Adrenal epinephrine and norepinephrine release to hypoglycemia measured by microdialysis in conscious rats

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Vollmer, Regis R., Judi th Joyce Balcita, Alan F. Sved, and David J. Edwards. Adrenal epinephrine and norepinephrine release to hypoglycemia measured by microdialysis in conscious rats. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1758–R1763, 1997.—Experiments were conducted in conscious male rats to determine whether hypoglycemia induced by insulin administration preferentially stimulated epinephrine (Epi) or norepinephrine (NE) adrenal medullary chromaffin cells. The release of Epi and NE from the adrenal medulla was continuously monitored using a microdialysis probe of novel design that had been inserted in the adrenal medulla 16 h before the administration of insulin. Following insulin, 3 U/kg iv, blood glucose declined and dialysate Epi levels rose. No measurable increase in dialysate NE was obtained. Similarly, plasma Epi increased with no detectable change in NE. Patterns of dialysate and plasma catecholamine changes were similar in two groups of animals that had been fed or fasted overnight before insulin treatment. However, the magnitude of the Epi increase was greater in the fasted animals. After recovery of the blood glucose concentration to preinsulin levels, dialysate and plasma catecholamine concentrations returned to control values. These experiments clearly demonstrate that adrenal medullary chromaffin cells that produce Epi are preferentially stimulated in response to insulin-induced hypoglycemia.

Adrenal medulla; chromaffin cells; sympathetic nervous system; insulin

EPINEPHRINE (Epi) and norepinephrine (NE) are secreted from two distinct populations of adrenal medullary chromaffin cells, which exist in the proportion of roughly four Epi-containing cells per one NE-containing cell in rats and humans (5). The amount of each catecholamine released is primarily governed by preganglionic neurons whose activity is controlled by tracts of neurons descending to the spinal cord from supraspinal centers (17). This neural input to the adrenal medulla is adjusted in response to a variety of physiological and pharmacological stimuli (4, 15). As a result, Epi and NE secretion into the circulation is modified, and it is thought that the circulating catecholamines assist in the restoration of homeostasis.

The adrenal medullary response to hypoglycemia is an example of a neurally mediated stimulation of catecholamine release. Interestingly, evidence is accumulating in support of the hypothesis that the Epi population of chromaffin cells may be preferentially stimulated compared with NE-containing cells during hypoglycemia (1, 2, 18). This viewpoint is supported by reports that the rise in blood Epi is disproportionately greater than the rise in NE even when the ratio of Epi to NE content of the adrenal medulla is taken into account (14, 18). As yet, however, the concept of differential control of Epi- and NE-containing cells has not been definitively established. In part, this is due to limitations in the methodology available. The interpretation of changes in blood catecholamine concentrations is confounded by the fact that blood NE originates from both the adrenal medulla and sympathetic neurons and the fraction contributed by each source cannot be readily determined.

Thus it would be useful to directly assess the extent to which the Epi- and NE-containing cells are stimulated. One approach is to measure the influence of a hypoglycemic challenge on the total glandular content of Epi and NE. In fact, it has been shown that total glandular content of Epi is reduced in response to hypoglycemia with no detectable depletion of NE (1, 2, 12, 18). However, this method is relatively insensitive because a large hypoglycemic challenge must be used to evoke sufficient catecholamine release to produce a measurable depletion of adrenal catecholamines, particularly in light of the tremendous capacity of the adrenal to synthesize new catecholamines to replenish those that have been released. Also, measurements of content are made at a single time point on the conclusion of the experiment and do not reflect the time course of release.

To overcome these limitations to assessing the extent to which Epi- and NE-containing cells have been activated, the present study was conducted utilizing an intra-adrenal microdialysis technique to evaluate the time course of changes in secretion from Epi and NE chromaffin cells in response to hypoglycemia. Other investigators have determined that microdialysis may be used to measure the release of steroids, neuropeptides, and catecholamines from the adrenal medulla (6–9). However, the technique has not been specifically employed to determine whether hypoglycemia or any other stimulus produces a selective activation of one or the other population of chromaffin cells.

