Plasma Dopamine–Beta-hydroxylase Activity and Catecholamine Levels in Anesthetized Dogs Following Acute Hemorrhage

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Because dopamine–beta-hydroxylase (DBH) is released from storage vesicles in adrenergic nerves and the adrenal medulla along with catecholamines, determination of circulating levels of this enzyme might serve as an index of sympathoadrenal activity. This hypothesis has been studied in dogs anesthetized with cyclopropane, isoflurane, and thiopental that were subjected to a single acute hemorrhage and followed for 5 hours. Plasma DBH activity and catecholamine levels were determined before and every 30 minutes after the hemorrhage. Changes in DBH activity did not correlate well with changes in levels of circulating catecholamines in the dog in response to this form of stress. (Key words: Sympathetic nervous system, dopamine–beta-hydroxylase; Sympathetic nervous system, epinephrine; Sympathetic nervous system, norepinephrine; Hemorrhage, sympathetic nervous system.)

Determination of the response of the sympathoadrenal system to stress has been hindered by the difficulty of accurate determination of plasma catecholamine levels and by the rapid disappearance of circulating catecholamines caused by enzymatic degradation and tissue uptake.¹ It has been suggested that the measurement of serum levels of proteins that are released from vesicular storage sites along with catecholamines might be a more sensitive indicator of sympathoadrenal function.² Dopamine–beta-hydroxylase (E.C. 1.14.17.1, DBH), the enzyme that catalyzes the conversion of dopamine to norepinephrine, is one of these releasable vesicular proteins.³ DBH that is biochemically and immunochemically the same as the enzyme in the adrenal medulla and sympathetic nerves is found in plasma, and it has been suggested that the determination of plasma DBH activity might extend our ability to monitor the release of catecholamines.⁴ Although several studies in which plasma DBH has been measured in man and in experimental animals have been carried out, plasma catecholamine levels have been concurrently measured in very few. In the experiments described below, the hypothesis that plasma DBH is a useful measure of catecholamine release has been investigated in anesthetized dogs by the simultaneous measurement of plasma catecholamine concentrations and plasma DBH activity following acute hemorrhage. The results indicate that plasma DBH activity does not correlate well with changes in circulating catecholamines in the dog in response to acute hemorrhage.

Materials and Methods

Experimental Protocol

Eighteen unpremedicated, fasted dogs (mean weight ± SEM, 16.6 ± 2.3 kg) were maintained at MAC-1 levels of cyclopropane (17.5 per cent), isoflurane (1.48 per cent), or thiopental anesthesia. Ventilation via an endotracheal tube was mechanically controlled by means of either a Harvard pump or a Bird ventilator (Mark IV–VIII). Minute volume (V) and FiO₂ were adjusted to provide a PaCO₂ of

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40 ± 2 mm Hg, and a PaO2 of 150 ± 10 mm Hg initially and throughout the control period. Body temperature was maintained at 37°C by external means. Catheters were placed in the carotid and pulmonary arteries for sampling of arterial and mixed venous blood. A femoral artery was cannulated to provide a source for the acute hemorrhage.

Samples of mixed venous blood for determination of DBH activity were obtained from each dog prior to anesthesia. Following blood volume determination (R125I HSA, Volometer), control determinations of arterial blood gases, pH, hemoglobin, hematocrit, cardiac output (Q), catecholamines, and DBH were made. A predetermined percentage of each dog’s measured blood volume was then withdrawn over a 15-minute period. Eight dogs were bled 40 per cent, six dogs were bled 50 per cent, and four dogs were bled 60 per cent of their blood volumes. Following the acute hemorrhage, measurements of plasma catecholamines, DBH, and hemoglobin were made every 30 minutes for 5 hours. All blood taken for sampling was replaced volume for volume with the previously shed blood.

CATECHOLAMINE ASSAY PROCEDURE

Plasma epinephrine, norepinephrine, and total catecholamines were determined by the trihydroxyindole method described elsewhere.8

DBH ASSAY PROCEDURE

DBH activity was measured by the enzymatic radiochemical procedure of Molinoff et al.7 as modified for the measurement of enzymatic activity in plasma.2 The assay was carried out as previously described with tyramine, 1 mM final concentration, as substrate, except that optimal conditions of plasma dilution, pH, and concentration of CuSO4 (to inhibit the actions of endogenous tissue inhibitors of DBH) were determined for the measurement of enzymatic activity in dog plasma. Samples were diluted one part with seven parts of ice-cold glass-distilled water. The optimal concentration of CuSO4 for the determination of DBH activity in dog plasma was found to be 20 μM (final concentration).

