Is Physiologic Sympathoadrenal Catecholamine Release Exocytotic in Humans?

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In cultured cells and isolated perfused organs, catecholamines are coreleased with chromogranin A (CgA) from adrenal chromaffin cells and sympathetic neurons. The corelease suggests that exocytosis is the mechanism of catecholamine secretion. To investigate whether physiologic catecholamine secretion is exocytotic in humans, we measured plasma norepinephrine, epinephrine, and CgA responses to differentiated stimuli of sympathoadrenal discharge. The CgA radioimmunoassay antibody recognized authentic CgA in normal human adrenal chromaffin vesicles. Insulin-induced hypoglycemia and caffeine ingestion, in decreasing order of potency, selectively stimulated epinephrine release from the adrenal medulla. During hypoglycemia, plasma levels of epinephrine and CgA rose, and peak plasma levels of epinephrine and CgA correlated, suggesting that gradations in epinephrine release represented gradations in exocytosis. However, significant increments in plasma CgA were not observed after caffeine ingestion. Furthermore, the rise of CgA levels during hypoglycemia lagged 60 minutes behind those of epinephrine. A less-pronounced temporal dissociation between CgA and epinephrine release was also shown in isolated chromaffin cells in vitro. Selective adrenal vein catheterization suggested a barrier to CgA transport across the adrenal capillary wall. Short-term, high-intensity dynamic exercise, assumption of the upright posture, prolonged low-intensity dynamic exercise, and smoking, in decreasing order of potency, stimulated norepinephrine release from sympathetic nerve endings. Only the first sympathetic neuronal stimulus resulted in significant increments in plasma CgA, increments considerably less than those attained during adrenal medullary activation by insulin hypoglycemia. During high-intensity exercise, peak plasma norepinephrine and CgA levels correlated, suggesting that gradations in norepinephrine release represented gradations in exocytosis. The human adrenal medulla was a far more prominent tissue source of CgA than human sympathetic nerves—adrenal medullary homogenates contained 97-fold more CgA (μg/g) than sympathetic nerve homogenates. In conclusion, catecholamine secretion during selective stimulation of either sympathetic nerves or the adrenal medulla is, at least in part, exocytotic. Furthermore, stimulation of the former results in comparatively modest changes in plasma CgA compared with changes attained during stimulation of the latter. CgA appears to be transported by a route different from that of catecholamines from adrenal medullary chromaffin cells to the circulation in vivo. (Circulation 1990;81:185–195)

Catecholamine release in isolated cells and organs is exocytotic from both adrenal medullary chromaffin cells and sympathetic neurons, as judged by its calcium dependence and by corelease of the catecholamine storage vesicle core constituents chromogranin A (CgA) and dopamine β-hydroxylase (DBH) but not by the cytosolic enzyme lactate dehydrogenase. Is the same true under physiologic circumstances in humans?

All editorial decisions for this article, including selection of reviewers and the final decision, were made by a guest editor. This procedure applies to all manuscripts with authors from the University of California San Diego or UCSD Medical Center.

From the Department of Medicine, University of California, and Veterans Administration Medical Center, San Diego.

Supported by the Veterans Administration, the National Institutes of Health (DK-36-400 and HL-35-018), the American Heart Association (D.T.O., Established Investigator), and the National Kidney Foundation (M.A.T., Fellow).

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Received May 17, 1989; revision accepted September 5, 1989.
Measurements of plasma DBH have been difficult to interpret. In humans, variation in the normal activity of plasma DBH is largely determined by genetic factors rather than by the extent of sympathoadrenal activity. Furthermore, because of its substantial circulating pool, acute stresses that evoke notable sympathoadrenal discharges fail to induce increments in its plasma concentration.

CgA is the major protein in the soluble core of catecholamine storage vesicles in the sympathoadrenal system. In essential hypertension, plasma CgA is modestly elevated, suggesting an excess of basal exocytotic sympathoadrenal activity in this disorder. The origin of this elevation in circulating CgA immunoreactivity, whether from a sympathetic neuronal or an adrenal source, is, however, not yet known. In addition, plasma CgA is elevated in patients with pheochromocytoma, suggesting that catecholamine secretion from the tumor is, at least in part, exocytotic in mechanism.

