

Antecedent Short-Term Central Nervous System Administration of Estrogen and Progesterone Alters Counterregulatory Responses to Hypoglycemia in Conscious Male Rats

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Abstract

The aim of this study was to test the hypothesis that antecedent short-term administration of estradiol or progesterone into the central nervous system (CNS) reduces levels of neuroendocrine counterregulatory hormones during subsequent hypoglycemia. Conscious unrestrained male Sprague-Dawley rats were studied during randomized 2-day experiments. Day 1 consisted of an 8hr lateral ventricle infusion of estradiol (1 μ g/ μ l; n=9), progesterone (1 μ g/ μ l; n=9) or saline (0.2 μ l/min, n=10). On day 2, a 2hr hyperinsulinemic (30pmol/kg/min) hypoglycemic (2.9 \pm 0.2mM) clamp was performed on all rats. Central administration of estradiol on day 1 resulted in significantly lower plasma epinephrine levels during hypoglycemia compared to saline. Whereas central administration of progesterone resulted in increased levels of plasma norepinephrine and decreased levels of corticosterone both at baseline and during hypoglycemia. Glucagon responses during hypoglycemia were unaffected by prior administration of estradiol or progesterone. Endogenous glucose production following day 1 estradiol was significantly lower during day 2 hypoglycemia, and consequently, the glucose infusion rate to maintain the glycemia was significantly greater after estradiol administration compared to saline. These data suggest that 1) CNS administration of both female reproductive hormones can have rapid effects in modulating levels of counterregulatory hormones during subsequent hypoglycemia in conscious male rats, 2) forebrain administration of reproductive hormones can significantly reduce pituitary adrenal and sympathetic nervous system drive during hypoglycemia, 3) reproductive steroid hormones produce differential effects on sympathetic nervous system activity during hypoglycemia, and 4)

reduction of epinephrine resulted in significantly blunted metabolic counterregulatory responses during hypoglycemia.

Introduction

Women, compared to men, have reduced autonomic responses to psychological stress (22), exercise (8, 19, 40) and hypoglycemia (14). We have previously shown that women taking estrogen replacement therapy had reduced autonomic counterregulatory activity to insulin induced hypoglycemia compared to age and BMI matched men and women not taking estrogen replacement therapy (35). Thus, suggesting that chronic elevations in estradiol may be an important mechanism for sexually dimorphic responses to stress.

Previous studies have demonstrated that estrogen can have rapid, biologic effects in the brain and periphery (15, 25, 31, 41). Whether these rapid effects are mediated through the classical estrogen receptors (estrogen receptor α and/or β) or non-genomic mechanisms is not understood. In vitro estradiol increases intracellular signaling within minutes (23). In vivo, both centrally and peripherally administered estrogen increases intracellular signaling within the hypothalamus at 6 and 24h after injection. These signaling changes may mediate a variety of physiological changes. Acute short-term (within 2h) estradiol treatment, similar to administration for 2weeks, significantly increased neurological dysfunction during insulin bolus induced hypoglycemia in female ovariectomized rats (1, 42). Glycogen content of cardiac muscle after exhaustive exercise was similarly preserved following 1hr or 6 days of E2 treatment in male rats (1, 20, 42). Local microinjection of estrogen into the insular cortex reduced renal sympathetic nerve activity in response to middle cerebral artery occlusion within 10min (33). Although much needs to be elucidated regarding the signaling pathways that mediate these effects, it is clear that estradiol can have short-term peripheral and central

affects on responses to differing types of stress. However it remains unknown whether short-term elevations of estradiol or progesterone, another important female sex steroid, (that has been found to have opposite effects on estrogen's ability to alter fat and carbohydrate metabolism (4-6, 16), can affect neuroendocrine or ANS counterregulatory drive during hypoglycemia. Thus, the aim of this study was: 1) to determine the role of short-term CNS elevations of estradiol and progesterone on counterregulatory activity during subsequent clamped moderate hypoglycemia in conscious, unrestrained rats.

Research Design and Methods

Animals. Twenty-nine male Sprague-Dawley rats (300-350g) bred and purchased from Harlan (Indianapolis, IN) were studied. The rats were housed and individually caged in the Vanderbilt University Animal Care Facility under controlled conditions (12:12 light-dark cycle, 50-60% humidity, 25°C) with free access to food and water. All procedures for animal use were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Animal Preparation. At least one week prior to each study, each rat had catheters placed in the carotid artery (for blood sampling) and the external jugular vein (for infusions) under a general anesthesia mixture (5mg/kg acepromazine, 10mg/kg xylazine, and 50mg/kg ketamine). Catheter lines were kept patent by flushing with 150U/ml of heparin. Immediately after the catheter placements, rats were placed on a stereotaxic frame (KOPF Instruments, Tujunga CA) for placement of a 6mm stainless steel guide cannula at stereotaxis coordinates corresponding to the lateral cerebral ventricle (-0.9mm anteroposterior, +1.4mm mediolateral, and -4.5 dorsoventral from bregma according to the atlas of Paxinos and Watson (27). The intracranial cannulae were held in place with

cranioplastic cement and three skull screws. Rats had free access to rat chow the days prior to surgery and experiments. Seven days post-surgery, only rats with greater than 90% of their pre-surgery body weight were used for the 2-day experiments.