Acetate buffer, 1 M, pH 4.9, was substituted for the tris-HCl buffer, 1 M, pH 6.0, used in the original procedure. The use of this buffer system resulted in a final reaction pH of 5.4, the optimal pH for the determination of DBH activity in this tissue (fig. 1). The DBH portion of the reaction was allowed to proceed for 1 hour.

Tissue samples that were heated to 95°C for 5 minutes served as blanks. Forty nanograms of octopamine HCl were added to a separate plasma sample in every assay as an internal standard for the portion of the reaction catalyzed by phenylethanolamine-N-methyltransferase. In some studies purified bovine adrenal DBH was also added to samples to eliminate possible artifacts caused by activation or inhibition of the enzyme. One unit of DBH activity represented the formation of 1 nmole of octopamine per milliliter of plasma per hour of incubation at 37°C.

PURIFICATION OF BOVINE ADRENAL DBH

DBH was purified from fresh bovine adrenal glands by the method of Geffen et al.8

ANALYSIS OF DATA

All data were recorded on computer punch cards and data analysis was performed with a
TABLE 1. Plasma Epinephrine, Norepinephrine, and
 DH Betaline (before Hemorrhage)

<table>
<thead>
<tr>
<th></th>
<th>Epinephrine</th>
<th>Norepi-</th>
<th>DBH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/ml)</td>
<td>nephrine</td>
<td>Units</td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>1.5 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>6 dogs</td>
<td>6 dogs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoflurane</td>
<td>1.0 ± 0.2</td>
<td>0.2 ± 0.0</td>
<td>2.9 ± 0.7</td>
</tr>
</tbody>
</table>

* No significant difference between means.

Results

BASELINE DBH ACTIVITY

Plasma DBH activity was measured in mixed venous blood obtained from 15 conscious unanesthetized dogs. The activity found in the blood of these animals was 2.8 ± 1.2 units (mean ± SD), with a range of 1 to 5.8 units. It was previously reported that plasma DBH activity in quadrupeds is approximately two orders of magnitude lower than that found in human plasma. Although the plasma DBH values reported here, obtained in blood from dogs, are comparable to those present in the bloods of other experimental animals, they are relatively low even as compared with enzymatic activity in rat blood. The average plasma DBH activity in adult Sprague-Dawley rats under assay conditions comparable to those described here is approximately 15 units (Olukotun and Weinsilboum, unpublished observation).

RESPONSE TO ACUTE HEMORRHAGE

Three of the dogs from which 60 per cent of the predetermined blood volume was removed died during the course of the experiment—two of them 20 minutes after hemorrhage before any data could be obtained and one 180 minutes after hemorrhage. Therefore, the data from these animals were excluded from the results presented below. Only two animals were treated with thiopental. Their baseline levels of plasma DBH and catecholamines and the responses of enzymatic activity and amine levels in blood were similar to those found in animals treated with cyclopropane and isoflurane. Because the data from animals anesthetized with thiopental were too few for statistical analysis, they are not presented separately below, although they were included in the calculations of the overall responses of all animals.

There was no statistically significant difference in the mean values of plasma epinephrine, norepinephrine, and DBH between control samples from dogs anesthetized with cyclopropane and those anesthetized with isoflurane (table 1). Serial concentrations of epinephrine, norepinephrine, and DBH in blood of dogs from which either 40 or 50 per cent of the blood volume had been removed were compared. In animals from which 40 per cent of the blood volume had been withdrawn, there were initial significant elevations of plasma concentrations of both epinephrine and norepinephrine, followed by a return to levels that did not differ significantly from values prior to hemorrhage. The elevation in norepinephrine concentration was more prolonged than that of epinephrine. There was no significant change in plasma DBH activity. These data are summarized in table 2.

The dogs from which 50 per cent of the blood volume had been removed showed quantitatively larger and more persistent elevations in epinephrine and norepinephrine concentrations in the blood than did animals from which 40 per cent of the blood volume was removed (table 3). Once again, there was no significant elevation in plasma DBH activity.

The overall response of all 14 anesthetized dogs to acute hemorrhage is shown in figure 2. Epinephrine concentration was elevated significantly in blood obtained from these animals for 150 minutes, while norepinephrine remained significantly elevated throughout the 300 minutes of the study. The only significant elevation in DBH activity occurred 120 minutes after hemorrhage.

Although the responses of the dogs anesthetized with cyclopropane were greater than those of the dogs receiving isoflurane, the differences were not statistically significant.