We measured plasma catecholamine responses, along with those of CgA, to various known selective physiologic stimuli of either sympathetic neuronal or adrenal medullary chromaffin cell discharge. Our findings provide further insight into the mechanism of catecholamine release in humans in vivo and into the potential sources of circulating CgA.

Methods

In Vivo Studies

The study group consisted of 11 female and 34 male volunteers, 23–68 years old. All subjects were studied in the morning after an overnight fast. Blood samples for determination of CgA and catecholamines (see below) were drawn through a heparin-lock inserted into a forearm vein 30 minutes before study.

Adrenal medullary chromaffin cell stimuli. Insulin hypoglycemia. Twelve healthy subjects, six men and six women, aged 31±1 years, underwent insulin hypoglycemia. Hypoglycemia was induced, with subjects in the supine position, by administering regular 0.15 μ/kg body wt i.v. insulin (Lilly, Indianapolis, Indiana) as a bolus. Baseline blood samples were drawn twice at 15-minute time intervals. Additional blood samples were obtained 30, 60, 90, and 120 minutes after insulin administration.

Caffeine. Blood pressure and pulse rate were measured, and blood was sampled in five subjects, 30–46 years old, before and 60 minutes after the oral ingestion of 200 mg caffeine (Bristol Myers, New York), with subjects in the sitting position.

Sympathetic neuronal stimuli. Short-term, high-intensity dynamic exercise. Twelve male subjects, aged 55±3 years, rested in the sitting position for 20 minutes and then exercised on a bicycle ergometer (Monark, Sweden) using a protocol consisting of 3-minute stages. The initial energy expenditure was 50 W for 3 minutes, increasing by 50 W for each 3-minute stage of the protocol, until the study goal (200 W) was achieved. Blood pressure and pulse rate were measured, and blood was sampled immediately before exercise, at the end of the exercise period, and 2 and 5 minutes after the exercise had stopped.

Prolonged, low-intensity dynamic exercise. Six healthy male subjects, aged 33±3 years, exercised on a bicycle ergometer for 60 minutes while maintaining a constant energy expenditure of 50 W. Pulse rate was measured, and blood was sampled at 30-minute intervals before and during exercise.

Posture. Blood pressure, pulse rate, plasma catecholamine levels and CgA levels were measured in six male subjects, aged 32±3, in the supine position for 30 minutes and after assuming the upright posture for an additional 30 minutes.

Smoking. Four seated subjects, aged 39±3 years, were studied before, during, and after smoking cigarettes (one cigarette every 15 minutes for 60 minutes). Blood pressure, pulse rate, plasma catecholamine levels and CgA levels were measured.

Selective adrenal vein catheterization. Vena caval catheterizations were performed in two patients with hypertension (ultimately diagnosed as essential hypertension) and in one patient with Conn’s syndrome (unilateral left adrenal aldosterone secreting adenoma) to localize the tumor preoperatively. Blood samples were drawn through the tip of the catheter from the level of the infrarenal inferior vena cava and then again after the catheter was introduced into the left adrenal vein. Collected blood was assayed for CgA and catecholamines.