Experimental Design. Three groups of male rats were studied during a 2-day experimental protocol. Day 1 consisted of lateral cerebral ventricular infusion of estrogen (1mg/ml; E2; n=9), progesterone (1mg/ml; P4; n=9) or saline (SAL; n=10). Water soluble estrogen and progesterone (0.01g, Sigma, St. Louis, MO) was diluted with 10 ml of 0.9% saline and infused at a rate of 0.06 mg/h during the morning and afternoon glycemic clamps. Using saline avoids any potential confounding side effects of the dissolving medium. On day 2, all rats were exposed to a 2h hyperinsulinemic hypoglycemic clamp. Rats were fasted overnight prior to each day of the two-day studies and remained conscious and unrestrained throughout the experimental protocols. To prevent a fall in hematocrit, after each blood draw, washed red blood cells plus normal saline were re-infused through the carotid cannula of the rat. The morning of the study, extensions were placed on the exteriorized catheters for ease of access and were removed between day 1 and day 2 studies.

Day 1 procedures. At time 0 min, rats were moved to the experimental cage and the ICV infusion of saline, estrogen, or progesterone was started. Plasma measurements of glucose were taken at time 0, 240, 360, and 480 minutes of ICV infusion. At the conclusion of Day 1 procedures, rats were fed 5-8g of rat chow.

Day 2 procedures. At time 0 min, rats were moved to an experimental cage and allowed to acclimate to the surroundings. The experiment consisted of a basal period (time 90-120) and an experimental period (time 120-240 min) during which a hyperinsulinemic

hypoglycemic clamp (described below) was performed. To measure glucose kinetics during the clamp a primed, (10 μ Ci) constant (0.1 μ Ci/min) infusion of HPLC purified [3-³H] glucose (Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) at time 0 min and continued through 240min. During the experimental period, blood was drawn every 5-15 minutes for measurements of plasma glucose, every 10 minutes during the basal period and every 15 minutes during the experimental periods for 3-³H-glucose, and at time 110, 120, 180, 210, and 240 for counterregulatory hormones. Rats were euthanized after day 2 procedures and placement of ICV (by injection of cresyl violet), carotid, and jugular cannulae was verified.

Glycemic Clamping Procedures. On day 2, from time 120-240min, a primed (60pmol/kg/min) continuous (30pmol/kg/min) infusion of insulin (Eli Lilly, Indianapolis, IN) containing 9.7% (vol/vol) of rat plasma was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA). After the start of insulin, glucose levels were allowed to fall (reaching nadir in ~30minutes) and a 20% dextrose infusion was adjusted to maintain glucose at ~2.9mM for 90 minutes.

Tracer Calculations. Rates of glucose appearance (Ra), EGP, and glucose utilization were calculated according to the methods of Wall et al. (43) and as described previously (37).

Analytical Methods. Plasma glucose was measured in duplicate by the glucose oxidase technique on a Beckman Glucose analyzer. Catecholamines were determined by high-pressure liquid chromatography (HPLC) with an interassay CV of 12% for both epinephrine and norepinephrine as described previously (36). Corticosterone (ICN

Biomedicals Inc, Irvine, CA; interassay CV=7%), insulin (interassay CV=11%) and glucagon (Linco Research Inc, St. Louis, MO; interassay CV=15%) were all measured using radioimmunoassay techniques described previously (36).

Statistical Analysis. Data are expressed as mean±SE and was analyzed using standard, parametric, two-way analysis of variance (ANOVA) and with repeated measures where appropriate. A Tukey's post hoc analysis was used to delineate statistical significance. A p value of ≤0.05 was accepted as statistical significance.

Results

Glucose and Insulin

All rats remained euglycemic during day 1 LV infusions (6.5 ± 0.1 mmol/l) and did not change from baseline glucose levels. Glucose (2.9 ± 0.1 mmol/l) and insulin (50 ± 8 at baseline and 945 ± 61 pmol/l) levels were also similar among all groups during day 2 hypoglycemic clamps (Figure 1).

Counterregulatory Hormones

Plasma norepinephrine levels were significantly greater at baseline and during the final 30 minutes of hypoglycemia in the P4 group compared to both SAL and E2 (0.5 ± 0.05 vs. 0.3 ± 0.06 , and 0.2 ± 0.02 at basal and 0.7 ± 0.05 vs. 0.5 ± 0.07 , and 0.4 ± 0.08 nmol·l⁻¹, for P4 vs. SAL and E2 respectively; $p < 0.05$; Figure 2). Basal levels of Day 2 plasma epinephrine were similar between the groups, (0.2 ± 0.03 , 0.2 ± 0.03 and 0.2 ± 0.05 for SAL, E2, and P4 groups, respectively). However, plasma epinephrine levels were significantly lower in E2 vs. both SAL and P4 at 210 and 240 minutes of hypoglycemia ($p < 0.05$; Figure 2). Plasma glucagon levels at baseline and during hypoglycemia were similar among the three groups across all time points (Figure 3). Plasma corticosterone

levels were reduced at baseline (2 ± 1 vs. 5 ± 2 , and 8 ± 2 $\text{nmol}\cdot\text{l}^{-1}$; $p<0.05$; Figure 3) and during the final 30min of day 2 hypoglycemia (15 ± 1 vs. 21 ± 2 , 22 ± 1 , $\text{nmol}\cdot\text{l}^{-1}$; $p<0.05$; Figure 3) in P4 vs. both SAL and E2 ($p<0.05$; Figure 3).

