efferent cardiac sympathetic nerve stimulation is shown in figure 2. The relatively high left atrial pressures seen in the absence of stellate stimulation in this preparation were not infrequently encountered in the acutely sympathectomized heart. During sympathetic stimulation at a constant stroke volume, pulse pressure widened with little change in mean aortic pressure, mean left atrial pressure declined, and coronary venous flow increased. With cessation of stimulation, there was narrowing of the pulse pressure, elevation of mean left atrial pressure, and return of coronary venous flow to control levels.

Data from the 21 early experiments, in which only the technique of Weil-Malherbe and Bone (EDA) was used, demonstrated an increase in EDA-condensing material in coronary venous blood during the stimulation of cardiac sympathetic nerves (fig. 2). However, in the poststimulatory period, there frequently appeared in coronary venous blood an increased concentration over the control levels of a substance with the same fluorescent peak as norepinephrine. This observation, plus the fact that the presence in the plasma of this poststimulatory "norepinephrine" was not associated with any of the hemodynamic changes characteristic of the response to catechol amines, prompted us to compare the results of analyses by both the EDA and THI techniques in the same experiment.

Two representative examples of the 15 such comparisons made are shown in figures 3A and 3B. In addition to the quantitative difference between the two methods (the EDA method showing higher values), there was also a marked qualitative disparity. In the sympathectomized animal shown in figure 3A, the EDA technique indicated a significant coronary venous level of catechol amines during control and poststimulatory periods; the THI method revealed a coronary venous level only during cardiac sympathetic nerve stimulation. During norepinephrine infusion (fig. 3B), although coronary venous catechol amine levels

\textsuperscript{*}1-(3, 4) (dichloroisoproterenol) 2-isopropylamine ethanol HCl, Lilly no. 20522, Lot no. 021-337-42, was kindly supplied by Dr. Irwin Slater of the Eli Lilly Research Laboratories.
Release of coronary venous catechol amines in response to cardiac efferent sympathetic nerve stimulation. Weil-Malherbe method alone. Stimulus, 5 volts at 5 per second. Aortic pressure (AP); mean left atrial pressure (LA); coronary venous flow (CF); coronary catechol amines (coronary CA); plasma catechol amines (plasma CA); input (I); output (O); arterial (A); coronary venous (CV); cardiac output (CO); heart rate (HR); trihydroxyindole (THI); Weil-Malherbe (W-M); were present in all samples by the EDA method, the THI technique revealed coronary venous catechol amines only in the stimulation and poststimulation periods. Furthermore, whereas the EDA method showed positive arterial levels prior to infusion (fig. 3B), the THI technique revealed that arterial catechol amines appeared only after the infusion had begun. The differences between values for total catechol amines measured by the two methods was at times large, especially in samples collected during and immediately after efferent cardiac sympathetic nerve stimulation. Not only did the values obtained for norepinephrine by the EDA method not bear a direct relationship to those obtained by the THI method, but neither did this determination relate to the observed hemodynamic events as did the latter analysis. In the 21 experiments in which the more specific THI method was used and the sympathetics were stimulated, it was determined that stimulation of the efferent cardiac sympathetic nerves was accompanied by an increased minute output of coronary venous catechol amines. This was uniformly accompanied by a positive inotropic effect.

The release of catechol amines into coronary venous blood appears to be a function of the frequency and voltage of the stimulation applied to the cardiac sympathetics and is, in general, proportional to the hemodynamic responses obtained. As shown in figure 4A, at a constant voltage, increasing the frequency of stimulation of the cardiac sympathetic efferents produced stepwise increments in the quantity of coronary venous catechol amines. This was accompanied by a graded increase in the inotropic response and coronary venous flow. As shown in figure 4B, at a constant frequency, increments in voltage also resulted in increases in the inotropic response as well as in coronary venous flow. These were paralleled, with one exception, by increments in the quantity of coronary venous catechol amines.