In Vitro Studies

Catecholamine release. Primary cultures of bovine adrenal medullary chromaffin cells were prepared and maintained in monolayer culture as described by Livett in 24-well plastic plates at a density of 2.5×10⁵ cells/well. After at least 3 days in culture, cells were rinsed three times in 1 ml release medium (0.15 M NaCl, 0.005 M KCl, 0.01 M Na HEPES, pH 7.0), and then stimulated to secrete into 1 ml release medium at 25°C. Catecholamine and CgA were released as a function of extracellular calcium (0 or 2 mM CaCl₂), membrane depolarization by extracellular potassium (5 or 50 mM KCl, with appropriate adjustment of sodium chloride to conserve ionic strength of the medium), the nictinic cholinergic agonist carbamylcholine chloride (carbachol, from 10⁻⁸ to 10⁻² M), and time (from 0 to 64 minutes). At the conclusion of the release period, supernate and cells were separated, and the cell pellet was lysed with 0.1% Triton X-100 so that release results could be expressed as a percentage of cell total stores (supernate/supernate+cell). A portion of each fraction was acidified with 0.2N perchloric acid (for catecholamine preservation and protein precipitation), and both neutral and acidic fractions were stored at −70°C before assay. Bovine chromogranin A was measured in neutral fractions, whereas catecholamines were measured in perchloric acid extracts. All experiments and controls were run in quadruplicate (four wells).
CHROMOGRANIN A AND CATECHOLAMINES IN ADRENAL TISSUES. Human chromaffin cell homogenates were prepared from adrenal medulla dissected from four patients at autopsy. Human sympathetic nerve homogenates were prepared from sympathetic axons dissected from autopsy vas deferens. The tissue was minced, homogenized with a Tissuemizer (Tekmar Co., Cincinnati, Ohio) in ice-cold 10 mM Na phosphate, pH 6.5 (at 1:10 ratio of tissue:buffer), frozen and thawed, and centrifuged at 10,000g for 10 minutes to sediment debris, whereupon the supernatant was frozen at −70°C before assay. Tissue homogenates were assayed for CgA and catecholamines.\textsuperscript{14} Catecholamines (norepinephrine and epinephrine) are stable in human postmortem tissues in situ at 4°C.\textsuperscript{15} The homogenization buffer was chosen to hypotonically lyse catecholamine storage vesicles while retaining both catecholamines and CgA undegraded.

**Immunoblotting.** Chromaffin vesicles were isolated from normal human surgical adrenal medulla by sucrose density gradient centrifugation as previously described.\textsuperscript{10,16} Chromaffin vesicles were lysed in hypotonic buffer (1 mM Na phosphate, pH 6.5), and the membranes were removed by preparative ultracentrifugation (100,000g, 60 minutes), leaving soluble vesicle core constituents (chromaffin vesicle lysate) in the supernatant.

Chromaffin vesicle lysate proteins were denatured in 2% sodium dodecyl sulfate (SDS) (Sigma Chemical Co., St. Louis, Missouri) in the presence of 20 mM dithiothreitol (Sigma Chemical), electrophoresed through 10% SDS-polyacrylamide gel electrophoresis (PAGE) slab gels, transferred electrophoretically to nitrocellulose, and stained either for total proteins with amido black,\textsuperscript{17} or for immunoreactive CgA by immunoblotting\textsuperscript{17} visualizing with an avidin-biotin complex bridge (Vectastain, Vector Labs., Inc., Burlingame, California).

The primary antibodies used were 1) rabbit anti-human pheochromocytoma chromogranin A\textsuperscript{12} (the antibody used for the human chromogranin A radioimmunoassay\textsuperscript{12,18}), and 2) rabbit anti-bovine chromogranin A synthetic C-terminal 16-mer.\textsuperscript{19}

**Assays.** Human chromogranin A was quantified by a rapid modification\textsuperscript{18} of the previously described homologous double-antibody equilibrium radioimmunoassay.\textsuperscript{1} The assay had intra-assay and interassay coefficients of variation of 4.2% and 8.2%, respectively. Bovine chromogranin A (for release from cultured chromaffin cells) was measured by homologous radioimmunoassay as previously described.\textsuperscript{10} Plasma catecholamines (norepinephrine and epinephrine) from in vivo human experiments were measured radioenzymatically.\textsuperscript{20} Plasma samples collected during insulin hypoglycemia were assayed for norepinephrine and epinephrine by radioimmunoassay after conversion to their methoxy (i.e., metanephrine) derivatives.\textsuperscript{21} This assay does correlate \((r=0.96)\) with radioenzymatic determinations of plasma and tissue catecholamines;\textsuperscript{21} however, it detects conjugated as well as free catecholamines\textsuperscript{22} and may thus underes-

**Statistical Analysis**

All results were expressed as mean ± SEM. Data were analyzed by ANOVA for repeated measures. Statistical significance was defined at the 0.05 level.

