Basal Levels of Plasma Epinephrine and Norepinephrine in the Dog

PATRICIA A. BRIDLE, B.S., K. BRIDGET BROSNIHAN, PH.D., ROBERT C. SPETH, PH.D., MARLENE GOORMASTIC, M.P.H., AND CARLOS M. FERRARIO, M.D.

SUMMARY Conscious (n = 62) and anesthetized (n = 34) dogs were studied to establish basal levels and ranges for plasma epinephrine (E) and norepinephrine (NE) in this species. Trained conscious dogs were familiarized to recording conditions and personnel for 2 to 3 weeks and acclimated to the laboratory for at least 15 minutes prior to blood sampling from a chronically implanted catheter. Their basal values were 65 ± 47 pg/ml for E and 145 ± 58 pg/ml for NE, which were significantly lower (p < 0.05) than values in a second group of conscious dogs trained in the same manner but sampled soon after arrival to the laboratory (E = 144 ± 93 pg/ml; NE = 193 ± 86 pg/ml). Catecholamine levels in dogs anesthetized with one of three different regimes commonly used in cardiovascular studies were shown to be similar to the basal values found in conscious dogs acclimated to the laboratory. The weak correlations found between basal plasma catecholamines and hemodynamic variables in all groups of conscious dogs reflect the complexity of factors interacting with the sympathetic nervous system in the maintenance of arterial pressure. These results document the variability that can be expected when using catecholamine levels as an index of sympathetic nervous system activity and the necessity of standardizing conditions for sample collection.

KEY WORDS • catecholamines • canine • heart rate • blood pressure • sympathetic nervous system activity

ACCURATE determination of basal levels of catecholamine concentrations in plasma is essential to assess the physiological significance of an experimental challenge in any species particularly when these measurements are used to gauge the degree of sympathetic nervous system activation. In dogs, reported values for basal catecholamine levels are variable,1,2 and this may reflect methodological inconsistencies in either sampling, in assay procedures, or both. We report here a population study conducted in dogs assessing basal plasma levels of E and NE from blood samples collected over a 3-year period. A sensitive radioenzymatic assay was performed on plasma samples collected in association with rigorously standardized animal training and blood sampling techniques.

From the Section of Cardiovascular Neurobiology, Department of Cardiovascular Research, Cleveland Clinic Foundation, Cleveland, Ohio.

Supported in part by Grants HL-6835 and HL-24100 from the National Institutes of Health, NHLBI, and a grant from the Division of Research Resources, NIH for PROPHET computers.

Address for reprints: Robert C. Speth, Ph.D., Research Division, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44106.

Methods

Physiological Procedures

Ninety-six normal male mongrel dogs weighing between 16 and 30 kg were used in these experiments. Five groups were compared.

Group 1

Conscious, trained, resting-recumbent dogs (n = 17) were trained according to conditions established in the laboratory for the chronic characterization of resting arterial pressure.1-3 Experiments were started 2 to 3 weeks after the dogs were instrumented with a catheter chronically positioned into the lower abdominal aorta via an iliac artery. During the recording and sampling session, dogs were housed in a pen in a dimly lit laboratory and shielded from ambient visual and auditory stimuli. Blood pressure and heart rate (HR) were recorded for 60 to 90 minutes. The dogs were acclimated to recording conditions at least 15 minutes prior to blood sampling. In all cases, when samples were taken dogs were in the recumbent position. Subsets of this group included 11 dogs that were aware of the trainer’s presence during blood sampling, and six dogs unaware of the trainer’s presence due to remote sampling by an extended catheter advanced through the top of a shielded cage.
Group 2

Conscious, trained, alert-standing dogs (n = 45) were trained as described above for Group 1. In most cases, however, samples were obtained from the dogs soon after arrival to the laboratory.

Group 3

Dogs (n = 18) were anesthetized with pentobarbital (30 mg/kg body weight), and the left adrenal gland and kidney were exposed through a left flank incision; catheters were then placed in femoral arteries and veins. Arterial blood samples were taken at least 1 hour after surgical procedures were completed.

Group 4

Group 4 dogs (n = 11) received an injection of morphine (2 mg/kg i.m.) as a preanesthetic procedure, followed by a-chloralose anesthesia (66 mg/kg i.v.). Surgical preparation and sampling procedures were the same as for Group 3.

Group 5

Group 5 dogs (n = 5) received an injection of morphine (2 mg/kg i.m.) followed by pentobarbital anesthesia (15 mg/kg i.v.). Surgical preparation and sampling procedures were the same as for Groups 3 and 4.