sample time (S); left ventricular end-diastolic pressure (LVED).
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The release of catechol amines from the heart, in response to efferent cardiac sympathetic nerve stimulation, does not appear to be dependent on increases in rate or stroke volume, since it occurs when these are held constant. It also takes place when coronary flow is returned to the control level during cardiac sympathetic stimulation by reducing arterial pressure by bleed-off from the femoral artery into the venous reservoir at a constant cardiac output. Figure 5 shows one of two such experiments. In this experiment, mean arterial pressure was reduced 60 mm. Hg to maintain a coronary flow during cardiac sympathetic stimulation equal to that in the control state. Even though coronary arterial catechol amine levels rose, presumably because of reflex adrenal medullary release secondary to carotid sinus hypotension, a marked myocardial production of catechol amines continued. That this myocardial catechol amine release can occur even when arterial levels do not rise was demonstrated by a similar experiment in adrenalectomized animals in which arterial levels were maintained by a constant norepinephrine infusion.

Of additional interest is the finding, also demonstrated here, that even before bleed-off had activated the presumed carotid-adrenal reflex release of catechol amines, there was a small elevation in the arterial catechol amine level. This may be secondary to the very large coronary venous release of catechol amines occurring in this experiment during stimulation of the cardiac sympathetic nerves. When the quantity of coronary venous catechol amines released is smaller, there is usually a fall in the arterial catechol amine level (fig. 6).

In the absence of efferent sympathetic nerve stimulation, the sympathectomized heart maintains an arteriovenous difference of catechol amines across the coronary bed. Thus, at low arterial levels, catechol amines were frequently not found in coronary venous blood before or after sympathetic nerve stimulation (fig. 6). However, either endogenously or by the intravenous infusion of norepinephrine, an arterial catechol amine level can be reached at which the heart does not completely clear the coronary arterial blood so that detectable amounts of catechol amines are present in coronary venous blood in the absence of cardiac sympathetic nerve stimulation (fig. 7).

The magnitude of the myocardial catechol amine extraction in the unstimulated state appears to be a function of the quantity of catechol amines delivered per minute to the myocardium. Figure 8 shows the control myocardial extraction from 18 experiments, in mg. per minute per gram of myocardium on the ordinate, plotted against the coronary arterial input of catechol amines, in mg. per minute per gram of myocardium on the abscissa. An increase in the myocardial cate-

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catechol amine input was accompanied by an increased myocardial catechol amine extraction. Within this general relationship, there were two patterns of extraction. In many instances, the relation of input to extraction is a linear function. This is shown in figure 8 by the circles connected by the line. Frequently, however, this function departs from linearity, as shown by the remaining circles in figure 8. The fact that differences in extraction can occur at all input levels suggests that factors other than the amount delivered per unit time may play a role.

The possibility that the amount of catechol amines already present in the heart may be important in this regard is suggested by contrasting the myocardial catechol amine extraction in the poststimulation period when the arterial level is low and when it is high. An example of the former is shown in figure 6; extraction was complete in the immediate poststimulation period. By way of contrast, when the arterial levels were maintained at or achieved a high level, the pattern of extraction in the immediate period after stimu-
lation was substantially changed. Examples of this are shown in figures 7 and 9.

It was also noted that with a given stimulus, the output of catechol amines in coronary venous blood diminished with time. As shown in figure 5, with the same stimulus voltage and frequency applied throughout, the output of coronary venous catechol amines declined in the later samples. That this diminution was not related to the reduction in coronary blood flow during the period of bleed-off was demonstrated by the fact that the release of catechol amines was still reduced after coronary flow was returned to the higher level. This depletion response was found in all of the four experiments in which a single stimulus was maintained for any substantial period of time. It appeared to be more prominent at the higher range of frequencies.

