Cardiac and Pulmonary Norepinephrine Release and Removal in the Dog

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SUMMARY. Norepinephrine extraction and spillover rates were determined in the heart and lungs of anesthetized dogs under resting conditions, during sympathetic stimulation, and during epicardial pacing. The fractional extraction of norepinephrine across the coronary and pulmonary vascular beds was measured from the venoarterial difference in tritiated norepinephrine after infusion of a tracer dose to a steady state level. Cardiac extraction averaged 0.299 ± 0.03 and pulmonary extraction averaged 0.215 ± 0.014; extraction was unaffected by sympathetic stimulation or pacing. Norepinephrine spillover from sympathetic nerve terminals in the heart and lungs was measured from the venoarterial difference in endogenous norepinephrine and plasma flow after correction for the extraction component. Cardiac norepinephrine spillover increased linearly with increasing frequency of sympathetic stimulation to 7.44 times resting levels at 2 Hz. During pacing, there was no change in cardiac norepinephrine spillover despite marked changes in heart rate. Norepinephrine spillover was demonstrated under resting conditions in the lung and was greater than observed in the heart. Pulmonary norepinephrine spillover increased with sympathetic stimulation to 4.15 times resting levels at 2 Hz. It is possible to separate the contributions of norepinephrine extraction and spillover to measured venoarterial differences of norepinephrine under physiological conditions in the dog. (Circ Res 53: 688–694, 1983)

UNDER resting conditions, most circulating plasma norepinephrine (NE) is derived from diffusion or "spillover" of NE released at sympathetic nerve terminals, with only a minor contribution from the adrenal medulla (Kopin et al., 1978; Brown et al., 1981). The level of resting plasma NE varies markedly when sampled in the venous drainage of different organs and in arterial blood, and most have considered that this reflects differences in organ NE spillover rate, with venoarterial NE difference providing a measure of sympathetic neurogenic tone (Cousineau et al., 1977; Manhem et al., 1978; Watson et al., 1979; Yamaguchi and Garceau, 1980). However, venous plasma NE depends on two factors: (1) spillover and (2) the amount of NE extracted from arterial blood during passage through the organ. The measurement of NE spillover rate from venoarterial difference therefore requires correction for the amount of NE extracted from arterial blood.

The aim of these experiments was to investigate the contributions of these two opposing processes to the venoarterial NE difference in the pulmonary and coronary circulations, both under resting conditions and during graded sympathetic stimulation. To our knowledge, these processes have not previously been measured simultaneously under both sets of conditions. The heart actively extracts NE administered through the coronary artery inflow (Iversen and Langer, 1969; Cousineau et al., 1980; Graefe, 1981), but coronary sinus plasma NE at rest is the same or higher than in aortic blood and increases markedly during sympathetic stimulation (Levy and Blattberg, 1976a; Miura et al., 1976). These changes reflect NE spillover, but it is not known whether extraction of circulating NE is affected by sympathetic stimulation. The venoarterial difference in plasma NE at rest and during sympathetic stimulation may therefore underestimate the true spillover rate. In contrast, in the pulmonary circulation which also extracts circulating NE (Hughes et al., 1969; Nicholas et al., 1974), pulmonary venous levels of plasma NE are lower than in pulmonary arterial blood at rest (Sole et al., 1979), and pulmonary sympathetic activity has been thought to make little contribution to the level of pulmonary venous NE.

The study was performed under approximate steady state conditions in anesthetized dogs. Stellate ganglion stimulation was used to increase sympathetic activity and the metabolic effects of changes in heart rate on NE kinetics assessed by pacing the heart to the same rates as observed during stellate ganglion stimulation. Extraction of NE by the heart and lungs was measured from venoarterial differences in the concentration of tritiated NE infused intravenously, and NE spillover was determined from the endogenous venoarterial differences in plasma NE after correction for extraction.