Biochemical Procedures

Arterial blood (2 to 3 ml) was collected from the iliac catheter into evacuated tubes containing EGTA and glutathione. Samples were cooled on ice and centrifuged at 1000 g at 4°C within 15 minutes of collection. The plasma was separated and stored at −70°C until assayed for catecholamines.

A modification of the radioenzymatic assay of Peuler and Johnson was used to assess plasma levels of unconjugated NE and E. Briefly, catecholamines in a 50 μl aliquot of plasma were radiolabeled by O-methylation during a 1-hour incubation at 37°C with 3 μCi of a 3H methyl donor (3H-S-adenosyl methionine, 15 Ci/m mole, Amersham Corporation, Arlington Hts., Illinois TRK. 236) in the presence of 10 μl of catechol-0-methyl transferase (COMT), obtained and partially purified from rat liver by the method of Axelrod and Tomchick. The incubation medium also contained 6 μl of a 0.3 M solution of MgCl$_2$, 2 μl of Tris: EGTA (1.0M:20 mM) buffer, pH 8.4 at room temperature, and 10 μl of distilled water or catecholamine standards in distilled water to yield a final volume of 100 μl. Blanks and plasma samples were assayed in duplicate, and a third tube containing 100 pg of freshly diluted catecholamines was included as an internal standard for each sample. The internal standard was added to each sample because of the variability in O-methylation of catecholamines between samples. It has been suggested by Comoy and Bohuon and many others that inhibitors of COMT are present in plasma. An aliquot of a standardized dog plasma was run with each assay for interassay comparison. After the extraction steps, the remaining aqueous phase (approximately 85–90 μl) was manually spotted onto TLC plates having a preabsorbent area (JT Baker Chem Corporation, Phillipsburg, New Jersey, Si 250F, PA 19C) in two successive applications of 245 μl separated by a drying period. The plates were then developed with chloroform/methanol/70% practical ethylamine (16:3:2) for 1 hour. The appropriate zones for 3H-metanephrine and 3H-normetanephrine were separately scraped into 7 ml scintillation vials, extracted into 0.5 ml of 0.15 M NH$_4$OH, and converted to vanillin by the addition of 30 μl of NaIO$_4$ followed 5 minutes later by the addition of 30 μl of glycerin (10%). The samples were then acidified with 0.5 ml of 0.3 M acetic acid and 5 ml of scintillation fluor (Research Products International Corporation, Mount Prospect, Illinois, 3a20) was added. The samples were vigorously mixed and counted by liquid scintillation spectrometry.

The sensitivity for radioenzymatic assays has been defined as twice the blank value. Applying this criterion to our assay, we found that the average sensitivity ± 1 SD of 70 assays used in this study was 33 ± 13 pg/ml for E and 45 ± 15 pg/ml for NE. For purposes of statistical evaluation, all values, including 21 values for E and 11 values for NE which were below this sensitivity limit, are included in the results. The intrassay coefficient of variation (CV) was obtained by eight repeated measurements of the same control sample in one assay. Both a dog plasma sample taken under standard conditions and a human plasma sample were tested to provide results at two different catecholamine concentrations. The values for dog plasma were 241 ± 16 pg/ml for E and 296 ± 27 pg/ml for NE, yielding a coefficient of variation (CV) of 6.7% and 9.0% for E and NE, respectively. The values for human plasma were 783 ± 40 pg/ml for E and 906 ± 70 pg/ml for NE, yielding a CV of 5.1% and 7.7% for EP and NE, respectively. The interassay CV was determined by measuring the same plasma sample in nine different assays. The values for E were 198 ± 17 pg/ml, and the values for NE were 311 ± 23 pg/ml, yielding a CV of 8.4% and 7.3% for E and NE, respectively. The linearity of the assay has been documented over the range of 0.5 to 1000 pg.

Statistical Analysis

Data are expressed as means ± sd. Comparisons within the five groups of animals were assessed by analysis of variance followed by Duncan’s multiple comparison test. Values are considered significant when $p < 0.05$.