INHIBITION STUDIES OF DBH ACTIVITY

Plasma, like other tissues, contains endogenous inhibitors of DBH activity. It is
thought that these inhibitors interact with copper that is present in the enzyme. Therefore, cupric sulfate is added to the reaction mixture in order to overcome the effects of these inhibitors. Because of the possibility that any change in DBH activity seen in the course of this study might have been secondary to changes in the concentrations of circulating inhibitors of the enzyme, purified bovine adrenal DBH was added to samples of plasma obtained at 0 time and at 120 minutes (the one time at which there was a significant increase in DBH activity), and the recovery of exogenously added enzyme was determined. Samples were chosen from dogs that showed the greatest changes in concentration of DBH after hemorrhage. As can be seen in table 4, there was no significant difference between recoveries of exogenously added DBH from samples with low and high DBH activity (before and after hemorrhage). Therefore, the changes seen in DBH activity in the course of this study do not reflect alterations in the concentrations of a circulating enzyme inhibitor or activator.

### Table 3. Plasma Concentrations of Epinephrine, Norepinephrine, and DBH for Six Dogs with 50 Per Cent Blood Loss

<table>
<thead>
<tr>
<th>Time after Blood Loss (Min)</th>
<th>Epinephrine nM/mL (M ± SEM)</th>
<th>Norepinephrine nM/mL (M ± SEM)</th>
<th>DBH Units/mL (M ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.8 ± 0.2</td>
<td>0.2 ± 0.1</td>
<td>2.3 ± 0.6</td>
</tr>
<tr>
<td>30</td>
<td>6.7 ± 1.3*</td>
<td>1.5 ± 0.3*</td>
<td>4.0 ± 1.1</td>
</tr>
<tr>
<td>60</td>
<td>5.6 ± 1.2*</td>
<td>1.5 ± 0.3*</td>
<td>4.4 ± 1.3</td>
</tr>
<tr>
<td>90</td>
<td>4.4 ± 1.1*</td>
<td>1.4 ± 0.3*</td>
<td>4.8 ± 1.5</td>
</tr>
<tr>
<td>120</td>
<td>3.7 ± 0.9*</td>
<td>1.5 ± 0.3*</td>
<td>6.5 ± 2.1</td>
</tr>
<tr>
<td>150</td>
<td>2.8 ± 0.6*</td>
<td>1.3 ± 0.3*</td>
<td>5.0 ± 1.5</td>
</tr>
<tr>
<td>180</td>
<td>2.2 ± 0.4*</td>
<td>1.2 ± 0.3*</td>
<td>5.1 ± 1.3</td>
</tr>
<tr>
<td>210</td>
<td>2.0 ± 0.1*</td>
<td>1.6 ± 0.2*</td>
<td>4.3 ± 0.9</td>
</tr>
<tr>
<td>240</td>
<td>1.8 ± 0.3*</td>
<td>1.0 ± 0.2*</td>
<td>4.0 ± 0.9</td>
</tr>
<tr>
<td>270</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2*</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>300</td>
<td>1.5 ± 0.5</td>
<td>1.1 ± 0.3*</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

* Significantly different from concentration at time zero (P < 0.05).

### Table 4. Recovery of Purified Bovine Adrenal DBH* from Dog Plasma

<table>
<thead>
<tr>
<th>Time of Samples, Number</th>
<th>Baseline DBH Activity Units/mL (M ± SEM)</th>
<th>Additional DBH* Activity Recovered (M ± SEM)</th>
<th>Percent Activity Recovered (M ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-hemorrhage, 6</td>
<td>3.20 ± 0.50</td>
<td>6.77 ± 0.51</td>
<td>81.5 ± 6.0†</td>
</tr>
<tr>
<td>Post-hemorrhage, 16</td>
<td>8.55 ± 2.11</td>
<td>6.65 ± 0.63</td>
<td>79.8 ± 7.5†</td>
</tr>
</tbody>
</table>