Methods

Experimental Preparation

Experiments were performed in 10 greyhounds weighing 23–31 kg, anesthetized with intravenous sodium pentobarbitone, bolus 35 mg/kg and infusion 6 mg/kg per
hour. After endotracheal intubation, positive pressure respiration was maintained with a Harvard Apparatus respiration pump. Polyvinyl catheters were inserted into the common carotid artery, veins in both forelimbs, and the pulmonary artery via the jugular vein, and were kept patent by flushing with heparinized saline (50 U/ml); bilateral vagotomy was performed in the neck. After midline sternotomy, the heart was suspended in a pericardial incision in the right atrium, placement of the catheter was maintained with a purse-string suture in the right atrial wall. Electromagnetic flowmeters (Biotronex Laboratory) were placed around the aortic root and left circumflex coronary artery for recording cardiac output and coronary blood flow. Arterial and pulmonary artery pressures were measured using Statham P23Db gauge pressure transducers, and heart rate was recorded from a Devices cardiograph. All circulatory variables were recorded using a Devices M-19 recorder.

The right stellate ganglion was exposed and decentralized, and bipolar platinum electrodes were placed around the decentralized ganglion. Pacing wires were sutured to the right atrial appendage. Stellate ganglion stimulation and pacing stimuli were delivered from a Grass SD9 stimulator. Arterial blood gases (Po2, PCO2, pH) and body temperature were monitored at regular intervals during the experiment and maintained within a physiological range.

### Tritiated Norepinephrine Kinetics

In preliminary experiments in three dogs, intravenous bolus kinetics of a tracer dose of l-NE[7, 8-3H] (3 µCi/kg, 40 Ci/mmol; Amersham) were determined and could be adequately described by a biexponential function. Intravenous bolus kinetics are summarized in Table 1. A loading dose and infusion rate were calculated from these data using standard pharmacokinetic techniques (Gibaldi and Perrier, 1977) to obtain a steady state pulmonary artery plasma level of tritiated NE of approximately 2000 dpm/ml plasma for the duration of the experiment, corresponding to the addition of 4 pg/ml of NE to the circulating level which averaged 80 pg/ml. The stable plasma levels of tritiated NE in pulmonary arterial, aortic, and coronary sinus blood obtained between 60 and 150 minutes of continuous infusion after an initial bolus in four dogs in which no interventions were carried out are shown in Figure 1. The steady state concentrations of tritiated NE declined progressively from pulmonary artery to aorta to coronary sinus. Whereas the level of tritiated NE in these three sites was constant from 60 to 150 minutes, the concentration of total tritium in plasma increased over the same period (Fig. 1). At the end of 150 minutes, only about 50% of the radioactivity in plasma was contributed by tritiated NE.

#### Protocol

After completion of all surgical procedures, a bolus followed by infusion of tritiated l-NE was administered into a forelimb vein, and measurements were begun 1 hour later. Resting circulatory variables were recorded and baseline blood samples were collected simultaneously from the pulmonary artery, aorta, and coronary sinus for plasma assay of tritiated NE, endogenous NE, and hematocrit. The stellate ganglion was stimulated supramaximally at frequencies of 0.5, 1, and 2 Hz (10V, 10 msec) in random order for periods of 2 minutes, with 30 minutes between stimulation periods, and circulatory variables and blood samples were collected during the last 30 seconds of stimulation when the circulatory response was stable. Fifteen minutes after each period of stimulation, when the circulatory response had returned to resting conditions, the heart was paced to the same heart rate as had been set.
observed during the previous stellate stimulation for a period of 2 minutes, and circulatory variables and blood samples from pulmonary artery, aorta, and coronary sinus were collected during the last 30 seconds of pacing. A total of 210 ml of blood were withdrawn during the experiment, and intravascular volume was replaced with Haemaccel (Hoechst).

Norepinephrine Assays

Plasma levels of tritiated and endogenous NE were assayed according to previously described methods (Peuler and Johnson, 1977; Esler et al., 1979). Briefly, tritiated NE was assayed by alumina adsorption with acid elution and counting of tritium in the acid eluate. Dihydroxy metabolites of tritiated NE in the acid eluate were searched for on several occasions by preliminary ethyl acetate extraction, but no evidence could be found for the formation of these metabolites under the present experimental conditions. Endogenous plasma NE was measured by radioenzymatic O-methylation of NE by tritiated-S-adenosylmethionine (74 Ci/mmol; Amersham) with subsequent extraction and thin layer chromatographic separation of the resulting tritiated normetanephrine. This assay was not affected by the tracer levels of tritiated NE present in plasma, since radioenzymatic O-methylation of NE in plasma always resulted in a greater than 20-fold increase in total counts.