Results

Table 1 shows the means, standard deviations, and ranges for plasma E and NE concentrations, heart rate (HR), and mean arterial blood pressure (MAP) in the five groups of dogs. Values for total catecholamines (E + NE) are also included, since it has been suggested that they may be an important indicator of sympathoadrenal activity. In dogs where more than one sample for catecholamine determination was taken, the average value for each variable was used. Analysis of variance and subsequent multiple comparison tests were
Table 1. Basal Values of Epinephrine (E), Norepinephrine (NE), E + NE, Heart Rate (HR), and Mean Arterial Pressure (MAP) from Conscious and Anesthetized Dogs under Different Conditions

<table>
<thead>
<tr>
<th>Dog group</th>
<th>Epinephrine</th>
<th>Norepinephrine</th>
<th>E + NE</th>
<th>HR</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pg/ml ± sd</td>
<td>range</td>
<td>pg/ml ± sd</td>
<td>range</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Conscious</td>
<td>122 ± 63</td>
<td>17-241</td>
<td>179 ± 82</td>
<td>53-405</td>
<td>302 ± 129</td>
</tr>
<tr>
<td>1. Resting-recumbent</td>
<td>17 ± 65</td>
<td>17-210</td>
<td>145 ± 58</td>
<td>90-301</td>
<td>209 ± 82</td>
</tr>
<tr>
<td>Aware</td>
<td>11 ± 75</td>
<td>21-210</td>
<td>132 ± 38</td>
<td>90-208</td>
<td>207 ± 78</td>
</tr>
<tr>
<td>Unaware</td>
<td>6 ± 46</td>
<td>17-87</td>
<td>168 ± 82</td>
<td>91-301</td>
<td>214 ± 96</td>
</tr>
<tr>
<td>2. Alert-standing</td>
<td>45 ± 144</td>
<td>40-241</td>
<td>193 ± 86</td>
<td>53-405</td>
<td>336 ± 127</td>
</tr>
<tr>
<td>Anesthetized</td>
<td>34 ± 55</td>
<td>5-257</td>
<td>90 ± 66</td>
<td>29-352</td>
<td>145 ± 96</td>
</tr>
<tr>
<td>3. Pentobarbital</td>
<td>18 ± 75</td>
<td>12-257</td>
<td>98 ± 52</td>
<td>29-223</td>
<td>173 ± 94</td>
</tr>
<tr>
<td>5. Morphine/ pentobarbital</td>
<td>5 ± 34</td>
<td>5-102</td>
<td>52 ± 23</td>
<td>30-86</td>
<td>86 ± 51</td>
</tr>
</tbody>
</table>

*All conscious dogs were trained according to conditions established for this laboratory for chronic recording of arterial pressure. Resting recumbent dogs were acclimated to the testing laboratory for at least 15 minutes prior to blood sampling; subsets of this group include dogs aware and unaware of the trainer’s presence. Alert, standing dogs were subjected to sampling soon after arrival to the laboratory. Values are means ± 1 SD. Statistical analysis of these data is presented in figures 1 and 2.

Conscious Dogs

The levels for MAP (93 ± 9 mm Hg) and HR (79 ± 10 bpm) in all conscious dogs reported in this study compare well with data reported from past studies in this laboratory and result from systematic conditioning of the animal before experimental procedures are performed. Corresponding levels of resting plasma NE and E for all conscious dogs (n = 62) were 179 ± 82 and 122 ± 63 pg/ml respectively.

The different sampling conditions between the two groups of conscious dogs influenced plasma catecholamine levels. Plasma NE and E levels were 193 ± 86 pg/ml and 144 ± 55 pg/ml for Group 2 animals where sampling occurred soon after the animal’s arrival to the laboratory, in most cases while the dogs were standing. Animals acclimatized to the recording surroundings for at least 15 minutes and allowed to assume a recumbent position (Group 1) had significantly lower levels of both plasma NE (145 ± 58 pg/ml) and E (65 ± 47 pg/ml) when compared to the corresponding values found in Group 2 (figs. 1 and 2). Within Group 1, there were no significant differences in catecholamine values in animals aware of the trainer’s presence.

Figure 1. Comparison of plasma epinephrine (E) concentrations found in conscious and anesthetized dogs. Values are means ± sd. Parentheses indicate the number of dogs. The overall analysis of variance was highly significant (F = 17.18, p < 0.0001). To assess differences among groups, Duncan’s multiple comparison test was used. Groups with different symbols ( , , ) were significantly different at p < 0.05; groups sharing the same symbol were not significantly different.

Figure 2. Comparison of plasma norepinephrine (NE) concentrations found in conscious and anesthetized dogs. Values are means ± sd. The number of dogs is in parentheses. The overall analysis of variance was highly significant (F = 9.42, p < 0.0001). To assess differences among groups, Duncan’s multiple comparison test was used. Groups with different symbols were significantly different at p < 0.05; groups sharing the same symbol were not significantly different.
during blood sampling when compared to those unaware (table 1).

