An in vivo microdialysis technique was used to study adrenal medullary function in 6 euglycemic and 6 hyperglycemic anesthetized Sprague-Dawley rats exposed to hypoxia. After stabilization of an adrenal dialysis probe, dialyzable adrenal epinephrine and norepinephrine were measured in response to 15 min of 7.8% oxygen breathing in both groups. In euglycemic rats, hypoxia increased epinephrine and norepinephrine by 650 and 320% above baseline, respectively. During hyperglycemia, (mean plasma glucose level 30.0 mM) epinephrine and norepinephrine rose only 119 and 104%, respectively. The catecholamine increase in the hyperglycemic rats was significantly attenuated in comparison to euglycemic controls (epinephrine, $P = 0.0232$; norepinephrine, $P = 0.0079$). These data demonstrate that acute hyperglycemia has the capacity to suppress the normal adrenal medullary response to hypoxemia. Diabetes 43:645-648, 1994

**RESEARCH DESIGN AND METHODS**

Twelve virgin female Sprague-Dawley rats weighing 250-300 g (Charles River, Kingston, NY) were used as experimental animals. The rats were housed in an animal facility approved by the American Association for Accreditation of Laboratory Animal Care with constant temperature and 12-h light/dark cycles. Purina Rat Chow and water were fed ad libitum before the experiments. The rats were anesthetized with sodium barbital, 80 mg/kg intraperitoneally (Steris Laboratories, Phoenix, AZ). A rectal thermistor probe was inserted and connected to an animal temperature controller (CMA/150, Carnegie Medicin, Stockholm, Sweden) that maintained core temperature during the experiment at 37.5-38.0°C by regulating a heating pad placed under the rat. Polyvinyl catheters were placed into the left femoral vein and the left jugular vein. The trachea was cannulated, and the rat was ventilated with a tidal volume of 1.5 ml at a rate of 100/min with a volume-controlled rodent respirator (model 683, Harvard Bioscience, South Natick, MA). The left adrenal gland was identified through a subcostal incision, and a CMA-12 microdialysis probe (Carnegie Medicin) with a 2-mm membrane was inserted into it. The probe was perfused with heparinized Ringer’s solution delivered by a Carnegie Medicin CMA/100 microinjection pump at a constant rate of 0.5 μl/min. The probe effluent was collected by a CMA/140 microfraction collector into glass tubes containing sufficient perchloric acid to result in a final concentration of 0.1 M in the total collection volume.

**Biochemical methods.** Immediately after collection the effluent was injected directly into a high-performance liquid chromatographic system with an amperometric detector (BAS 200A, Bioanalytical Systems, West Lafayette, IN). The detector potential was set at 0.65 mV with a gain of 10 nA/V. A 100 x 3.2 mm BAS phase II ODS 3 μ column was used. The content of norepinephrine and epinephrine in injected microdialysis fluid was determined by peak area calculation on a chromatographic integrating data processor (CR501 Chromatopac, Shimadzu, Kyoto, Japan) with comparison to external standards. The mobile phase consisted of monochloroacetic acid (0.076 M), disodium EDTA (0.5 mM), sodium octyl sulfate (1.0 mM), and acetonitrile (1-2%) at pH 3.0, which was delivered at a flow rate of 1.0 ml/min. In all instances catecholamine concentrations were well above the detection threshold for this system, which was 0.08 pmol of epinephrine and 0.09 pmol of norepinephrine in a 20-μl injectate. The inter- and intraassay coefficients of variation were <10%. Plasma glucose was measured with a hexokinase method (procedure 115, Sigma, St. Louis, MO).