**Results**

**In Vivo Studies**

**Adrenal medullary stimulation.** INSULIN HYPOGLYCEMIA. Changes in mean blood glucose, plasma norepinephrine, epinephrine, and CgA concentrations after insulin administration are shown in Figure 1. Mean blood glucose concentrations reached a nadir of 20±2 mg/dl by 30 minutes \((p<0.001)\). Plasma epinephrine levels rose 14-fold \((p<0.001)\), peaking 30–60 minutes after insulin injection, whereas plasma norepinephrine levels remained unaltered. Plasma CgA levels peaked only later at 90–120 minutes, rising 1.7-fold \((p<0.001)\). Basal levels of CgA, norepinephrine, and epinephrine were not significantly correlated. By 30 minutes, however, CgA and epinephrine plasma concentrations correlated \((r=0.64, n=12, p=0.034)\). This correlation was maximal at 60 minutes \((r=0.877, n=12, p<0.001)\) and persisted at 90 minutes \((r=0.754, n=12, p=0.007)\) and at 120 minutes \((r=0.801, n=12, p=0.005)\). Normal men and women did not differ in basal values, degree of hypoglycemic stimulus, or degree or timing of norepinephrine, epinephrine, and CgA responses.

**CAFFEINE.** Plasma epinephrine levels rose twofold \((p<0.05)\) 60 minutes after caffeine ingestion, whereas plasma norepinephrine and CgA were not significantly altered (Figure 2). There was also a significant rise in diastolic and systolic blood pressures at 60 minutes \((p<0.05)\).

**Sympathetic Neuronal Stimulation**

**Short-term, high-intensity dynamic exercise.** Pulse rate rose significantly in response to dynamic exercise, reaching a maximum of 152±5 beats/min at the end of exercise. Exercise resulted in a threefold rise in plasma norepinephrine \((p<0.001)\), peaking 2 minutes after exercise, without a significant change in plasma epinephrine (Figure 3A). Plasma CgA levels displayed a small but significant \((p<0.01)\) 1.2-fold rise in response to exercise. Basal levels of CgA correlated with neither norepinephrine nor epineph-
rime levels. However, levels of CgA and norepinephrine displayed a positive correlation at their peak, 2 minutes after exercise ($r=0.7$, $n=12$, $p=0.02$). As depicted in Figure 3B, the relative increases in plasma albumin, CgA, and norepinephrine levels were 14%, 21%, and 303%, respectively. The basal plasma epinephrine levels in this study (Figure 3A), though measured radioenzymatically, were somewhat higher than the basal values in the other studies we conducted (Figures 1, 2, and 4–6).

**Prolonged, Low-Intensity Dynamic Exercise.** Pulse rate was significantly elevated throughout the exercise period, reaching a maximum of $116 \pm 8$ beats/min (Figure 4). Plasma norepinephrine levels rose 1.5-fold ($p<0.05$), whereas plasma epinephrine and CgA levels remained unaltered. Basal and stimulated levels of CgA, epinephrine, and norepinephrine were not significantly correlated.

**Posture.** Plasma catecholamine levels, as shown in Figure 5, were increased by standing, whereas CgA levels remained unaltered. Plasma norepinephrine levels were significantly elevated within 5–10 minutes of assuming upright posture, rising 2.6-fold ($p<0.001$) after 30 minutes of ambulation. In contrast, plasma epinephrine levels rose only later and less prominently (1.4-fold) 30 minutes after standing. Both diastolic blood pressure and pulse rate rose significantly ($p<0.001$).

**Smoking.** Smoking did not significantly alter blood pressure or pulse rate (Figure 6). Plasma epinephrine and CgA levels were unchanged by cigarette smoking, whereas there was a small but significant
1.4-fold rise in plasma norepinephrine at 60 minutes ($p<0.05$).

Selective adrenal vein catheterizations. By mass (ng/ml), CgA was more abundant than catecholamines in the inferior venal cava and the adrenal vein. The mass ratios of epinephrine to norepinephrine in the left adrenal vein and the inferior vena cava were 4.4±1.6 to 1 and 0.6±0.5 to 1, respectively. There was a marked step up in plasma epinephrine and norepinephrine concentrations (158±50- and 18±15-fold, respectively) from the inferior vena cava to the left adrenal vein (Table 1). In contrast, plasma CgA levels were essentially unchanged.