The quantity of catechol amines released by cardiac sympathetic nerve stimulation at a given frequency and voltage was increased by the prior infusion of norepinephrine, even after the hemodynamic parameters altered by this infusion had returned to preinfusion levels (fig. 10). In this experiment, two periods of sympathetic nerve stimulation were carried out within 10 minutes of each other. A third period of stimulation was performed as a time control 20 minutes later and after the addition of 200 ml. of blood. An additional 200 ml. of blood were given, and then a 446-μg. infusion of norepinephrine was begun. Approximately 14 minutes after the termination of the hemodynamic response to this infusion, two periods of stimulation were again performed with an interval of 10 minutes between them. The coronary venous catechol amine output was greater than the previously obtained values.

The action of dichloroisoproterenol (DCI) in modifying the response to cardiac sympathetic stimulation was helpful in elucidating the possible sites of action of catechol amines. With cardiac output maintained constant, the initial dose of DCI produced a fall in arterial and LVED pressures, a slight increase or no change in heart rate, and a transient increase in coronary outflow. Thereafter, coronary flow fell to a lower level (figs. 11 and 12). Additional doses of DCI produced little or no further changes. When sympathetic nerve
stimulation was carried out after DCI, there was a substantial diminution in the hemodynamic response. In contrast to the large increases in coronary flow during sympathetic nerve stimulation prior to the administration of DCI, after the administration there was an initial transient decrease with the initiation of sympathetic stimulation. With continued stimulation, coronary venous flow achieved a steady state at a value slightly less than or equal to the control level. When efferent cardiac sympathetic stimulation was terminated after DCI, coronary flow increased.
Figure 11 shows that the hemodynamic alterations produced by the administration of DCI were not associated with a change in either the arterial or coronary venous levels of catechol amines in the unstimulated, control state. When the afferent cardiac sympathetic nerves were stimulated following the administration of DCI, catechol amines appeared in coronary venous blood, as they had in the period prior to blocking, although the hemodynamic effects of the sympathetic stimulation were either diminished or nearly abolished (figs. 11 and 12). Also, both the arterial catechol amine levels and the coronary venous output of catechol amines increased during the stimulation period.

The increase in the arterial level of catechol amines during stellate stimulation after DCI did not appear to represent an alteration in the peripheral metabolism or adrenal release of catechol amines, since mixed venous (excluding coronary) blood did not show an elevated level (fig. 12).

Discussion

Comparison of THI and EDA Methods

Price and co-workers18–20 have previously shown that good agreement exists between the THI and bioassay methods for the determination of catechol amines, whereas the EDA determinations showed poor agreement with both these methods. The present experiments show that qualitative as well as quantitative discrepancies exist between the EDA and THI methods when the same eluate is used to assay catechol amines in canine plasma. The above data demonstrate a relationship between coronary venous catechol amines as determined by the THI method and a positive inotropic effect during stimulation of the cardiac sympathetic nerves. Such a relationship was not observed with the EDA method. In a separate group of three experiments, not presented above, large volumes of blood were obtained by means of a Morawitz cannula from the coronary sinus during control periods and periods of cardiac sympathetic nerve stimulation, and the plasma from such collections was analyzed chromatographically by Dr. J. Richard Crout. The ethylenediamine-condensing material in excess of the total catechol amines, measured by the trihydroxyindole method, was not retained by a cationic exchange resin and thereby was demonstrated not to be catechol amine in nature. There remains the possibility that either a neutral catechol or a catechol acid may account for this difference. These findings suggest the need for re-evaluation of the results of experiments in which hemodynamic changes have been related to catechol amine levels analyzed by the EDA method.

Release of Myocardial Catechol Amines During Sympathetic Stimulation

These studies corroborate the work of Outshoorn and Vogt2 who demonstrated the release of norepinephrine on stimulation of the
nervi accelerantes in the dog. In addition, by maintaining controlled hemodynamic conditions, it has been demonstrated in these studies that the production of catechol amines by the heart in response to cardiac sympathetic nerve stimulation is a function of the frequency and voltage of the stimulus, occurs in the absence of alterations in heart rate and stroke volume, and takes place whether coronary blood flow is held constant or allowed to increase.