**Experimental protocol.** The adrenal probe was perfused continuously at 5.0 μl/min after insertion, and dialysate samples were collected every 5 min until the end of the experiment. Initially high levels of dialyzed catecholamines dropped rapidly and stabilized after about 2–3 h. Once stable baseline concentrations were present for at least 30 min, the experiment was started. During the first two samples, the rat was ventilated with room air. The inspired gas mixture was changed to 7.8% O₂ (balance nitrogen) for 15 min and then returned to room air for another 15 min, when the experiment was terminated. A 0.5-ml blood sample for plasma glucose determination was obtained during the control period and after 10 min of hypoxia. After each blood sample was withdrawn, an equal volume of warmed heparinized (1:10,000) Ringer’s solution was injected into the femoral vein catheter.
In 6 rats, hyperglycemia was induced at the same time as low oxygen breathing by infusing a 50% glucose solution into the jugular vein catheter by an electronically controlled pump (model 940, Harvard Bioscience) at a rate of 0.30 mmol/min for 0.75 min, then 0.15 mmol/min for 10 min, and finally 0.06 mmol/min until air breathing was reinstituted. This approach had been determined in some preliminary studies to result in a rapid rise of blood glucose to sustained levels of ~30 mM. The 6 control rats were infused with Ringer’s solution at the same rates.

**Data analysis.** All data are presented as means ± SE. Differences in the response of the euglycemic and hyperglycemic groups to hypoxia were sought with one-way (two-sample) repeated measures analysis of variance (ANOVA), with time the within factor, and catecholamine concentrations the dependent variables (SuperANOVA, Abacus Concepts, Berkeley, CA) (9). Our primary interest was to determine whether a difference occurred between the two glycemia groups in the degree of rise of catecholamines during hypoxemia. Therefore, we subtracted the mean of the two baseline values from each of the four stimulated values and analyzed these differences. In addition, we analyzed the raw data (two baseline and four hypoxic values) using the same repeated measures analysis. Differences between groups at individual time points were determined with the Student-Newman-Keuls test. The Huynh-Feldt e-factor for degree of freedom adjustment was used in the ANOVA.

**RESULTS**
The catecholamine data for the baseline samples and the first four samples after the onset of hypoxia are shown in Table 1. The mean plasma glucose level was not altered 10 min after onset of hypoxia in the euglycemic rats, although it did increase somewhat in 3 of the 6 rats. In the experimental group, glucose levels were increased about fourfold by the glucose infusion. In the euglycemic rats, dialyzable epinephrine and norepinephrine rose in response to hypoxia, achieving respective peak increases ~650 and 320% above baseline. Catecholamine concentrations in the dialysate also increased in the hyperglycemic rats, but peak increases were only 119 and 104% above baseline for epinephrine and norepinephrine, respectively. Levels of catecholamines in both groups decreased after the hypoxic stimulus was removed. The ANOVA for the analysis of the increases in catecholamines over baseline values indicated that epinephrine increased significantly in response to hypoxia in both hyperglycemic and euglycemic groups ($F = 4.70; P = 0.0083$), but a significant difference was observed in the magnitude of the epinephrine response between the two groups that depended on the collection time ($F = 3.66; P = 0.0232$). The group differences for norepinephrine were similar. Norepinephrine rose in both groups ($F = 13.19; P = 0.0001$), and a significant group × time interaction occurred ($F = 4.76; P = 0.0079$). When the raw data were analyzed, epinephrine again showed a significantly greater response to hypoxia in the euglycemic rats than in the hyperglycemic rats ($F = 7.90; P = 0.0184$). Norepinephrine showed a similar trend that was not statistically significant ($F = 1.38; P = 0.2667$). The percentage of change in catecholamine values in the two groups is demonstrated in Fig. 1.

**FIG. 1.** Percentage of baseline epinephrine (○, ●) and norepinephrine (□, ■) microdialysate concentrations in euglycemic (○, □) and hyperglycemic (●, ■) rats. Hypoxia induced a significant rise in catecholamines in both groups. The percentage of increase of both catecholamines in the hyperglycemic animals was significantly lower than in the euglycemic animals at 10, 15, and 20 min after the onset of low oxygen breathing.