Determination of Norepinephrine Extraction and Spillover Rates

Fractional extraction (E) of tritiated NE at steady state was determined from the relationship:

\[ E = \frac{(A-V)\text{HNE}}{A\text{HNE}} \]

where \( A\text{HNE} \) = arterial concentration of tritiated NE; \( V\text{HNE} \) = venous concentration of tritiated NE.

Organ removal of arterial NE was determined from:

\[ \text{Removal (pg/min)} = A\text{[NE]} \cdot E \cdot Qp \]

where \( A\text{[NE]} \) = arterial concentration of endogenous NE (pg/ml); \( Qp \) = organ plasma flow (ml/min).

If the organ extracts no arterial NE, then the NE spillover rate = \( Qp \cdot (V-A)\text{[NE]} \), and we have termed this expression "NE output." If there is extraction, the true spillover rate is underestimated by the amount removed by the organ. Hence, NE spillover rate

\[ \text{NE} \text{ } (pg/min) = Qp(V-A)\text{[NE]} + E \cdot A\text{[NE]} \]

or

\[ \text{NE spillover rate} = \text{NE output} + \text{NE removal}. \]

<table>
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<th>Table 2</th>
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<td>Resting Circulatory Variables Prior to Stellate Stimulation and Epicardial Pacing</td>
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<tr>
<td>Stellate stimulation</td>
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<td>MAP (mm Hg)</td>
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<td>CO (liters/min)</td>
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<td>HR (beats/min)</td>
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<td>PAP (mm Hg)</td>
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Data in the form of mean ± SEM. MAP = mean arterial pressure; CO = cardiac output; LCF = left circumflex coronary flow; HR = heart rate; PAP = pulmonary artery mean pressure.

Results

Circulatory Response to Stellate Stimulation and Pacing

There were no significant differences in resting circulatory variables prior to stellate stimulation and pacing (Table 2). Stellate ganglion stimulation resulted in increases in heart rate, cardiac output, and left circumflex coronary blood flow which were related to stimulation frequency (Fig. 2). These variables reached a stable level within 30 seconds of the onset of stimulation and remained unchanged until stimulation was terminated at the end of 2 minutes. During epicardial pacing at the same heart
Cardiac Norepinephrine Extraction and Spillover

Under resting conditions, the heart extracted 29.9 ± 3.0% (SEM) of tritiated NE present in arterial blood, resulting in removal of 0.539 ± 0.07 ng/min of endogenous NE in left circumflex coronary blood. During both stellate stimulation and pacing, there was no significant change in either NE extraction or removal (Fig. 3; Table 3).

Using the venoarterial difference in endogenous NE to calculate NE output without accounting for removal resulted in underestimation of the true spillover rate by 61% under resting conditions (Fig. 3). NE spillover rate increased monotonically with increasing frequency of stellate stimulation, and at 2.0 Hz, spillover was 7.44 times the observed resting values (Fig. 3). In contrast, epicardial pacing to the same heart rate as observed during stellate stimulation was not associated with a significant change in NE spillover rate, compared with resting.

The relationship between cardiac NE spillover rate and increase in heart rate during stellate stimulation was curvilinear over the range 0.5 to 2.0 Hz stimulation frequency (Fig. 4).

Pulmonary Norepinephrine Extraction and Spillover

Under resting conditions, the pulmonary circulation extracted 21.5 ± 1.4% of tritiated NE present in pulmonary artery blood, and extraction remained unchanged during both stellate stimulation and pacing (Table 3). Pulmonary NE extraction was significantly less than cardiac extraction (P < 0.025). Calculated pulmonary removal of NE under resting conditions was 18.07 ± 1.56 ng/min (Fig. 5), corresponding to 26.02 ± 2.25 μg/day.

Because the concentration of endogenous NE in aortic blood was always lower than in pulmonary artery blood, calculated NE output was negative. After subtraction of the NE removal component, significant NE spillover was measurable under resting conditions (10.56 ± 5.17 ng/min) (Fig. 5). During stellate stimulation at 2 Hz, the NE spillover rate increased to 4.15 times the resting spillover rate (Fig. 5). During epicardial pacing, there was no significant change in pulmonary NE spillover rate.