* Pure bovine DBH, with mean activity level of 8.32 ± 1.13 units/mL.
† No significant difference between means.
‡ Time of peak DBH level (120 minutes after hemorrhage).
control levels, and that the subsequent changes in hemoglobin concentrations reflect changes in plasma volume due to shifts of extracellular fluid into the intravascular space. It is also assumed that the erythrocyte volume does not change during the period of sampling. An estimate of plasma volume can then be made at each sampling period as follows:

$$[\text{Hb}]_1 \times (\text{PV}_1 + \text{CV}_1) = [\text{Hb}]_2 \times (\text{PV}_2 + \text{CV}_2)$$

assuming

$$\text{CV}_1 = \text{CV}_2$$

$$\text{PV}_2 = \frac{[\text{Hb}]_1 \times (\text{PV}_1 + \text{CV}_1) - [\text{Hb}]_2 \times \text{CV}_1}{[\text{Hb}]_2}$$

$$[\text{Hb}]_1 = \text{control concentration of hemoglobin}$$

$$[\text{Hb}]_2 = \text{hemoglobin concentration at time of sampling}$$

$$\text{PV}_1 = \text{plasma volume immediately after hemorrhage}$$

$$\text{PV}_2 = \text{estimated plasma volume at time of sampling}$$

$$\text{CV}_1 = \text{erythrocyte volume immediately after hemorrhage}$$

$$\text{CV}_2 = \text{erythrocyte volume at time of sampling}$$

These estimates of plasma volume for each dog at each time of sampling, along with the known concentrations of amines and DBH at these times, were used to calculate the total.
plasma content values. These data are shown in figure 3 and do not differ appreciably from the concentration data shown in figure 2.

**INDIVIDUAL VARIATIONS IN RESPONSE**

Although the overall results of this study showed that plasma DBH activity does not change significantly in response to acute hemorrhage, there were very large individual differences in the responses of this indicator of sympathetic nervous system and adrenal medullary function. Such variations have been reported to occur in previous studies in man.10,11 Figure 4 shows the response of one dog that died 180 minutes after removal of 60 percent of the blood volume with isoflurane anesthesia. There were dramatic increases in both concentration and total content of biogenic amines and DBH in the blood of this animal after hemorrhage. It is not clear whether the difference between this dog and other animals relates to the severity of stress, to the dog's individual response to stress, or to a biochemical variation that resulted in the release of a larger amount of DBH with the catecholamines.

**Discussion**

The catecholamines epinephrine and norepinephrine are thought to be released from their storage granule by exocytosis, a process whereby the soluble contents of the vesicle are extruded through an opening in the cell membrane.4,12 These soluble contents include the catecholamines themselves, ATP, DBH, and at least one other protein, chromogranin A.12 After its release from peripheral sympathetic nerve terminals, most of the norepinephrine is taken back into the nerve terminal by a neural membrane reuptake process.14 Some of the neurotransmitter is metabolized locally by the enzymes catechol-O-methyltransferase and monoamine oxidase, and only a small part of the amine finds its way into the circulation.14 Both epinephrine and norepinephrine released from the adrenal medulla are carried through the blood as hormones and are subject both to enzymatic metabolism and to neural membrane uptake at sites throughout the body.14 DBH is released together with the catecholamines from both the adrenal medulla and the sympathetic nerves.14,12 Some evidence that DBH is not taken back into the nerve terminal has accumulated.14 Little is known about the subsequent metabolism of this protein.

In theory, measurements of the circulating levels of this macromolecule might serve as a good index of sympathetic adrenal function. The results of studies in both man and animals in which this hypothesis has previously been tested have been conflicting. In most stressful situations in which catecholamine release is known to occur in both man and animals, small but statistically significant increases in circulating levels of DBH have been reported.16,17 In very few of these studies have catecholamine levels and DBH activity been determined simultaneously. In the study reported here, plasma DBH activity was not found to be an accurate indicator of sympathetic adrenal response as measured by simultaneous catecholamine determinations.

There are several possible explanations for the failure of plasma DBH activity to correlate well with plasma catecholamines in the population of animals studied in these experiments. The levels of enzymatic activity in dog blood are even lower than those found in bloods of other experimental animals. Since human plasma DBH activity is two to three orders of
magnitude higher than that of the dog, this species may not be an appropriate model for the situation in man. Furthermore, although the overall changes in plasma DBH activity in the studies described here were not striking, there were wide individual variations, and in some dogs there were dramatic elevations of plasma DBH content in response to acute hemorrhage which correlated well with changes in plasma levels of catecholamines (fig. 4). Previous studies have been done in animals such as Sprague-Dawley rats. These animals are more genetically homogeneous than are the dogs studied in these experiments. A great deal of evidence that there are genetically mediated differences in baseline human DBH activity has accumulated recently.\textsuperscript{16,17} Some humans have virtually no circulating enzymatic activity.

Whether the differences in the responses to the stress of acute hemorrhage seen here reflect variable severity of the stress itself or individualized reactions to stress (due to either genetic or environmental factors) is not known.

The authors thank Fredrick A. Raymond for his assistance with these studies.

References