METHODS

General. Male Sprague-Dawley rats (Zivic-Miller Laboratories, Zelienople, PA), weighing 250–275 g on arrival, were individually housed in a room maintained at 23 ± 1°C with a 12:12-h light-dark cycle. The animals were allowed a minimum of 1 wk to acclimate before being used in an experiment. Food and water were available ad libitum.

Microdialysis probe construction. The probe was constructed from a 1-cm length of 26-gauge stainless steel hypodermic tubing, 457 µm OD, 244 µm ID (Small Parts, Miami Lakes, FL), as illustrated in Fig. 1. With a hand-held grinder, an opening 2.5 mm in length was formed in the side of the tube, and one end of the tube was shaped to a sharp point. With the aid of a dissecting microscope, a 2-cm section of the tube, and one end of the tube was shaped to a sharp point. With the aid of a dissecting microscope, a 2-cm section
of regenerated cellulose dialysis tubing, 216 µm OD, 18,000 molecular weight cutoff (Spectrum, Houston, TX), was passed inside the probe tubing and secured to the blunt end of the probe shaft with epoxy cement. Cement was also used to form a seal between the probe shaft and the microdialysis fiber at each side of the window, leaving 2 mm of the microdialysis tubing exposed. The cement was allowed to dry for 24 h, and excess dialysis tubing extending from each end of the probe shaft was trimmed. A nub formed by heating PE-20 tubing was slid onto the blunt end of the probe and positioned 0.5 mm from the edge of the window. The perfusion inflow tubing consisted of a 10-cm section of silicone tubing, 635 µm OD, 304 µm ID (A-M Systems, Everett, WA), which was slid onto the blunt end of the probe until it made contact with the nub.

Probe insertion. Animals were anesthetized with pentobarbital sodium, 60 mg/kg ip, and surgery was performed using aseptic procedures. The left adrenal gland was approached retroperitoneally via an incision caudal to the ribs and parallel to the axis of the ribs. The gland was gently lifted, and the sharpened end of the probe was inserted in the center of the left lateral surface of the gland and passed through the gland to exit at the center of the right lateral surface. A 10-cm length of silicone tubing was used for the outflow from the probe and was advanced over the pointed end of the probe until it touched the capsule of the gland. A sleeve fashioned from heat-flared PE-90 tubing was slid over the Silastic probe and was advanced over the pointed end of the probe until it made contact with the nub.

Insulin-induced hypoglycemia. Dialysate samples were collected at 15-min intervals. Blood samples were taken at time 0 and at 30 min and 1, 2, 3, and 4 h after insulin. The dose of insulin was selected to produce a hypoglycemic effect from which recovery occurred within the 4-h experimental period. Blood samples (1 ml) were collected into ice-cold tubes containing 40 µl of a solution containing EDTA (5 mM) and sodium metabisulfite (3 mM). This blood was replaced immediately with an equal volume of heparinized blood that had been removed from a donor animal. Samples were centrifuged (5,000 g for 2 min), and the plasmas were separated and frozen (−70°C) for later analysis of glucose and catecholamine concentrations. Plasma glucose was determined spectrophotometrically using an assay kit (Sigma Chemical, St. Louis, MO) based on the glucose oxidase method of Trinder (16).