Knowing the regional extraction of arterial NE

<table>
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<tr>
<th>TABLE 3</th>
<th>Cardiac and Pulmonary Extraction of Tritiated Norepinephrine</th>
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<tr>
<td></td>
<td>Stellate stimulation</td>
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<td></td>
<td>Control</td>
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<tr>
<td>Cardiac extraction (% ± SEM)</td>
<td>29.9 ± 3.0</td>
</tr>
<tr>
<td>Pulmonary extraction (% ± SEM)</td>
<td>21.1 ± 1.4</td>
</tr>
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Epicardial pacing 0.5, 1.0, and 2.0 refers to pacing at same heart rate as during stellate stimulation at frequencies of 0.5 Hz, 1.0 Hz, and 2.0 Hz.
Figure 4. Relationship between changes in heart rate induced by stellate stimulation or pacing, and cardiac NE spillover.

Figure 5. Pulmonary NE spillover, output, and removal (mean ± SE) under resting conditions (control), during stellate stimulation, and pacing.

Figure 6. Components of plasma NE in venous drainage of heart (upper panel) and lungs (lower panel) derived from spillover from sympathetic nerve terminals (shaded) vs. that derived from arterial blood passing unchanged through the organ (unshaded). Determinations at rest, during stellate stimulation, and during epicardial pacing. The total height of histograms (spillover and inflow) represents total plasma NE at the two sites.

and NE spillover rate, the amount of NE in regional venous plasma which was derived from spillover at sympathetic nerve terminals within the organ vs. venous NE derived from arterial blood passing unchanged through the organ was calculated. This is shown in Figure 6. Under resting conditions, 13% of aortic plasma NE was derived from pulmonary sympathetic nerve terminals, whereas 41% of coronary sinus plasma NE was derived from cardiac sympathetic nerve terminals. During stellate stimulation, the amount of venous NE derived from arterial blood without removal remained relatively constant, but the component derived from spillover increased.

Discussion

This study has demonstrated that, under physiological conditions, the processes of NE extraction and spillover occur independently in the cardiac and pulmonary circulation, both at rest and during sympathetic stimulation. Previous studies have examined one or the other of these processes, but have not quantified their relative magnitudes in vivo.

Norepinephrine Extraction

We measured cardiac and pulmonary extraction of circulating NE by maintenance of a steady state tracer level of tritiated L-NE which increased plasma NE by 5% above resting levels (80 pg/ml). This level of plasma NE is far below that required for pharmacological activity (Silverberg et al., 1978) or for saturation of uptake mechanisms, the \( K_m \) of which in the lung is \( 10^{-6} \) M (Nicholas et al., 1974) and in the heart \( 0.26 \times 10^{-6} \) M (Iversen and Langer, 1969).
In other investigations of NE extraction, high concentrations of unlabeled NE (Hughes et al., 1969; Graefe, 1981) or the racemic isomer (Hughes et al., 1969; Iversen and Langer, 1969; Cousineau et al., 1980) have been used. The metabolism of NE is determined by the steric form of the substrate, with l-NE undergoing relatively more deamination and less O-methylation than racemic NE because of the differing optical specificities of monoamine oxidase and catechol-O-methyl transferase (Nicholas et al., 1974). Although no differences have been reported between the kinetics of pulmonary uptake of d- and l-NE (Iwasawa et al., 1973) or between dl- and l-NE (Nicholas et al., 1974) in short-term experiments (up to 12 minutes), we used the natural l-isomer because of possible changes in uptake related to different metabolic pathways during our longer term experiments. We also measured tritiated NE rather than total tritium in plasma (Cousineau et al., 1980) because preliminary experiments showed rapid metabolism, with only 50% of plasma tritium being accounted for by tritiated NE after 2 hours of infusion.

One possible source of error in measuring trans-organ extraction with tritiated NE, especially during sympathetic stimulation, is re-release of labeled NE from sympathetic nerve terminals resulting in spuriously high venous levels of tritiated NE. We feel it is unlikely that this occurred to a significant extent in our experiments, as there was no evidence of an elevation in tritiated NE in venous effluent during sympathetic stimulation. Furthermore, in the study of Yamaguchi et al. (1973) in which a bolus of tritiated NE was administered via intracoronary injection, the minimal frequency of stellate stimulation required to elicit measurable tritium release into coronary sinus blood was 3 Hz.