In Vitro Studies

Catecholamine release from cultured bovine chromaffin cells. Epinephrine and CgA could be released from chromaffin cells during a 20-minute period by either membrane depolarization with 50 mM K+ or by the nicotinic cholinergic agonist carbachol (Figure 7A). In either case, release was dependent on extracellular calcium. A dose-response curve for carbachol effects at 20 minutes of stimulation (Figure 7B) indicates that maximal carbachol effects on secretion are achieved at $10^{-4}$ M. In each 20-minute secretagogue stimulation, a consistently higher percentage of cell total of epinephrine was released than of CgA (Figure 7A and B).

To explore whether this disparity was time dependent, we examined the time course of release of CgA and epinephrine in response to $10^{-4}$ M carbachol (Figure 7C). Although both show progressive release with time, CgA release lags behind epinephrine release. The lag is maximal at 20 minutes (when epinephrine has been maximally released) but narrows as CgA and epinephrine percent release approach equivalence at 64 minutes (Figure 7C).
Immunoblotting. The antisera, including that used in the human chromogranin A radioimmunoassay, recognized an M, 70-75 kd CgA immunoreactive band in human adrenal medullary chromaffin vesicles (Figure 8) as well as lower molecular size bands (putative intravesicular cleavage products of the parent CgA molecule). The SDS-PAGE M, of human CgA is in the range of previous reports.

Chromogranin A and catecholamines in human adrennergic tissues. Sympathetic nerves contained 97-fold less CgA (μg/g wt) than adrenal medulla. Within the adrenal medulla, CgA and catecholamines were present in a mass stoichiometric ratio of 0.5±0.1 to 1 (μg/μg), epinephrine representing 97±0.8% of the total catecholamines (Table 2). The ratio of CgA to norepinephrine in sympathetic nerves was estimated at 0.9±0.3 to 1 (Table 2).

Discussion
In vitro, a large body of evidence suggests that catecholamines are secreted by exocytosis from adrenal chromaffin cells and sympathetic neurons. However, there is lingering controversy about whether physiologic catecholamine secretion is exocytotic. In vivo, either the adrenal medulla or sympathetic nerves can be selectively activated physiologically or pharmacologically. Although activation of sympathetic neurons results exclusively in norepinephrine release, activation of the adrenal medulla results predominantly in epinephrine release. Is it justi-
in descending order of potency, stimulate predominantly adrenomedullary epinephrine release, whereas dynamic exercise\textsuperscript{28} and assumption of the upright posture\textsuperscript{31,32} stimulate norepinephrine release from sympathetic nerves. Comparable increments in both norepinephrine and epinephrine levels have been observed in response to cigarette smoking.\textsuperscript{33}

As predicted, insulin-induced hypoglycemia resulted in a greater degree of adrenomedullary stimulation than did caffeine ingestion as reflected by a greater rise in epinephrine (Figures 1 and 2). No significant increments in plasma norepinephrine levels were detected, suggesting that neither test stimulated sympathetic nerves (Figures 1 and 2). CgA levels rose significantly during hypoglycemia, whereas they remained unchanged after caffeine ingestion (Figures 1 and 2), suggesting that physiologic catecholamine secretion is exocytotic during intense adrenomedullary discharge. This conclusion is reinforced by the significant correlation (at 60 minutes, \( r=0.877, n=12, p<0.001 \)) between stimulated levels of CgA and epinephrine observed during hypoglycemia. Thus, gradations of epinephrine release represent gradations of exocytosis. The rise of plasma CgA was slower than that of epinephrine. This temporal dissociation in the appearance of the plasma CgA peak (90–120 minutes) versus the epinephrine peak (30–60 minutes) (Figure 1) suggests different modes of transport of CgA and epinephrine from the chromaffin granule to the circulation.

Can CgA be transported across the adrenal capillary wall into the circulation? To explore further the temporal dissociation of CgA and epinephrine appearance, we determined the plasma concentrations of CgA and catecholamines in the left adrenal vein and the inferior vena cava (Table 1) as well as the ratio of CgA to catecholamines in normal human adrenal medullae (Table 2). The mass ratio of CgA to catecholamines in the adrenal medulla was 0.5±0.1 to 1. If soluble core constituents (CgA, catecholamines) of the chromaffin granule are released together and cotransported directly into the circulation, a proportional step up in CgA and epinephrine levels, in the ratio of 0.5 to 1, into the adrenal vein is predicted. However, a marked step up in plasma epinephrine (mean change, approximately 27 ng/ml, from the Table 1). This is consistent with what we observed during hypoglycemia.