Measurement of catecholamines. Catecholamines were assayed using high-performance liquid chromatography (HPLC) with electrochemical detection (Waters, Marlborough, MA) (10, 19). Dialysate samples required no purification before HPLC analysis. Plasma samples to which internal standard, 3,4 dihydroxybenzylamine, had been added and which had had their pH adjusted to 8.4 with 1 M tris(hydroxymethyl)aminomethane buffer were extracted with acid-washed alumina. Catecholamines were eluted from alumina with acetic acid (0.1 N) and were separated on a 5-µm, 3.9 × 150 mm C18 reverse-phase column (Waters). The mobile phase consisted of sodium acetate (50 mM), citric acid monohydrate (20 mM), sodium-1-octane-sulfonate (2 mM), di-n-butylamine (1.0 mM), disodium EDTA (0.1 mM), and methanol (4%). Catecholamines were oxidized during exposure to a glassy carbon electrode set at a potential of 0.6 V versus Ag/AgCl. Data were acquired with the use of ChromPerfect software (Justice Innovations, Mountain View, CA), and calculations were based on peak areas.

Statistics. Results are presented as means ± SE. Student’s t-test was used for comparison of two group means. The differences in multiple measurements taken over a period of time were assessed by analysis of variance (ANOVA) for repeated measures (SigmaStat; Jandel, San Rafael, CA). A statistically significant effect was accepted when P < 0.05.

RESULTS
Calibration of adrenal medullary dialysis probes. The amount of catecholamines recovered in dialysate was tested by placing probes into a beaker of Ringer solution containing 100 ng/ml NE and Epi. Ringer solution containing no catecholamines was pumped through the probes at different rates, and 15-min samples were collected and analyzed to calculate recov-
ery (Fig. 2). On the basis of this test, a perfusion flow rate of 4 µl/min was selected for use in the animal experiments because it provided a sufficient sample volume and catecholamine concentrations that were comfortably within the range of detection on our HPLC system. To ensure proper functioning, each probe was tested at a perfusion flow rate of 4 µl/min before insertion into an experimental animal. Recovery of both Epi and NE was ~2.0% (Table 1).

Effects of insulin-induced hypoglycemia. The dialysate samples collected immediately after probe insertion contained high concentrations of catecholamines, with the Epi concentration being 2.7 times greater than NE. The concentrations declined rapidly in the first 3 h, followed by a more gradual decline over the next 10 h (Fig. 3). Experiments to test the effects of insulin-induced hypoglycemia were conducted on the morning after probe insertion to ensure that dialysate catecholamines had achieved a relatively stable level. Insulin, 3 units/kg iv, was administered to two groups of animals that had been fed (n = 9) or fasted overnight (n = 6). Baseline values for dialysate and plasma catecholamines and blood glucose are summarized in Table 2. There were no significant differences in these parameters in the fed and fasted groups.

In fed animals, insulin produced a rapid decline in the blood glucose concentration, which reached a nadir at 1 h, followed by a gradual return to preinsulin levels by the end of the 4-h observation period (Fig. 4). Dialysate Epi rose to reach a level approximately twofold higher than baseline at 1.5 h after insulin and declined to baseline levels by 4 h. Plasma Epi concentration also increased significantly in response to insulin administration. In contrast to Epi, concentrations of NE in dialysate and plasma were not significantly changed in response to hypoglycemia.

The changes in plasma glucose concentration to insulin in overnight-fasted rats were similar to the fed animals. Although the baseline blood glucose concentra-

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<th>Table 1. Probe test to determine percentage recovery of catecholamines from a test solution with known concentrations of epinephrine and norepinephrine</th>
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<tr>
<td>Epinephrine, ng/ml</td>
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<tr>
<td>Test solution</td>
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<td>Dialysate</td>
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<td>Percent recovery</td>
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Values are means ± SE; n = 19. Flow rate was 4 µl/min at a room temperature of 22°C.

Fig. 2. Microdialysate catecholamine concentrations were measured at different flow rates in dialysate collected from probes immersed in a beaker of Ringer solution containing epinephrine and norepinephrine (100 ng/ml, n = 5). Pure Ringer solution was used as the perfusate. A: relationship between recovery of catecholamines in dialysate as a percentage of the concentrations of epinephrine and norepinephrine in the beaker solution. B: relationship between the total (absolute) amount of catecholamines present in the dialysate for each minute of collection.