The variability found within an individual dog was investigated in five dogs from Group 2 from which nine or more plasma samples were taken on different days during their control period. A summary of the mean values and coefficients of variation for HR, MAP, and catecholamines are shown in table 2. No consistent correlations were found between plasma E, NE, or E + NE levels and either HR or MAP within individual dogs, although plasma E levels were correlated with NE levels in three of the five animals studied (Dog 3: \( r = 0.72, p < 0.02 \); Dog 4: \( r = 0.90, p < 0.001 \); and Dog 5: \( r = 0.66, p < 0.05 \)).

Several significant correlations were found, however, in the group of conscious dogs where averages of the repeated measures for each dog were used (n = 62). A strong correlation was found between plasma E values and plasma NE values (\( r = 0.58, p < 0.01 \)). Other correlations include: 1) plasma NE and MAP (\( r = 0.42, p < 0.001 \)); 2) plasma NE and HR (\( r = 0.35, p < 0.01 \)); and 3) plasma E and MAP (\( r = 0.35, p < 0.01 \)). A negative correlation was found between body weight and both E (\( r = -0.38, p < 0.01, n = 55 \)) and NE (\( r = -0.28, p < 0.05, n = 55 \)).

### Anesthetized Dogs

As shown in figures 1 and 2, plasma catecholamines for each group of anesthetized dogs did not differ from one another in spite of differences in HR and MAP (table 1) associated with each anesthetic regime. In all three groups of anesthetized dogs, both plasma E and NE were significantly lower than in conscious, trained dogs in Group 2 (\( p < 0.05 \)). However, the levels of plasma catecholamines in anesthetized dogs were not significantly different from those of dogs acclimatized to the laboratory (Group 1), with the exception of reduced NE levels in morphine/pentobarbital anesthetized dogs (\( p < 0.05 \)).

### Discussion

The conditions for obtaining an unstressed control state for hemodynamic measurement in conscious, mongrel dogs with previously implanted catheters have been well established in this laboratory by Fer-rario et al.4,6 The level for MAP (93 ± 9 mm Hg) and HR (78 ± 10 bpm) in all conscious dogs reported in this study compares well with past studies.4,6 These basal levels of MAP and HR reflect the fact that 2 to 3 weeks of previous training occurred during which time animals became familiar with the recording procedures, personnel, and surroundings on a nearly daily basis and are suggestive of the low sympathetic tone present under these basal conditions.

Within the groups of conscious dogs, a number of sampling variations were shown to influence plasma catecholamines. In all trained dogs, arterial samples were taken from chronically implanted catheters, thus avoiding the stress associated with acute venipuncture. The values for the groups of all conscious dogs in our experiments are lower than the arterial values reported by Callingham2 and venous values reported by Porlier et al.11 for conscious dogs. The difference between our data and theirs probably reflects either a difference in the degree of training of the animals or the site of sampling. The presence of indwelling catheters or strain differences may have accounted for the lower values in our study as compared to the study of Buhler et al.1 where German Shepherds were trained for daily blood drawing by cubital venipuncture. Further subdivision of our conscious dog population revealed that, if animals were acclimatized to the recording surroundings for at least 15 minutes (Group 1), a significant lowering of plasma NE and E values was found when compared to the corresponding values found in Group 2, where sampling occurred before the recording period soon after the animals' arrival to the laboratory, and in most cases while the dogs were standing. Furthermore, in Group 1, there were no significant differences in catecholamine values in animals aware of the trainer's presence during sampling procedures as compared to those unaware (table 1), although there was a tendency for plasma E levels in the group that was sampled remotely to be the lowest of the entire conscious dog population. The values found in Group 1 compare well with the values in conscious trained dogs with previously implanted arterial catheters recently reported by Johnson et al.14 and Fater et al.15 These values are comparable to resting basal levels found in humans12 and are significantly lower than resting, basal values reported in monkeys16 and rats.17

### Table 2. Assessment of Day-to-Day Variation of Norepinephrine (NE), Epinephrine (E), E + NE, Heart Rate (HR) and Mean Arterial Pressure (MAP) in Five Dogs Where Repeated Samples Were Taken

<table>
<thead>
<tr>
<th>Dog</th>
<th>No.</th>
<th>HR (bpm) Mean ±sd CV</th>
<th>MAP (mm Hg) Mean ±sd CV</th>
<th>E (pg/ml) Mean ±sd CV</th>
<th>NE (pg/ml) Mean ±sd CV</th>
<th>E + NE (pg/ml) Mean ±sd CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>80±8 9%</td>
<td>98±7 7%</td>
<td>150±68 45%</td>
<td>255±69 27%</td>
<td>405±96 24%</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>68±5 7%</td>
<td>95±9 9%</td>
<td>139±89 64%</td>
<td>129±31 24%</td>
<td>267±104 39%</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>63±3 5%</td>
<td>89±4 5%</td>
<td>97±47 49%</td>
<td>78±15 19%</td>
<td>157±59 34%</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>80±7 9%</td>
<td>93±4 5%</td>
<td>122±46 37%</td>
<td>133±27 20%</td>
<td>256±70 28%</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>90±8 9%</td>
<td>96±11 12%</td>
<td>195±42 22%</td>
<td>227±49 22%</td>
<td>422±83 20%</td>
</tr>
</tbody>
</table>