![Figure 6](image-url)  
**FIGURE 6. Plot of mean (±SEM) pulse rate, blood pressure, plasma catecholamine (norepinephrine and epinephrine), and chromogranin A concentrations in association with cigarette smoking (n=4). *p<0.05.**

<table>
<thead>
<tr>
<th>Patient diagnosis</th>
<th>Chromogranin A</th>
<th>Epinephrine</th>
<th>Norepinephrine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ng/ml</td>
<td>pg/ml</td>
<td>pg/ml</td>
</tr>
<tr>
<td>Essential hypertension</td>
<td>39 ±6.5</td>
<td>15 ±238</td>
<td>624 ±52</td>
</tr>
<tr>
<td>Conn’s syndrome</td>
<td>17 ±5.5</td>
<td>25 ±20,160</td>
<td>465 ±7,008</td>
</tr>
<tr>
<td>Mean</td>
<td>26.7 ±0.96</td>
<td>285 ±158</td>
<td>519 ±52</td>
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<tr>
<td></td>
<td>IVC</td>
<td>LAV</td>
<td>LAV</td>
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<td></td>
<td>LAV/IVC</td>
<td>Ratio</td>
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<tr>
<td></td>
<td>90.9 ±0.03</td>
<td>254 ±50</td>
<td>2.29</td>
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Takiyyuddin et al Catecholamine Release In Vivo

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by guest on August 17, 2017

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TABLE 1. Selective Adrenal Venous Catheterizations. Plasma Norepinephrine, Epinephrine, and Chromogranin A Levels in the Left Adrenal Vein and Infracervical Inferior Vena Cava in Three Patients Who Underwent Venous Caval Catheterizations

<table>
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<tr>
<td></td>
<td>LAV/IVC</td>
<td>Ratio</td>
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<tr>
<td></td>
<td>90.9 ±0.03</td>
<td>254 ±50</td>
<td>2.29</td>
</tr>
</tbody>
</table>

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IVC, inferior vena cava; LAV, left adrenal vein.
or 158-fold, adrenal vein vs. inferior vena cava), as shown in Table 1, was unaccompanied by any increase in plasma CgA. Thus, a relative barrier does exist for the direct transit of CgA across the adrenal capillary wall.

A less-pronounced temporal lag between the appearance of CgA and catecholamines during monolayer chromaffin cell secretion (Figure 7C) is compatible with a slower diffusion of the anomalously large 77–80 Å Stokes radius CgA from the exo-

Figure 7. Catecholamine and chromogranin A release from primary cultures of bovine chromaffin cells. Panel A: Bar graph of calcium dependence of release by membrane depolarization (50 mM K+) or the nicotinic cholinergic agonist carbachol (10^{-4} M). n=4 wells/experiment. The release time is 20 minutes. Panel B: Plot of dose-response relation for stimulation of catecholamine and chromogranin A release from bovine chromaffin cells by the nicotinic cholinergic agonist carbachol (10^{-4} M). n=4 wells/experiment. The release time is 20 minutes. Panel C: Plot of time course of catecholamine and chromogranin A release from bovine chromaffin cells in vitro in response to the nicotinic cholinergic agonist carbachol (0.1 mM) during 0–64 minutes.
Axons

Vas

Human Norepinephrine

Epinephrine (%

Chromogranin A

TABLE 2. Chromogranin A Immunoreactivity, Epinephrine, and Norepinephrine in Human Adrenal Medullae and Sympathetic Axons (Vas Deferens) From Autopsy

<table>
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<th>Adrenal medulla homogenate (n=4)</th>
<th>Sympathetic nerve homogenate (n=4)</th>
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<tr>
<td>Chromogranin A (ng/g wt)</td>
<td>175.090±103,371</td>
<td>1,808±646</td>
</tr>
<tr>
<td>Epinephrine (ng/g wt)</td>
<td>277.292±136,626</td>
<td>–</td>
</tr>
<tr>
<td>Epinephrine (% total catecholamines)</td>
<td>97.4±0.8</td>
<td>–</td>
</tr>
<tr>
<td>Norepinephrine (ng/g wt)</td>
<td>5,809±2,827</td>
<td>1,817±95</td>
</tr>
<tr>
<td>Norepinephrine (% total catecholamines)</td>
<td>2.6±0.8</td>
<td>100</td>
</tr>
<tr>
<td>Chromogranin A/catecholamines (mass ratio)</td>
<td>0.5±0.1</td>
<td>0.9±0.3</td>
</tr>
</tbody>
</table>

Chromogranin A was measured by radioimmunoassay, whereas epinephrine and norepinephrine were measured fluorimetrically.