That there can be sufficient catechol amines released by cardiac sympathetic stimulation to produce a response in the sensitized cat nictitating membrane has been shown previously. That the canine heart can make a significant contribution to the circulating levels of catechol amines is shown by the experiments described above. In many experiments the arterial level fell during stellate stimulation (fig. 6); however, this could be explained on the basis of a reflex diminution of adrenal medullary secretion secondary to a carotid sinus activity increase. In other instances, such as that shown in figure 5, an elevated arterial level occurred during cardiac sympathetic nerve stimulation. However, it should be pointed out that arterial levels of catechol amines were elevated only during stimulation at the higher frequencies. The relevance of these data to the reflex sympathetic release of catechol amines in intact animals is not clear.

**Myocardial Extraction of Catechol Amines**

The observation that the acutely sympathectomized heart is able to extract catechol amines is compatible with the work of Raab and Gigee and also of Axelrod et al., who have demonstrated the remarkable ability of the innervated myocardium to bind catechol amines, and further suggests that functioning sympathetics are not a major requirement for this affinity. However, the necessity for the presence of undegenerated nerve endings for this myocardial ability to bind catechol amines is indicated by the work of Cooper et al. who have shown that the completely, chronically denervated heart has little or no myocardial catechol amine content, does not respond to tyramine, and is hypersensitive to infused norepinephrine. That a substantial part of the ability to bind catechol amines is independent of the capacity of the myocardium to respond to catechol amines is shown by the observation that DCI, in doses sufficient to block the hemodynamic response, fails to prevent myocardial extraction of catechol amines. The fact that DCI also fails to prevent the release of catechol amines by
the heart during sympathetic stimulation indicates that the neural mechanism for release is not blocked by this drug. It further suggests that if nerve endings play any role in binding catechol amines, this function is also unmodified by DCI.

It is unlikely that the presently known enzymatic pathways of catechol amine metabolism available to the heart, i.e., the O-methyltransferase and the amine oxidase enzymes, could be solely responsible for the myocardial extraction, since Crout et al.24 have demonstrated that there is no increase in myocardial catechol amine levels until 10 to 18 hours after complete inhibition of the amine oxidase system and no increase following O-methyltransferase inhibition. The limited capacity of these enzyme systems in the heart makes it improbable that they could be responsible for the major portion of the extraction of catechol amines by the heart, even after receptor blockade with DCI.

The observation that the heart does not always completely clear coronary arterial blood of catechol amines at the higher levels suggests that the quantity of catechol amines already present in the myocardial stores is important in determining the extent of extraction. Also, the coronary arterial level of catechol amine appears to influence the extent of the myocardial release during stimulation. When stimulation was done at low arterial catechol amine levels, the coronary venous catechol amine output rose above the coronary input levels (fig. 6), whereas at high arterial levels, the coronary venous catechol amine output attained a level only equal to or slightly above the coronary input (figs. 7 and 9). These observations are consonant with the suggestion that the storage of catechol amines by the myocardium is a selective25 process. This would permit the movement of catechol amines into these sites, even in the presence of low myocardial input levels, until the stores are saturated. It can be postulated that during stimulation, binding by the store is abolished so that the catechol amines previously bound move out of the stores and eventually into coronary venous blood. When the gradient between the storage sites and the plasma is large (low catechol amine input levels), the movement of the catechol amines released from these sites into the plasma is rapid, and large quantities of catechol amines can be seen early in the coronary outflow; when this gradient is small (high catechol amine input levels), the movement is slower. With the cessation of stimulation at low input levels, the storage of catechol amines occurs rapidly once again, because of the extensive depletion of the stores produced by the previous stimulation, and this is reflected in a return to complete extraction early in the poststimulation period. At high input levels of catechol amines, take-up by the stores following stimulation is slow, resulting in a delayed return to the prestimulation pattern of extraction. This delay is due to the higher myocardial catechol amine content of the stores, as a result of both the initial high level within the reservoir, as well as the hindered elaboration during stimulation. The observation that when arterial levels are ele-