Values are means ± sd. No. = number of repeated measurements for each dog. CV = coefficient of variation, calculated as the standard deviation divided by the mean times 100.
The large variation of catecholamine levels found within the same animals when repeated day-to-day samples were taken was not entirely unexpected. Plasma levels of NE and E have been shown to fluctuate rapidly under basal conditions. These fluctuations are dependent upon both rapid release and rapid metabolism of NE and E. In the monkey under undisturbed, basal conditions, assessment of samples taken at 2-minute intervals over a 45-minute period showed coefficients of variation ranging between 11% and 49%. In monkeys, these moment-to-moment variations averaged 19.2% ± 8.3% for NE and 22.8% ± 13.9% (mean ± sd) for E, values only slightly lower than the day-to-day variation observed in our study.

In these experiments we sought possible relationships between plasma catecholamines and MAP and HR. An important finding of the study was that plasma NE and E levels correlated well in three of the five dogs when repeated measures of individual dogs were assessed and in all conscious dogs (n = 62). This finding suggests that, in spite of the known divergence of control of the sympathetic nervous system and the adrenal medulla under various conditions, the overall response in this study was their common activation. Similar findings have been reported for humans and monkeys.

Other correlations found here were the positive ones between plasma NE and MAP (r = 0.42), plasma E, and MAP (r = 0.35), and plasma NE and HR (r = 0.35). While the correlations are weak, the data suggest that the sympathetic nervous system plays a role in the maintenance of blood pressure and HR in resting trained dogs. The findings agree with studies reported in humans in the supine but not upright position. Although in some studies no correlation between MAP and plasma catecholamines has been found. The weakness of the correlation reflects the complexity of factors that interact to maintain arterial pressure under resting conditions.

The effects of three anesthetic regimes on the plasma concentrations of NE and E were determined. The results demonstrate that there was no difference in the levels of catecholamines among the three types of anesthetics used. This is an unexpected finding, in view of the different cardiovascular effects normally associated with pentobarbital and α-chloralose anesthesia. Generally, MAP is elevated and tachycardia is present in animals given pentobarbital. On the other hand, morphine/chloralose is believed to cause less interference with cardiovascular reflexes and to approximate values of blood pressure and HR found in conscious animals. The cardiovascular changes in our study seen under pentobarbital and morphine/chloralose anesthesia were expected (table 1). In spite of these differences in cardiovascular activity, plasma NE and E levels of these two groups were not significantly different from plasma catecholamines found in conscious resting recumbent dogs. The addition of morphine as a preanesthetic with pentobarbital resulted in a significant lowering of plasma NE compared to both groups of conscious dogs. The lowering of plasma NE in morphine/pentobarbital dogs is consistent with the report of Taborsky et al., which demonstrated that morphine blocked the surgically induced rise of plasma venous catecholamines associated with pentobarbital anesthetized, laparatomized dogs.

In summary, this population study of trained conscious dogs emphasizes the importance of standardizing conditions for assessing basal levels of plasma catecholamines. Indwelling catheters, periods of acclimatization, familiarity to surroundings and trainer, shielded cages, quiet recording rooms, and attention to posture contributed to the relatively low basal levels of plasma catecholamines found. Administration of three kinds of anesthetic agents commonly used in cardiovascular investigations was shown to have minimal effects on plasma catecholamines when compared to plasma levels found in resting, recumbent conscious dogs.

Acknowledgments
We thank Susan M. Flack for her expert technical assistance and Dr. Juan M. Saavedra from NIH for scientific guidance in establishing the catecholamine assay in our laboratory.

References
6. Ferrario CM, Barnes KL, Bohonek N: Neurogenic hypertension produced by lesions of the nucleus tractus solitani alone or with sinoaortic denervation in the dog. Hypertension 3 (supp II): 11-12, 1981
Basal levels of plasma epinephrine and norepinephrine in the dog.
P A Bridle, K B Brosnihan, R C Speth, M Goormastic and C M Ferrario

*Hypertension*. 1983;5:V128
doi: 10.1161/01.HYP.5.6_Pt_3.V128

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1983 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/5/6_Pt_3/V128

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org/subscriptions/