Glucose Kinetics

Specific activity, listed in Table 1, was stable and not statistically different between groups during the basal and final 30 minutes of the hyperinsulinemic hypoglycemic clamps in all groups with an average CV of $8\pm 1\%$ for both periods. During the final 30 minutes of day 2 hypoglycemia, EGP in the E2 group was significantly less than SAL (24 ± 5 vs. 36 ± 4 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $p<0.05$; Figure 4), but neither group differed from P4 (28 ± 4 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Glucose rate of disappearance during the final 30 minutes of hypoglycemia was similar between all groups (Figure 5). The glucose infusion rate needed to maintain the glycemic level was significantly greater in E2 compared to SAL (31 ± 4 vs. 19 ± 4 , $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $p<0.05$; Figure 4).

Discussion

In the current study we have used unrestrained, conscious male rats to determine the short-term effects of CNS delivery of estradiol and progesterone on hormonal and metabolic responses to next day hypoglycemia. Our results demonstrate that estradiol in the CNS can rapidly induce blunting of epinephrine levels during next day hypoglycemia. These data are the first that we are aware of to demonstrate during clamped hypoglycemia in awake rats that estradiol administered centrally can rapidly mediate differences in sympatho-adrenal drive during hypoglycemia.

We have additionally determined the short-term effects of lateral ventricle infusion of progesterone (P4) in mediating differences in counterregulatory hormone

levels during hypoglycemia. We found that while E2 blunted epinephrine, and as a result EGP, P4 enhanced both basal and end of clamp norepinephrine levels. On the other hand, P4 blunted basal and end of clamp plasma levels of corticosterone. Therefore, the present results provide novel data that E2 and P4 can have rapid differential effects on key mechanistic pathways regulating counterregulatory hormone levels during hypoglycemia. Additionally, these findings support the concept that estrogen may be considered as a factor responsible for causing a form of hypoglycemia associated autonomic failure.

Previous studies in humans have demonstrated that women have blunted catecholamine, growth hormone, cortisol, and glucagon responses to hypoglycemia compared to men (2, 12, 14). We have also shown that post menopausal women taking E2 only replacement therapy have reduced epinephrine, glucagon and muscle sympathetic nerve activity compared to age and BMI matched women not taking E2 replacement therapy and to men (35). Adams, et al., have demonstrated that anesthetized 14-day, ovariectomized female rats had elevated epinephrine responses to insulin bolus induced hypoglycemia compared to non-ovariectomized controls. Conversely, epinephrine responses to hypoglycemia were significantly reduced in ovariectomized rats replaced with E2 (1). In the current study, short-term forebrain E2 infusion in male rats reduced epinephrine but not glucagon levels and forebrain P4 infusion blunted plasma corticosterone levels. Thus, our present data may explain why postmenopausal women only taking E2 replacement had preserved cortisol responses to hypoglycemia. Growth hormone was not measured due to a lack of increase during hypoglycemia in rats (unpublished observations). The lack of a signal to reduce glucagon in the present study

versus results in humans is interesting. One possibility is that the time course of the effects of E2 on plasma glucagon during hypoglycemia is different from that on sympatho-adrenal responses. In other words, more prolonged exposure to E2 is required to down regulate glucagon responses to hypoglycemia. On the other hand, glucagon responses to hypoglycemia have been shown to be regulated by both changes in insulin levels within the pancreatic islets (28) and direct autonomic nervous system regulation (18, 39) in rats and humans. Interestingly, E2 has also been shown to act directly on pancreatic alpha cells to reduce glucagon levels (31). Thus, E2 may act centrally to blunt epinephrine but peripherally to blunt glucagon levels during hypoglycemia.

E2 and P4 had differential affects on sympathetic nervous system drive during hypoglycemia. Lateral ventricle E2 blunted plasma epinephrine levels (sympatho-adrenal activation) but not plasma norepinephrine (sympatho-adrenal and sympathetic-neural activation), whereas lateral ventricle P4 increased norepinephrine levels but had no effect on epinephrine levels during hypoglycemia. The mechanism for these novel, divergent results with epinephrine and norepinephrine following the different steroid administration is unknown. It is well recognized that progesterone (P4) and estradiol (E2) can have opposite physiologic effects. For instance, P4 administration has been reported to reverse E2 mediated increases in insulin sensitivity (29, 38). Chronic (3wk) P4 plus E2 administration, resulted in enhanced counterregulatory responses to hypoglycemia in dogs (3). While one study has shown that the source of norepinephrine in humans is primarily adrenomedullary (11), data from animal models suggest that central regulation of sympathetic output may be more complicated and organ specific (21). For example, increased levels of norepinephrine have been found within the brain during

hypoglycemia, specifically in the paraventricular (9, 26) and ventromedial nuclei (10), and despite reduced systemic levels, these hypothalamic levels were not reduced after multiple daily episodes of hypoglycemia (9). The authors postulated that the mechanism for blunted sympatho-adrenal responses with repeated hypoglycemia occurs downstream from the hypothalamus. With regards to our data, it is possible that E2 and P4 alter sympathetic outflow in different areas of the brain leading to the divergent results. Thus, the elevated basal levels of norepinephrine following P4 administration are likely to be caused by increased sympathetic neural outflow, whereas the elevated norepinephrine levels during hypoglycemia were likely to be caused by both increased sympathetic neural and sympatho-adrenal activity.