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Figure 10
Influence of infusion of norepinephrine on quantitative release of coronary venous catechol amines in response to cardiac efferent sympathetic nerve stimulation. Stimulus, 6 volts at 5 per second. Abbreviations same as figure 2.
Depletion and Repletion of Myocardial Catechol Amines

The observation that continued stimulation of the cardiac sympathetic efferent nerves results in a diminution in the release of coronary venous catechol amines is compatible with the work of Raab and Humphreys who, using a colorimetric method which measured epinephrine as well as "related catechol compounds," reported that the concentration of chromogenic material in postganglionic fibers could be diminished by prolonged electrical stimulation. Although it has been suggested that nerves can synthesize catechol amines, the present data show that the myocardial stores can be partially depleted by continued sympathetic activity. It appears unlikely that the diminution in the quantity of coronary venous catechol amines released during prolonged stimulation of the efferent cardiac sympathetic nerves is due to the Wedensky inhibition described by Orias and explained by him as being a function of high-frequency stimulation of preganglionic cervical sympathetic fibers, since the frequencies used (1 to 7 impulses per second) are far below the critical level for ganglionic inhibition (approximately 20 impulses per second). Furthermore, the studies of Dye which have often been cited as evidence against depletion, clearly show that the response to continued stimulation never reached the initial level, even when stimulation fatigue had been compensated for by shifting to a fresh portion of the nerve. The frequencies used by this author were also well within the limits of the refractory periods for sympathetic nerve fibers and ganglia. It is possible that "stimulation fatigue" could account for some of the depletion response. Nevertheless, that some of the circulating catechol amines which have been bound can be released by nervous activity is shown by the capacity of infused norepinephrine to enable the increased release of catechol amines during successive sympa-
thetic nerve stimulations and, thus, to increase the response to a given intensity of stimulation. It seems unlikely that this potentiation of efferent sympathetic nerve stimulation by norepinephrine would be secondary to any effect at the ganglia or to a reversal of nerve fatigue.

The observation that previously infused norepinephrine can modify both the hemodynamic response to cardiac sympathetic nerve stimulation and the quantitative release of catechol amines in coronary venous blood would make it appear, therefore, that a portion of the exogenous norepinephrine which is bound by the myocardium is held in some form near or at nerve endings and that this norepinephrine can be released by subsequent nervous activity. These data are consistent with the early work by Raab who noted in rats that 14 days after bilateral adrenalectomy there was a 22 per cent reduction in the epinephrine-like chromogenic material found in the heart. Repeated studies in cats by Raab and Maes using the same chromogenic analysis, were inconclusive. Burn and Rand have recently reported that the threshold of sympathetic stimulation necessary to produce vasoconstriction in the isolated hind limb of the dog was reduced following an infusion of norepinephrine, even after the hemodynamic effects of the norepinephrine had disappeared. It would seem possible to extend to the heart Burn's speculation concerning peripheral sympathetic nerves, that at least part of the function of the circulating norepinephrine released by the adrenal may serve to fill the myocardial "stores" and thus potentiate the cardiac response to sympathetic stimulation.

Dichloroisoproterenol

Interest in the use of dichloroisoproterenol as a tool with which to investigate some of the aspects of the myocardial production and utilization of catechol amines has been prompted by the recent work of Moran and Perkins, who reported that this drug selectively blocks the positive inotropic and chronotropic effects of both infused catechol amines and stimulation of the postganglionic cardiac sympathetic nerves. Our observations confirm their findings and demonstrate further that the activity of this drug is limited primarily, if not completely, to the blockade of receptor sites. It does not prevent the release of catechol amines from the cardiac sympathetic nerve endings since, after the abolition by DCI of the cardiodynamic responses to stimulation of the cardiac sympathetic nerves,
there is still an increased quantity of catechol amines in coronary venous blood.