Figure 8. Immunoblots of chromogranin A (CgA) in chromaffin vesicle lysate (CVL) of normal human adrenal medulla. After sodium dodecyl sulfate–polyacrylamide gel electrophoresis of prestained protein size standards or CVL, proteins were electrophoretically transferred to nitrocellulose sheets and stained either for protein (amido black) or CgA, using antiserum either to purified human pheochromocytoma CgA (the antibody used in the human CgA radioimmunoassay) or to a synthetic CgA C-terminus (16-mer).

cytotic pore. CgA has also been reported to have an anomalously low diffusion coefficient for a molecule of its mass. The exocytotic pore barrier (Figure 7C) cannot entirely explain the approximate 60-minute time lag of CgA behind epinephrine in vivo (Figure 1). These findings suggest an additional relative barrier to transport of CgA into the circulation at the level of the capillary wall. This conclusion is in line with the findings of Carmichael et al, who observed that during feline adrenal medullary secretion after hypoglycemia, catecholamines were transported directly into the adrenal vein, whereas large mole-

cules such as CgA made their way from the adrenal medulla to the circulation at least in part through a lymphatic route ultimately involving the thoracic duct. Capillary walls are only very slowly permeable to large proteins. Endothelial intercellular junctions may only open to 50–60 Å whereas the Stokes radius of CgA is larger at 77–80 Å as mentioned. Even though adrenal capillary endothelial cells possess 500 Å fenestrae, such fenestrae apparently do not transport proteins. DBH also arrives in plasma, after sympathoadrenal stimulation, in part through the lymphatics and the thoracic duct.

Among the selective stimuli of sympathetic neuronal norepinephrine release, short-term, high-intensity dynamic exercise was the most potent (Figure 3A). CgA and norepinephrine were significantly correlated at their peak plasma concentrations 2 minutes after exercise (r=0.7, n=12, p=0.02); thus, gradations of norepinephrine release represent gradations of exocytosis. The postexercise decline in plasma norepinephrine was faster than that of CgA, suggesting a slower rate of plasma clearance or longer half life of CgA (Figure 3A). The relative increase in plasma CgA was greater than that of plasma albumin (21% vs. 14%), and CgA’s postexercise decline followed a time course different from that of albumin; thus, changes in plasma CgA levels were not simply the result of hemoconcentration (Figure 3B).

Selective stimulation of sympathetic nerves (Figure 3A) resulted in comparatively modest changes in
plasma CgA levels compared with changes attained during adrenomedullary activation (Figure 1). This observation is consistent with the greater abundance (97:1) of CgA in adrenal medullary compared with sympathetic neuronal tissue sources (Table 2).

Only rather intense stimulation of the adrenal medulla (Figures 1 and 2) and sympathetic neurons (Figures 3–6) measurably changed plasma CgA levels. Does this indicate that modest augmentations in sympathoadrenal outflow (Figures 2, 5, and 6) result in diffusional or otherwise nonexocytotic catecholamine secretion? Considering the small magnitude of plasma catecholamine increments during modest adrenal or sympathetic stimulations (40–240 pg/ml) (Figures 2, 5, and 6), the tissue source ratios of CgA to catecholamines (Table 2) and the relatively high basal concentration of CgA (20–50 ng/ml), one might predict that all-or-none (exocytotic, proportional) corelease of catecholamines and CgA under these circumstances would augment plasma CgA by less than 1 ng/ml, a change likely to be imperceptible by the CgA radioimmunoassay. The relatively high basal plasma CgA concentration may also be a function of its multiple potential endocrine tissue sources. ⁴¹ In any event, plasma CgA changes are not informative for modest changes of exocytotic catecholamine release within the physiologic range (e.g., Figures 2, 4–6).