**DISCUSSION**
Most studies of the mechanism of adrenal catecholamine secretion in response to glycemic changes (1-4,10,11) have not addressed the possibility that elevated glucose levels might modify epinephrine secretion. Our data demonstrate that acute hyperglycemia is associated with the suppression of adrenal catecholamine release during hypoxemia. The technique of microdialysis involves insertion into tissue of a continuously perfused thin probe tipped with a polycarbonate ether dialysis membrane. Compounds below the molecular weight threshold of the membrane (20,000 D in our system) diffuse from interstitial fluid across the membrane into the perfusate along a concentration gradient. High levels of measured substances are seen initially, the consequence of cellular injury from probe insertion. These generally fall rapidly and a stable uniform rate of diffusion is established in the undisturbed tissue. The technique has

**TABLE 1**
Catecholamine concentrations in serial dialysate samples during baseline and hypoxic periods

<table>
<thead>
<tr>
<th>Sample</th>
<th>Baseline 1</th>
<th>Baseline 2</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euglycemia ($n = 6$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine (pM)</td>
<td>139 ± 53</td>
<td>156 ± 68</td>
<td>240 ± 106</td>
<td>756 ± 466</td>
<td>1101 ± 525</td>
<td>1114 ± 432</td>
</tr>
<tr>
<td>Norepinephrine (pM)</td>
<td>47 ± 14</td>
<td>45 ± 12</td>
<td>50 ± 13</td>
<td>113 ± 36</td>
<td>170 ± 50</td>
<td>195 ± 48</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>—</td>
<td>7.5 ± 0.5</td>
<td>—</td>
<td>7.4 ± 0.8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hyperglycemia ($n = 6$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epinephrine (pM)</td>
<td>63 ± 17</td>
<td>70 ± 20</td>
<td>73 ± 20*</td>
<td>75 ± 21*</td>
<td>111 ± 27*</td>
<td>138 ± 30*</td>
</tr>
<tr>
<td>Norepinephrine (pM)</td>
<td>38 ± 13</td>
<td>39 ± 15</td>
<td>42 ± 16</td>
<td>50 ± 17</td>
<td>66 ± 24</td>
<td>79 ± 28</td>
</tr>
<tr>
<td>Plasma glucose (mM)</td>
<td>—</td>
<td>7.5 ± 0.6</td>
<td>—</td>
<td>30.0 ± 0.8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SE. Each sample represents a 5-min period of microdialysis probe perfusion. Hypoxia was induced after the second baseline sample. * Significantly different from euglycemic rats at corresponding time period.
been used successfully to study catecholamine activity in the CNS and in peripheral tissues, including the adrenal gland (12-15).

The approach is well suited to assessment of the adrenal medulla, the secretions of which are released by exocytosis into the extracellular space, from which they are readily dialyzed. Adrenal microdialysis is thus a technique that reflects adrenal medullary function directly and, like direct sampling of adrenal venous blood, has the advantage over peripheral blood sampling that dialysate catecholamines are not admixed with those released by peripheral sympathetic nerves. Also, microdialysis detects short-term changes in adrenal function that might be missed in individual plasma samples or in 24-h urinary analyses.

Our application of a microdialysis technique to in vivo study of the adrenal gland revealed a dramatic increase in adrenal epinephrine and norepinephrine analyte in response to hypoxia in euglycemic rats. Epinephrine was quantitatively the predominant catecholamine during baseline and hypoxic periods. Epinephrine increased more rapidly and reached higher relative and absolute levels than did norepinephrine. This pattern of adrenal catecholamine response to hypoxia is consistent with that observed by direct measurement of adrenal secretory rates (16). We chose 15 min of an ~8% inspired oxygen fraction because this was shown in a recent study (15) to be a uniformly nonfatal stress that induced a reproducibly large adrenal catecholamine response in a similar experimental system. The continued increase at the 20-min sample, after the termination of the hypoxic stimulus, is accounted for by the time it takes for dialysate that exits the probe to arrive in the collection vials.

The adrenal catecholamine response to the same hypoxic stress was markedly different in the hyperglycemic rats. Although both epinephrine and norepinephrine levels rose during hypoxia in this group, the increase was subdued markedly in comparison with the euglycemic rats. The mean peak increase over baseline values in epinephrine was ~500% lower and in norepinephrine 200% lower in the glucose infusion group compared with the control group.