The doses of E2 and P4 used in this present study are similar to previous reports (1, 3, 16). We do not know the volume of distribution of the E2 and P4 infused into the lateral ventricle of the brain. However, it is reasonable to assume that due to cerebrospinal fluid circulation, areas of the forebrain, midbrain and hindbrain could have been affected by the steroid action. These are all areas, specifically the paraventricular nucleus of the hypothalamus (13), the ventromedial hypothalamus (9, 17, 24) and the hindbrain (34), that have been implicated in regulating counterregulatory responses to hypoglycemia and contain E2 and P4 receptors. Central injections of E2 into hindbrain areas have been found to depress renal sympathetic drive and increase vagal nerve activity in male rats (30, 32). P4 receptors are also found in glucose sensing areas within the hypothalamus and hindbrain regions and within the pituitary (Haywood, 1999 #0). The decrease in corticosterone levels strongly suggests that P4 has effects on forebrain pathways to inhibit hypothalamo-pituitary adrenal drive. Several studies have

demonstrated the importance of hippocampal, thalamic and hypothalamic regulation of HPA axis responses to stress (26). However, the specific regions and receptors within the CNS that are responsible for the effects of E2 and P4 on counterregulatory responses to hypoglycemia remain unknown. Furthermore, we were unable to measure estradiol and progesterone levels in the plasma and cannot rule out potential peripheral actions of these sex steroids on counterregulatory hormones.

In summary, these data show that short-term (i.e. hrs) central administration of female reproductive hormones in male rats can have: 1) rapid significant effects on levels of ANS and neuroendocrine counterregulatory hormones during subsequent hypoglycemia, 2) differential regulation of plasma catecholamine levels during hypoglycemia, and 3) significantly blunted metabolic (glucose production) counterregulatory responses during subsequent hypoglycemia. The effect of E2 is consistent with data in humans suggesting that estradiol may be considered as a mechanism responsible for causing a partial subset of the condition known as hypoglycemia associated autonomic failure (7).

Acknowledgements

We thank Donna Tate, Eric Allen, Angelina Penaloza, Pam Venson, and Wanda Snead for their expert technical assistance.

This work was supported by a research grants from the NIH NRSA (DK065461-01), the National Institutes of Health (NHLBI;HL;5PO1 HL056693-10 and NIH/NIDDK; 5RO1 DK069803-03), and a Diabetes Research and Training Grant (5P60-DK020593-28).

References

1. **Adams JM, Legan SJ, Ott CE, and Jackson BA.** Modulation of hypoglycemia-induced increases in plasma epinephrine by estrogen in the female rat. *J Neurosci Res* 79: 360-367, 2005.
2. **Amiel SA, Maran A, Powrie JK, Umpleby AM, and Macdonald IA.** Gender differences in counterregulation to hypoglycemia. *Diabetologia* 36: 460-464, 1993.
3. **Batista MR, Smith MS, Snead WL, Connolly CC, Lacy DB, and Moore MC.** Chronic estradiol and progesterone treatment in conscious dogs: effects on insulin sensitivity and response to hypoglycemia. *Am J Physiol Regul Integr Comp Physiol* 289: R1064-1073, 2005.
4. **Campbell SE and Febbraio M.** Effect of ovarian hormones on mitochondrial enzyme activity in the fat oxidation pathway of skeletal muscle. *Am J Physiol Endocrinol Metab* 281: E803-E808, 2001.
5. **Campbell SE and Febbraio MA.** Effect of ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake. *Am J Physiol Endocrinol Metab* 282: E1139-E1146, 2001.
6. **Carrington LJ and Bailey CJ.** Effects of natural and synthetic estrogens and progestins on glycogen deposition in female mice. *Horm Res* 21: 199-203, 1985.
7. **Cryer PE.** Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes* 54: 3592-3601, 2005.
8. **Davis SN, Galassetti P, Wasserman DH, and Tate D.** Effect of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. *J Clin Endocrinol Metab* 85: 224-230, 2000.
9. **de Vries MG, Lawson MA, and Beverly JL.** Dissociation of hypothalamic noradrenergic activity and sympathoadrenal responses to recurrent hypoglycemia. *Am J Physiol Regul Integr Comp Physiol* 286: R910-915, 2004.
10. **de Vries MG, Lawson MA, and Beverly JL.** Hypoglycemia-induced noradrenergic activation in the VMH is a result of decreased ambient glucose. *Am J Physiol Regul Integr Comp Physiol* 289: R977-981, 2005.
11. **DeRosa MA and Cryer PE.** Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation. *Am J Physiol Endocrinol Metab* 287: E32-41, 2004.
12. **Diamond MP, Grainger DA, Rossi G, Connolly-Diamond M, and Sherwin RS.** Counter-regulatory response to hypoglycemia in the follicular and luteal phases of the menstrual cycle. *Fertil Steril* 60: 988-993, 1993.
13. **Evans SB, Wilkinson CW, Gronbeck P, Bennett JL, Taborsky GJ, Jr., and Figlewicz DP.** Inactivation of the PVN during hypoglycemia partially simulates hypoglycemia-associated autonomic failure. *Am J Physiol Regul Integr Comp Physiol* 284: R57-65, 2003.
14. **Galassetti P, Neill RA, Tate D, Ertl AC, Wasserman DH, and Davis SN.** Sexual dimorphism in counterregulatory responses to hypoglycemia after antecedent exercise. *J Clin Endocrinol Metab* 86: 3516-3524, 2001.
15. **Garcia-Segura LM, Olmos G, Tranque P, and Naftolin F.** Rapid effects of gonadal steroids upon hypothalamic neuronal membrane ultrastructure. *J Steroid Biochem* 27: 615-623, 1987.