Fig. 3. Changes in microdialysate catecholamine levels after probe implantation. Average concentrations of catecholamines appearing in dialysate beginning immediately after probe implantation and over the next 24 h are shown; n = 3.
Table 2. Baseline values for concentrations of plasma glucose, dialysate catecholamines, and plasma catecholamines in fed and overnight-fasted animals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fed (n = 6)</th>
<th>Fasted (n = 6)</th>
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<tr>
<td>Plasma glucose, mg/dl</td>
<td>99.7 ± 7.0</td>
<td>85.3 ± 6.9</td>
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<tr>
<td>Dialysate catecholamines, ng/ml</td>
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<td></td>
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<tr>
<td>Epinephrine</td>
<td>2.51 ± 0.45</td>
<td>1.96 ± 1.03</td>
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<tr>
<td>Norepinephrine</td>
<td>1.09 ± 0.16</td>
<td>1.49 ± 0.88</td>
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<tr>
<td>Plasma catecholamines, pg/ml</td>
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<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>142.4 ± 49.7</td>
<td>90.6 ± 22.8</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>311.7 ± 65.7</td>
<td>241.6 ± 59.8</td>
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Values are means ± SE. No significant differences were detected between fed and fasted group means when compared with Student’s t-test.

Fig. 4. Effects of insulin-induced hypoglycemia in fed animals on microdialysate (top) and plasma catecholamine concentrations (middle). Plasma glucose concentration is plotted at bottom. Insulin, 3 U/kg, was administered intravenously at time 0. *Significant main effect for epinephrine concentration over time by repeated-measures analysis of variance, P < 0.001.

Fig. 5. Effects of insulin-induced hypoglycemia in fasted animals on microdialysate (top) and plasma catecholamine concentrations (middle). Plasma glucose concentration is plotted at bottom. Insulin, 3 U/kg, was administered intravenously at time 0. *Significant main effect for epinephrine concentration over time by repeated-measures analysis of variance, P < 0.001.
in conscious rats under resting and stimulated conditions, thereby permitting a detailed appraisal of the time course of catecholamine release.

In our experiments, dialysate Epi levels increased as blood glucose fell and returned toward baseline as blood glucose recovered to preinsulin levels. This pattern was similar in fed animals and overnight-fasted animals; however, the increment in Epi release was greater in the fasted animals. In both the fed and fasted groups there was no evidence that NE-containing cells were stimulated. Although fasted animals tended to have a lower blood glucose concentration before insulin administration, this difference was not statistically significant and the nadir obtained during the peak hypoglycemia was similar in the two groups of animals. It is not clear why a more robust stimulation of Epi occurred in fasted animals, because it is generally accepted that adrenal neural stimulation is coupled to the sensation of blood glucose levels. Perhaps the reduction in energy stores during the overnight fast period somehow augmented the sensitivity of glucose sensors or central integrative sites.

The most important conclusion to be drawn from these experiments is that Epi chromaffin cells were selectively stimulated during hypoglycemia, with no measurable activation of NE-containing cells. Moreover, the results are consistent with the conclusion that the central nervous system integrative centers are capable of providing a selective increment in neural input to the Epi-containing cells without simultaneously stimulating NE-containing cells. Recent anatomic investigations in the cat provide evidence for the existence of two distinct groupings of preganglionic sympathetic neurons that separately innervate Epi and NE chromaffin cells (3).

An advantage of the microdialysis method is that adrenal catecholamine release could be measured independently from NE released from sympathetic neurons. Baseline concentrations of NE in the dialysate of resting fed animals was 1.09 ± 0.16 ng/ml. Testing of our microdialysis probes in a solution containing known amounts of NE indicated that the percentage recovery was 2.11 ± 0.15%. Thus the concentration of NE in the extracellular fluid would be 47 times greater than dialysate, 52 ng/ml, a concentration that is 167 times the plasma concentration of 312 ± 66 pg/ml. Thus any possible contribution of NE secreted from postganglionic sympathetic neurons to the dialysate NE would be negligible.