Nijima (5,17,18) proposed that a neurally mediated feedback loop exists that contributes to the regulation of blood glucose by altering firing frequency in adrenal branches of the splanchnic nerve in response to a broad spectrum of blood glucose levels. He demonstrated that central hypoglycemia increased and hyperglycemia suppressed adrenal nerve activity. Cohn et al. (8) reported that plasma catecholamine concentrations increased in euglycemic and hyperglycemic fetal monkeys in response to similar degrees of hypoxemia; however, a trend was found to a more muted catecholamine response in the hyperglycemic fetuses. Santiago et al. (4) showed that plasma epinephrine increased when blood glucose was decreased from hyperglycemic (11.1 mM) to euglycemic (5.6 mM) levels in men. A greater epinephrine increase occurred with a fall from normal to hypoglycemic concentrations.

Although these findings may be interpreted to reflect a spectrum of adrenal counterregulatory responses to decreasing blood glucose levels, no obvious homeostatic advantage to maintain supraphysiologic levels is apparent under most circumstances. The rise in plasma epinephrine when the glucose fell from high to normal may have represented release of suppression, rather than stimulation. Indeed, the resting plasma epinephrine levels found by Santiago et al. (4) in hyperglycemic men were lower than those in euglycemic men. These findings are consistent with the possibility that a high blood glucose level might actively suppress epinephrine release. Other observations made in humans have suggested the potential for hyperglycemia to alter adrenal epinephrine release. Trunet et al. (6) found a significant decrease in plasma epinephrine, but not norepinephrine, after hyperglycemia induced by ingestion of 100 g of glucose. Similar observations were made by Schwartz et al. (7) in response to a high carbohydrate meal, and Kleinbaum and Shamoong (19) found a slight drop in plasma epinephrine during the peak of glycemia after 75 g of oral glucose. However, neither Tse et al. (20) nor Welle et al. (21) found suppression of plasma epinephrine after 75 or 100 g of oral glucose, respectively.

Our data were obtained in barbiturate-anesthetized animals, and the possibility that the anesthetic influenced results requires consideration. Both Joyce et al. (22) and Russell et al. (23) found no changes in plasma epinephrine during barbiturate induction of anesthesia in humans, suggesting that thiopental and pentobarbital had no acute effect on the adrenal medulla. Pentobarbital anesthesia also did not alter plasma epinephrine levels in rats (24). Other investigators showed, however, that under certain conditions pentobarbital suppressed catecholamine release in the isolated cow adrenal (25) and that it blocked the epinephrine-releasing effects of a serotonin receptor agonist (26). Also, anesthesia and sympathetic nervous system responses to surgical trauma may alter insulin-glucose homeostasis (25,27). We cannot rule out effects of drugs or surgical stress in our experiments, but we think it unlikely that the observed adrenal suppression was a consequence of anesthesia or surgery. This is because the medullary responses of the euglycemic hypoxic animals, which received the same quantity of anesthesia as the hyperglycemic rats, were apparently not suppressed, because findings similar to our own were suggested in the unanesthetized fetus (8) and because hyperinsulinemia has not been demonstrated to suppress adrenal catecholamine release (28).

A number of physiological changes accompany acute hyperglycemia. These include increased insulin secretion, reduced hepatic glucose output, alterations in gastrointestinal peptide hormones, and secondary effects of insulin, such as increased amino acid uptake into muscle, stimulation of fatty acid synthesis, and changes in serum electrolytes. Our experimental design did not allow us to determine with certainty whether the observed suppression of adrenal catecholamine release was a direct consequence of the rise in blood glucose or was caused by another factor provoked by hyperglycemia.

We may conclude nevertheless that acute hyperglycemia is associated with an alteration in the adrenal response to superimposed hypoxemia in rats. We speculate that the presence of high blood glucose levels might impair the adaptive response to oxygen deprivation or other stresses that normally prompt exuberant adrenal catecholamine release. Our findings have implications with regard to understanding the physiology of glycemic control as well as the pathophysiology of hyperglycemic events. Glucose-mediated suppression of adrenal function could help explain some of the morbidity associated with adult hyperglycemic states such as diabetic ketoacidosis and hyperosmolar coma, as well as fetal morbidity during pregnancies in diabetic women with poor glycemic control.
REFERENCES