16. **Hansen FM, Fahmy N, and Nielsen JH.** The influence of sexual hormones on lipogenesis and lipolysis in rat fat cells. *Acta Endocrinol (Copenh)* 95: 566-570, 1980.
17. **Hasselbach SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, and Paulson OB.** No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. *Diabetes* 48: 1915-1921, 1999.
18. **Havel PJ and Ahren B.** Activation of autonomic nerves and the adrenal medulla contributes to increased glucagon secretion during moderate insulin-induced hypoglycemia in women
1. *Diabetes* 46: 801-807, 1997.
19. **Horton TJ, Gayles EC, Prach PA, Koppenhafer TA, and Pagliassotti MJ.** Female rats do not develop sucrose-induced insulin resistance. *Am J Physiol* 272: R1571-1576, 1997.
20. **Kendrick ZV.** Effect of estradiol on tissue glycogen metabolism and lipid availability in exercised male rats. *J Appl Physiol* 71: 1694-1699, 1991.
21. **Kreier F, Kap YS, Mettenleiter TC, van Heijningen C, van der Vliet J, Kalsbeek A, Sauerwein HP, Fliers E, Romijn JA, and Buijs RM.** Tracing from fat tissue, liver, and pancreas: a neuroanatomical framework for the role of the brain in type 2 diabetes. *Endocrinology* 147: 1140-1147, 2006.
22. **Litschauer B, Zauchner S, Huemer KH, and Kafka-Lutzow A.** Cardiovascular, endocrine, and receptor measures as related to sex and menstrual cycle phase. *Psychosom Med* 60: 219-226, 1998.
23. **Malyala A, Kelly MJ, and Ronnekleiv OK.** Estrogen modulation of hypothalamic neurons: activation of multiple signaling pathways and gene expression changes. *Steroids* 70: 397-406, 2005.
24. **McCrimmon RJ, Fan X, Ding Y, Zhu W, Jacob RJ, and Sherwin RS.** Potential role for AMP-activated protein kinase in hypoglycemia sensing in the ventromedial hypothalamus. *Diabetes* 53: 1953-1958, 2004.
25. **McEwen BS.** Non-genomic and genomic effects of steroids on neural activity. *Trends Pharmacol Sci* 12: 141-147, 1991.
26. **Pacak K, Palkovits M, Kvetnansky R, Yadid G, Kopin IJ, and Goldstein DS.** Effects of various stressors on in vivo norepinephrine release in the hypothalamic paraventricular nucleus and on the pituitary-adrenocortical axis. *Ann N Y Acad Sci* 771: 115-130, 1995.
27. **Paxinos G and Watson C.** *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press, Inc, 1986.
28. **Raju B and Cryer PE.** Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes* 54: 757-764, 2005.
29. **Raudaskoski T, Tomas C, and Laatikainen T.** Insulin sensitivity during postmenopausal hormone replacement with transdermal estradiol and intrauterine levonorgestrel. *Acta Obstet Gynecol Scand* 78: 540-545, 1999.
30. **Ronnekleiv OK and Kelly MJ.** Diversity of ovarian steroid signaling in the hypothalamus. *Front Neuroendocrinol* 26: 65-84, 2005.