Plasma Epi levels increased in agreement with the dialysate measurements, which indicated a stimulation of Epi-containing cells. The finding that plasma NE was not altered by hypoglycemia by itself does not allow us to make the conclusion that sympathetic neurons were not significantly stimulated by insulin-induced hypoglycemia because it is possible that there were offsetting changes in sympathetic neuronal and adrenal medullary contributions to plasma NE. However, we have ruled out a change in adrenal medullary NE secretion because we measured NE release in dialysate and found it to be unaltered. Therefore, we may definitively conclude that in our experiments the activity of sympathetic noradrenergic neurons also did not change after insulin. The conclusion that the adrenal gland but not sympathetic neurons are stimulated during hypoglycemia concurs with the observation of Young et al. (20).

Although only plasma Epi secretion increased in response to insulin-induced hypoglycemia in our experiments, others have reported that plasma NE also increases (11, 13, 14). In two studies, conscious rats that had been fasted overnight were given insulin (13, 14), and the subsequent increases in plasma NE were much smaller than the increases in Epi. Even when the fact is taken into account that the ratio of Epi to NE in the adrenal medulla of the rat is 3:1, there is clearly a preferential increment in Epi. Furthermore, when the adrenal medullas were removed from a group of rats before insulin administration, the increment in plasma NE still occurred, prompting the authors to conclude that the source of the NE during insulin-induced hypoglycemia was the sympathetic neurons and not the adrenal medulla (13). In rats anesthetized with equithesin, insulin raised only Epi during the first 30 min but raised both Epi and NE thereafter, which the authors attribute to a nonneural component of release (11).

Thus, although the other investigators have reported increases in plasma NE whereas we did not, there is agreement between those studies and ours that 1) insulin produces a preferential increase in plasma Epi and 2) insulin appears to cause no release of NE from the adrenal gland. That small increments in plasma NE are seen by some investigators is likely due to differences in methodology such as dose, route of administration, and presence of anesthesia.

The microdialysis probe used in our experiments differs substantially from flexible probes described in previous reports (7, 8). The rigid probe provides the advantage that the width of the tract of damage to the adrenal is limited to the width of the 26-gauge stainless steel tubing shaft of the probe. In contrast, the damage tract produced by insertion of flexible probes is greater than the diameter of the outflow tubing, which is typically pulled through the gland after being stretched over a 22-gauge needle (7, 8). The rigid probe design also prevents lateral movement of the microdialysis fiber once the probe is in place. With the flexible probes, the microdialysis tubing is smaller than the tract of damage produced during its placement. This may lead to movement of the fiber during experimentation. Because of their small size, dialysis fibers easily bend and twist, a problem that is prevented with the rigid probe. Furthermore, the plastic cuffs at each end of the probe hold the dialysis window in a fixed position.

It is important to consider when using the microdialysis technique to measure adrenal release that the initial high catecholamine levels in the dialysate samples collected immediately after probe insertion preclude the use of this technique for short-duration experiments that do not allow adequate time for the establishment of a stable baseline. These high concentrations can be attributed to the disruption of chromaf-
Perspectives

The present study supports the contention that hypoglycemia activated the adrenal medulla without significant stimulation of sympathetic neurons. Furthermore, the adrenal medullary response consists of a selective stimulation of Epi-containing cells without activation of NE-containing cells. The microdialysis probe used in this study is suitable for the measurement of neurally elicited changes in adrenal catecholamine release. Of particular significance is that direct probing of the adrenal medulla allows for measurement of NE release from chromaffin cells that is not confounded by NE release from sympathetic neurons. The finding that Epi- and NE-containing cells can be selectively stimulated provides impetus for future investigations of patterns of adrenal secretion that may be associated with a broad range of physiological stimuli that are known to activate the sympathoadrenal system. Moreover, it will be useful to ascertain the neural pathways that underlie the ability to selectively activate populations of adrenal medullary cells.

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REFERENCES