The work of Ahlquist indicates that there are two kinds of adrenergic receptors, the alpha, mediating excitatory responses such as vasoconstriction, myometrial relaxation, etc., and the beta, mediating vasodilatation and the cardiac excitatory effects of positive chronotropism and inotropism. Moran and Perkins suggested, in view of the lack of response to exogenous catechol amines, that DCI blocked the beta adrenergic receptor sites. The increase in arterial catechol amine levels during direct cardiac sympathetic nerve stimulation after the injection of dichloroisoproterenol is not due to a reduced extraction by peripheral receptors or to adrenal medullary release, since venous levels do not increase. Therefore, it must be secondary to myocardial and/or pulmonary or aortic inability to accept, at beta receptor sites, the endogenously released catechol amines. It follows, therefore, that the quantity of catechol amines responsible for elevating the arterial level probably bears a relation to that amount of catechol amines which would be utilized by the myocardium (and also possibly the pulmonary vessels) during a normal hemodynamic response to that intensity and duration of stimulation. It further indicates that in the unblocked heart, the quantity of catechol amines appearing in coronary venous blood at any time is correspondingly diminished by that fraction attributable to utilization. However, because in the unstimulated state, when arterial levels are low, the concentration of catechol amines at receptor sites is also low, the quantity utilized is probably small. Only when input is high or when sympathetic stimulation releases catechol amines in the immediate proximity of the receptor sites, would the quantity of catechol amines removed from coronary blood by utilization become significant enough to be detectable by the method employed in this study.

It is tempting to attribute the initial coronary vasodilation observed after the first dose of DCI to a preliminary stimulation of beta receptors prior to their blockade, a possibility consonant with the work of Fleming and Hawkins. Hashimoto and his colleagues have recently reported on the effects of infused norepinephrine on coronary vascular resistance in the perfused fibrillating dog heart before and after the administration of dichloroisoproterenol. These workers attempted to relate the coronary vasodilatation during norepinephrine infusion to local vascular hypoxia in the myocardium secondary to the increased oxygen consumption produced by this drug. They suggested that the only direct action of catechol amines on coronary vasculature was that of constriction. However, the dangers of attempting to measure oxygen consumption and coronary flow in the fibrillating heart, independent of changes in contractility, as an index of drug action seem obvious, since the very nature of the fibrillation is significantly altered by infusion of catechol amines, being coarser and more forceful after the drug is administered. That there are cardiac receptor sites in the functioning myocardium capable of mediating alpha adrenergic responses is confirmed by our observations that when the predominating beta receptor sites are blocked with DCI, cardiac sympathetic nervous stimulation with its demonstrated release of catechol amines produces an initial coronary vasoconstriction (figs. 11 and 12), the subsequent rise in coronary flow being attributable to metabolic factors which overcome the constrictor stimulus.

Summary

Stimulation of the cardiac sympathetic efferent nerves is accompanied by a release of catechol amines in coronary blood. This release is a function of stimulation intensity and occurs even when stroke volume and coronary outflow are maintained constant. Comparison between the ethylenediamine and the trihydroxyindole methods for the analysis of plasma catechol amines shows both quantitative and qualitative differences, with only the results with the latter method showing a consistent relationship to the hemodynamic changes observed during cardiac sympathetic stimulation. The acutely sympathectomized
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heart extracts catechol amines from coronary blood in the absence of nervous stimulation.
The patterns of extraction observed indicate that the quantity of catechol amines in the myocardium can modify the extent of extraction in the unstimulated state as well as the extent of release during stimulation. Continued stimulation of the cardiac sympathetics is accompanied by at least a partial depletion of the myocardial catechol amine stores. These stores appear to be repleted by circulating catechol amines, since the response to any given intensity of stimulation can be potentiated by a prior norepinephrine infusion. Dichloroisoproterenol prevents neither the myocardial extraction of catechol amines in the unstimulated state nor the release of catechol amines during sympathetic stimulation; it does lessen or abolish the cardiodynamic effects of sympathetic stimulation. Data are presented which indicate that a direct coronary vasoconstriction is one of the consequences of cardiac sympathetic nerve stimulation, the vasodilatation usually observed being due, at least in part, to overriding metabolic factors.

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