31. **Ropero AB, Soria B, and Nadal A.** A nonclassical estrogen membrane receptor triggers rapid differential actions in the endocrine pancreas. *Mol Endocrinol* 16: 497-505, 2002.
32. **Saleh MC, Connell BJ, and Saleh TM.** Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res* 879: 105-114, 2000.
33. **Saleh TM, Connell BJ, and Cribb AE.** Estrogen in the parabrachial nucleus attenuates the sympathoexcitation following MCAO in male rats. *Brain Res* 1066: 187-195, 2005.
34. **Sanders NM and Ritter S.** Repeated 2-deoxy-D-glucose-induced glucoprivation attenuates Fos expression and glucoregulatory responses during subsequent glucoprivation. *Diabetes* 49: 1865-1874, 2000.
35. **Sandoval DA, Ertl AC, Richardson MA, Tate DB, and Davis SN.** Estrogen blunts neuroendocrine and metabolic responses to hypoglycemia. *Diabetes* 52: 1749-1755, 2003.
36. **Sandoval DA, Ping L, Neill RA, Gong B, Walsh K, and Davis SN.** Brain region-dependent effects of dexamethasone on counterregulatory responses to hypoglycemia in conscious rats. *Am J Physiol Regul Integr Comp Physiol* 288: R413-419, 2005.
37. **Sandoval DA, Ping L, Neill RA, Morrey S, and Davis SN.** The effects of dehydroepiandrosterone sulfate on counterregulatory responses during repeated hypoglycemia in conscious normal rats. *Diabetes* 53: 679-686, 2004.
38. **Sites CK, L'Hommedieu GD, Toth MJ, Brochu M, Cooper BC, and Fairhurst PA.** The effect of hormone replacement therapy on body composition, body fat distribution, and insulin sensitivity in menopausal women: a randomized, double-blind, placebo-controlled trial. *J Clin Endocrinol Metab* 90: 2701-2707, 2005.
39. **Taborsky GJ, Jr., Ahren B, and Havel PJ.** Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired alpha-cell responses in type 1 diabetes. *Diabetes* 47: 995-1005, 1998.
40. **Tarnopolsky L, MacDougall S, Atkinson S, Tarnopolski M, and Sutton J.** Gender differences in substrate for endurance exercise. *J Appl Physiol* 68: 302-308, 1990.
41. **Toran-Allerand CD, Singh M, and Setalo G, Jr.** Novel mechanisms of estrogen action in the brain: new players in an old story. *Front Neuroendocrinol* 20: 97-121, 1999.
42. **Verma S, Srivastava RK, Sood S, and Sharma S.** Effect of estrogen on hypoglycemia-induced neurological impairment in ovariectomized rats. *Methods Find Exp Clin Pharmacol* 27: 405-409, 2005.
43. **Wall JS, Steele R, DeBodo RD, and Altszuler N.** Effect of insulin on utilization and production of circulating glucose. *Am J Physiol* 189: 43-50, 1957.

Table 1. Glucose specific activity (dpm/mmol) at baseline and final 30min during day 2 hyperinsulinemic hypoglycemia (2.8 ± 0.1 mmol) in conscious rats.

Group	Time (min)					
	100	110	120	210	225	240
SAL	650 \pm 183	649 \pm 178	631 \pm 165	1047 \pm 126	1057 \pm 72	1026 \pm 89
E2	1139 \pm 200	1175 \pm 195	1045 \pm 177	1020 \pm 208	943 \pm 175	973 \pm 53
P4	708 \pm 87	617 \pm 94	611 \pm 52	818 \pm 81	745 \pm 98	733 \pm 122

Figure 1. Plasma glucose and insulin levels during day 2 exposure to hyperinsulinemic hypoglycemia after either antecedent ICV infusion of saline (SAL), estradiol (E2), or progesterone (P4).

Figure 2. Plasma norepinephrine and epinephrine responses to day 2 hyperinsulinemic hypoglycemia after either antecedent ICV infusion of saline (SAL), estradiol (E2), or progesterone (P4) in conscious, unrestrained rats. * $p < 0.05$ vs. SAL and P4. Norepinephrine levels were significantly elevated across all time points in P4 vs. both SAL and E2 (main effect, group; $p < 0.05$).

Figure 3. Plasma glucagon and corticosterone responses to day 2 hyperinsulinemic hypoglycemia after either antecedent ICV infusion of saline (SAL), estradiol (E2), or progesterone (P4) in conscious, unrestrained rats. * $p < 0.05$ vs. SAL and E2. Corticosterone levels were significantly decreased across all time points in P4 vs. both SAL and E2 (main effect, group; $p < 0.05$).

Figure 4. Glucose infusion rate, glucose rate of disappearance, and endogenous glucose production during the final 30 minutes of day 2 hyperinsulinemic hypoglycemia after either antecedent ICV infusion of saline (SAL), estradiol (E2), or progesterone (P4) in conscious, unrestrained rats. * $p < 0.05$ vs. SAL.