We found that extraction of tritiated NE was unaffected by either stellate stimulation or epicardial pacing. These results are consistent with autoradiographic evidence that most circulating NE is taken up into endothelial or smooth muscle cells rather than sympathetic nerve terminals in the lung (Hughes et al., 1969; Nicholas et al., 1974) and, probably, in the heart, since it has been suggested that, during sympathetic stimulation, neuronal NE uptake is decreased (Håggendal and Malmfors, 1969).

Our results for pulmonary extraction are similar to those obtained by other workers using both infusion (Ginn and Vane, 1968) and bolus (Gillis et al., 1972) techniques. We found a lower fractional extraction of NE by the heart than was reported by Cousineau et al. (1980) in dogs, but these workers used racemic NE in higher concentrations than used in the present experiments. Although the lung extracts a smaller percentage of circulating NE than the heart, its metabolic role in the disposition of circulating NE and, thus, in the maintenance of systemic NE levels, is 33 times that of the heart, because of the large differences in blood flow between the organs.

Norepinephrine Spillover

Having determined NE removal by the heart and lungs, it was possible to correct NE output as indicated by the venoarterial difference in plasma NE to obtain a direct measurement of NE spillover rate under resting conditions and during sympathetic stimulation. A stimulation period of 2 minutes was chosen because coronary sinus NE levels are maximal between 1 and 2 minutes of stellate stimulation, declining with continued stimulation at high frequencies (Levy and Blattberg, 1976a). Under resting conditions, there was significant NE spillover from the heart, although we only measured spillover in the left circumflex coronary artery distribution which accounts for about 40% of total coronary blood flow (Blair, 1961; Gregg et al., 1965). During stellate stimulation, there was a marked increase in cardiac NE spillover and heart rate and from the slope of the stimulus-response curves over the range of stimulation frequencies used, there was no evidence of exhaustion of neurotransmitter or saturation of cardiac \( \beta \)-receptors. Minor changes in the fractional drainage of the circumflex coronary artery into the coronary sinus during stellate stimulation are unlikely to have affected the measured NE spillover rate significantly. In contrast, epicardial pacing did not affect cardiac NE spillover rate, indicating that the metabolic effects of changes in heart rate and coronary blood flow were without effect on cardiac sympathetic activity. These findings are unlike those of Schwartz et al. (1979), who observed increases in coronary sinus NE during endocardial pacing in man. However, they did not measure NE spillover rate, and activation of cardiac reflexes may have reduced cardiac vagal efferent activity which modulates NE release by cardiac sympathetic nerves (Levy and Blattberg, 1976b). This was prevented in our study by bilateral vagotomy. It is also possible that endocardial pacing depolarizes larger numbers of sympathetic nerve terminals than does epicardial pacing.

Cardiac NE spillover probably is derived mainly from diffusion through capillary walls of transmitter released at sympathetic nerve terminals on cardiac muscle. Terminals in the smooth muscle of coronary arteries and arterioles may also make a contribution.

The present study is the first demonstration of pulmonary NE spillover into circulating blood under resting conditions without inhibition of NE uptake and metabolism (Tong et al., 1978). Although the venoarterial difference in NE due to spillover was small in the pulmonary circulation, NE spillover rate was greater than in the heart because of the high blood flow. Using our technique, stellate ganglion stimulation resulted in an increase in pulmonary NE spillover rate.

The major sites of sympathetic innervation in the lung are the bronchial smooth muscle and pulmonary arteries (Holzbauer and Sharmon, 1972), and most spillover into the pulmonary circulation prob-
ably originates from the latter source since the blood supply to the bronchi is derived from the systemic circulation and would not have been measured as a pulmonary artery-left atrial difference under the conditions of this study. Since left atrial pressure was not measured in our study, we were unable to measure possible changes in pulmonary vascular resistance during stellate stimulation or pacing.

In conclusion, we have shown that venoarterial differences in NE may be separated into a component due to extraction and a component due to NE spillover from sympathetic nerve terminals. With this technique, more precise assessment of sympathetic function in vivo is possible, and the effect of various pathophysiological states on both extraction and spillover of NE may be studied in individual organs in the intact animal.

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