It could also be argued that mild sympathetic neuronal activation results in selective exocytosis from CgA-poor small dense core vesicles rather than CgA-rich large dense core vesicles. ⁴² However, there are no known mechanisms for selective exocytotic mobilization of small over large dense core vesicles. Furthermore, large dense core vesicles are relatively abundant in human sympathetic neurons. ⁴³ Indeed, electrical stimulation of human sympathetic axons in vitro mobilizes large and small dense core vesicles to morphologic exocytosis. ⁴³ Finally, in the adrenal medulla, there is no CgA-poor population of chromaffin granules or chromaffin cells ⁴⁴–⁴⁶ from which catecholamines could be mobilized by exocytosis without CgA during modest adrenal medullary activation (Figure 2).

Larger absolute (ng/ml) changes in CgA than in catecholamines during intense sympathoadrenal stimulation (Figures 1 and 3) suggest a longer plasma half-life, or delayed plasma removal rate, for CgA compared with catecholamines. This explanation is supported by an inspection of the relative rates of decline of CgA and catecholamines after stimulation (Figures 1 and 3). We have determined plasma CgA immunoreactivity’s half-life after sympathoadrenal deactivation to be 18.4 minutes, ¹² whereas the half-life for plasma norepinephrine is about 2 minutes. ⁴⁷ Plasma CgA’s disposition or removal may in part depend on the kidney. ¹⁸

In conclusion, the most parsimonious interpretation of our data is that physiologic catecholamine release in humans, from either the adrenal medulla or sympathetic neurons, is exocytotic. This conclusion is especially evident at extremes of sympathoadrenal activity (Figures 1 and 3).

Acknowledgments

We appreciate the technical assistance of Ms. Annie Chen and Mr. James Peirce, and the secretarial support of Ms. Ann Chavez.

References

18. O’Connor DT, Pandian MR, Carlton E, Cervenka JH, Hsiao RJ: Rapid radioimmunoassay of circulating chromogranin A: In vitro stability, exploration of the neuroendocrine character...
of neoplasia, and assessment of the effects of organ failure.  

19. O’Connor DT, Takiyuddin MA: Synthetic peptide epitopes: Clues to the structure, conformation, distribution and processing of chromogranin A (CgA) (abstract).  


22. Aron DC, Bravo EL, Capka LP: Erroneous plasma norepi-


26. Shah SD, Tse TF, Clutter WE, Cryer PE: The human symp-

27. Young JB, Rosa RM, Landsberg L: Dissociation of sympa-

28. Cryer PE: Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system.  


34. Smith AD, Winkler H: Purification and properties of an acidic protein from chromaffin granules of bovine adrenal medulla.  

35. Carmichael SW, Stoddard SL, O’Connor DT, Yaksh TL, Tyce GM: The secretion of catecholamines, chromogranin A, and neuropeptide Y from the adrenal medulla of the cat via the adrenolobar vein and the thoracic duct: Different anatomic routes based on size.  


37. Palade GE, Simionescu M, Simionescu N: Structural aspects of the permeability of the microvascular endothelium.  

38. Elfvén LG: The ultrastructure of the capillary fenestrae in the adrenal medulla of the rat.  

39. Renkin EM: Relation of capillary morphology to transport of fluid and large molecules: A review.  

40. Ngai S, Dairman W, Marchelle M, Specter S: Dopamine-

41. O’Connor DT, Deftos LJ: Secretion of chromogranin A by peptide-producing endocrine neoplasms.  


45. Lloyd RV, Blaivas M, Wilson BS: Distribution of chromogranin and S-100 protein in normal and abnormal adrenal medul-

46. Wilson BS, Lloyd RV: Detection of chromogranin in neuro-


**KEY WORDS**  
chromogranin A  •  exocytosis  •  adrenal medulla  •  nervous system, sympathetic
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M A Takiyyuddin, J H Cervenka, P A Sullivan, M R Pandian, R J Parmer, J A Barbosa and D T O'Connor

Circulation. 1990;81:185-195
doi: 10.1161/01.CIR.81.1.185
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/81/1/185

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