Figure 1

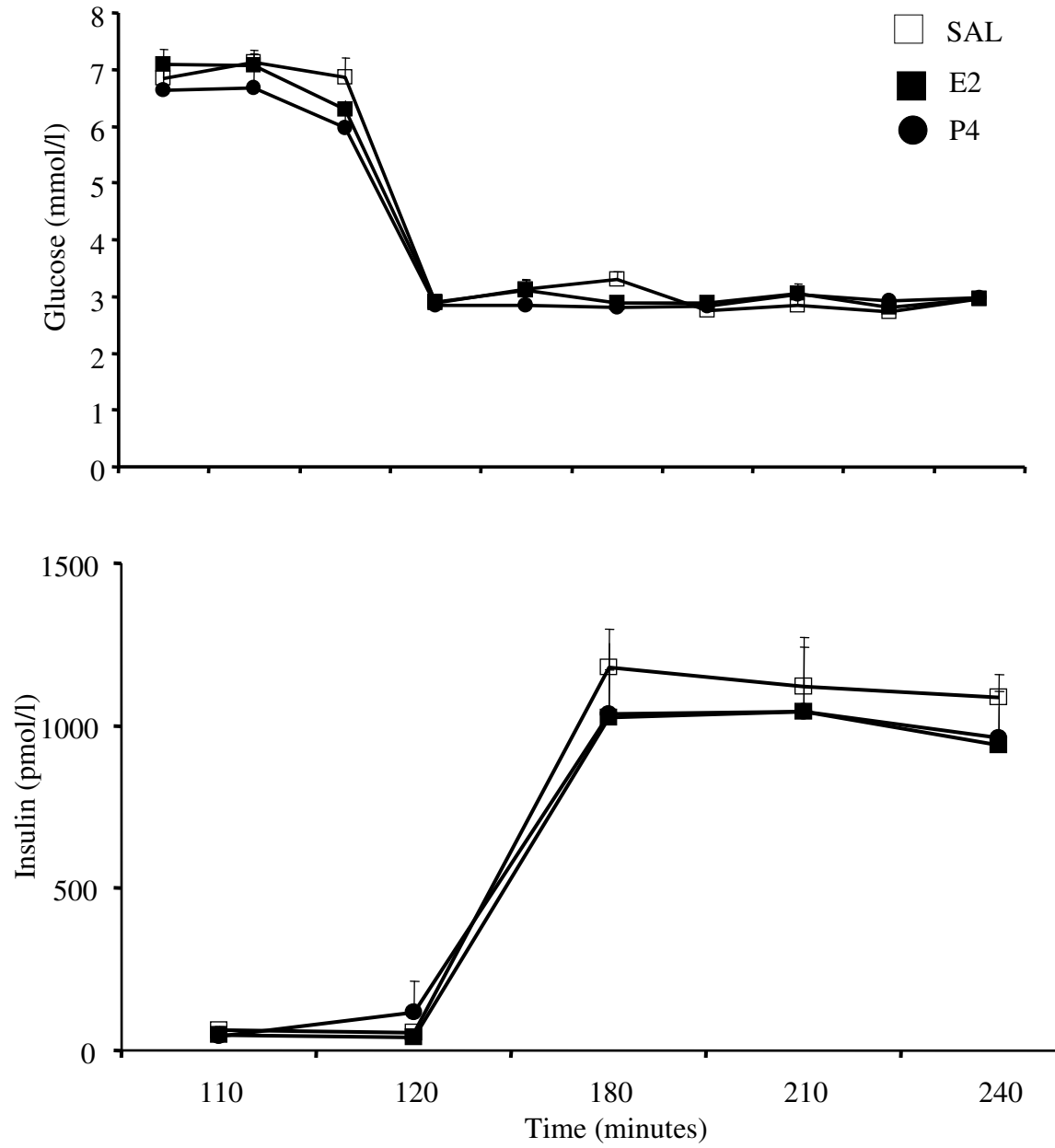


Figure 2

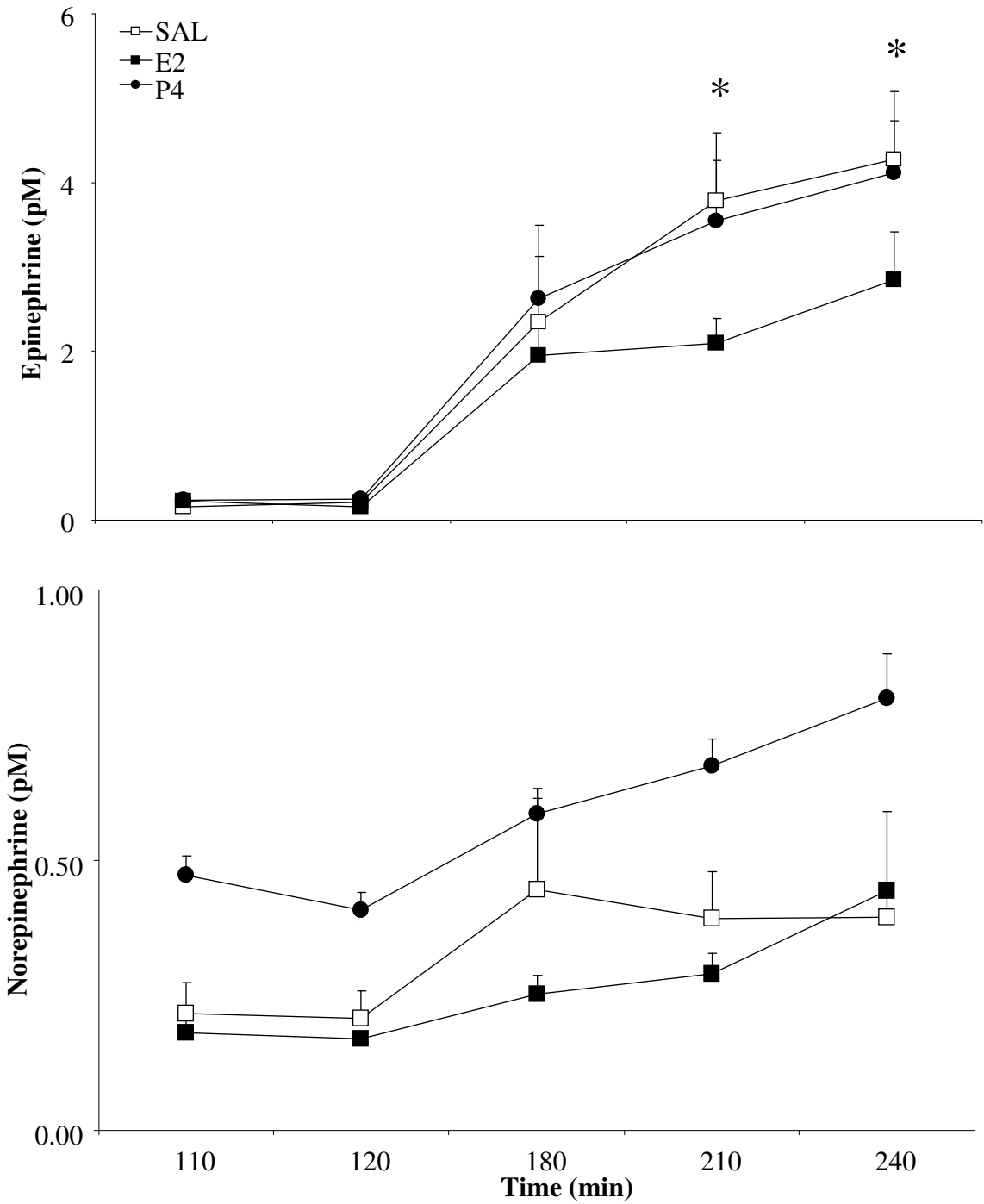


Figure 3

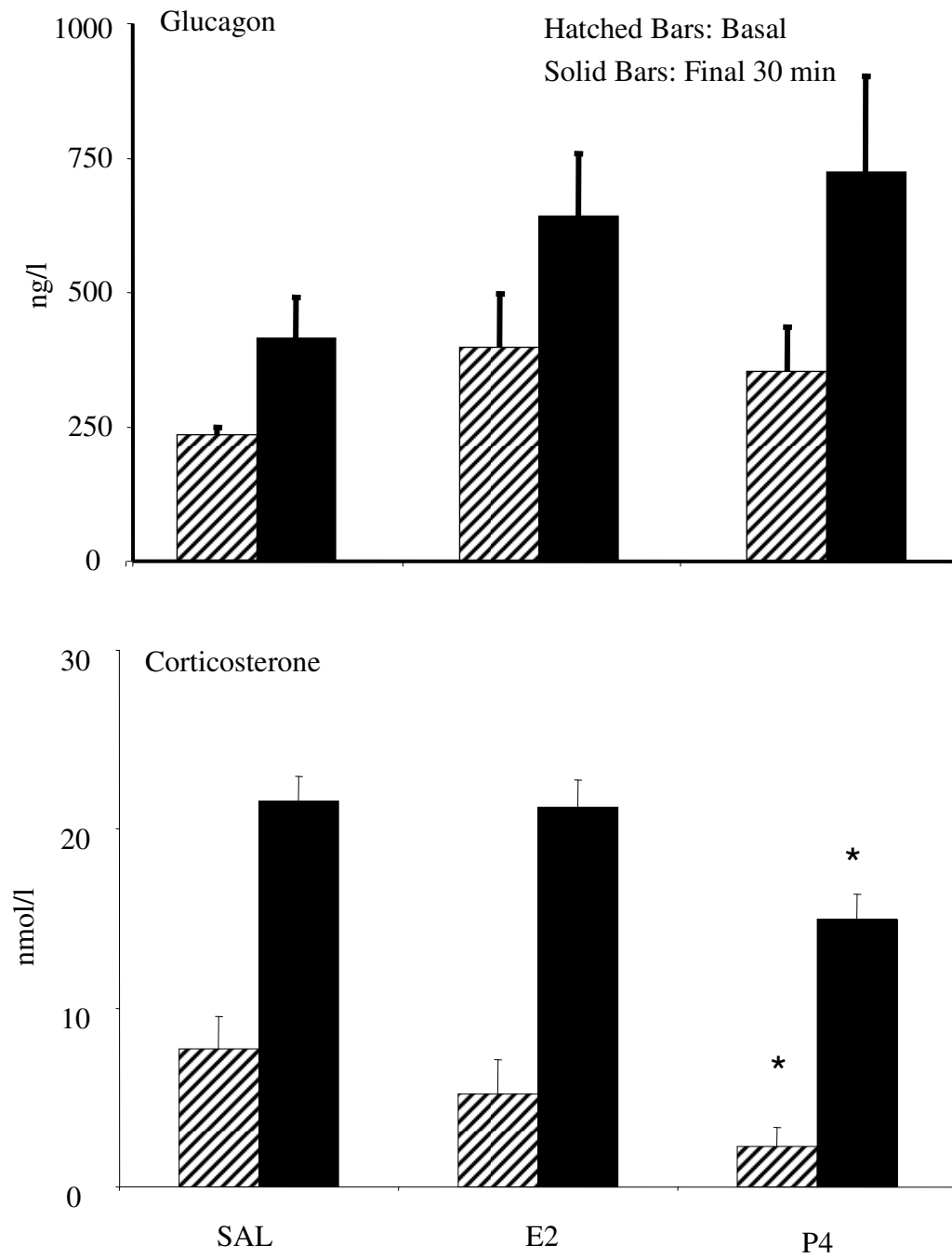


Figure 4

