Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1623 – 1628
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Forebrain and hindbrain effects of ethanol on counterregulatory responses to hypoglycemia in conscious rats

Darleen A. Sandoval*, Bin Gong, Stephen N. Davis

Department of Medicine, Vanderbilt University School of Medicine and, Nashville Veterans Affairs Medical Center, Nashville, TN 37232, USA

Received 21 October 2006; accepted 6 June 2007

Abstract

Ethanol (OH) has been reported to blunt counterregulation and increase insulin sensitivity, thereby causing greater hypoglycemia in patients with diabetes mellitus. The aim of this study was to determine if fore- or hindbrain sensing of OH differentially affects autonomic and metabolic counterregulatory responses to hypoglycemia. Forty-one Sprague-Dawley rats received 5% OH or isotonic sodium chloride solution (or normal saline; SAL) infused peripherally or into the lateral (forebrain) or fourth cerebral ventricles (hindbrain) from time 0 to 120 minutes. From time 120 to 240 minutes, rats were exposed to a hyperinsulinemic (5 mU/[kg min]) hypoglycemic (2.9 ± 0.1 mmol/L) clamp. The 4 groups of rats studied were as follows: SAL (n = 8), peripheral alcohol (POH) (n = 10), lateral ventricle alcohol (LVOH) (n = 12), and left ventricle alcohol (4VOH) (n = 11). After OH, basal levels of norepinephrine were lower in the POH and 4VOH groups ($P < .05$). Epinephrine and norepinephrine responses to hypoglycemia were significantly lower in POH, 4VOH, and LVOH vs SAL. However, the magnitude of blunting was significantly greater in POH and 4VOH vs LVOH. Other counterregulatory hormones and glucose kinetics were not significantly different among all groups during hypoglycemia. In summary, peripheral and central nervous system OH infusion blunted autonomic nervous system counterregulatory (epinephrine, norepinephrine) responses to subsequent hypoglycemia. The greater impact of 4VOH compared with LVOH administration suggests that OH exerts its effects to blunt autonomic nervous system counterregulatory responses during hypoglycemia primarily by actions on the hindbrain.

1. Introduction

Although The Diabetes Control and Complications Trial determined that maintaining blood glucose toward normal levels delays the development and/or progression of microvascular complications of type 1 diabetes mellitus (T1DM) [1], the benefit is offset by a 3-fold increase in severe hypoglycemia [2]. The mechanism for this increase is unclear and is not solely due to excessive insulin. Factors such as sex, exercise, and recurrent hypoglycemia all reduce counterregulatory responses to falling glucose levels, predisposing the diabetic patients to further hypoglycemia.

Another factor that is believed to contribute to the increased incidence of hypoglycemia in patients with T1DM is alcohol ingestion. Two prospective studies have suggested that alcohol ingestion is responsible for a fifth of all episodes of severe hypoglycemia admitted to emergency departments [3,4] and that it may reduce hypoglycemic symptom awareness [5]. In addition, other studies have suggested that the effects of alcohol-induced hypoglycemia is delayed from 2 hours [6] up to the morning after [7-9] and that alcohol impairs recovery from hypoglycemia in T1DM [10]. However, the neuroendocrine mechanisms by which alcohol causes hypoglycemia in T1DM have not been fully defined. Growth hormone [8,11], cortisol, glucagon [11,12], epinephrine, and glucagon [12] and stress-induced increases in norepinephrine release [13] have all been found to be reduced by alcohol exposure during or after hypoglycemia in humans. Because glucagon responses to hypoglycemia are lost after only a few years of disease duration in T1DM [14] and are significantly attenuated in type 2 diabetes mellitus, epinephrine is the essential counterregulatory hormone in DM. Therefore, the purpose of this study was 2-fold: (1) to determine whether peripheral alcohol administration blunts

* Corresponding author. University of Cincinnati, Cincinnati, OH 45237, USA. Tel.: +1 513 558 1601; fax: +1 513 558 8990.
E-mail address: darleen.sandoval@uc.edu (D.A. Sandoval).

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.031

counterregulatory responses to hypoglycemia and (2) to determine whether regional sensing of alcohol by the central nervous system (CNS) regulates counterregulatory responses to hypoglycemia. To achieve this aim, 5% ethanol (OH) was infused peripherally, into the lateral cerebral ventricle, or into the fourth cerebral ventricle of the brain in conscious, unrestrained Sprague-Dawley rats; and the responses to subsequent clamped hyperinsulinemic hypoglycemia were determined.

2. Materials and methods

2.1. Experimental animals

Forty-one male Sprague-Dawley rats (300-350 g) 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 water. All procedures for animal use were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

2.2. Animal preparation

One week before the study, in all rats, under a general anesthesia mixture (5 mg/kg acepromazine, 10 mg/kg xylazine, and 50 mg/kg ketamine), catheters were placed in the carotid artery (for blood sampling) and the external jugular vein (for infusions). Immediately after this surgery, rats had a 6-mm stainless steel cannula placed in either the lateral or fourth ventricle of the brain. Rats were placed in a stereotaxic frame (KOPF Instruments, Tujunga, CA) for placement of the guide cannula at stereotaxis coordinates of −0.9 mm anteroposterior, +1.4 mm mediolateral, and −4.5 dorsoventral from bregma for lateral ventricle cannulation and at −2.2 mm anteroposterior, midline, and −7 mm from lambda for fourth ventricle cannulation as determined via the atlas of Paxinos and Watson [15]. The intracranial cannulas were held in place with cranioplastic cement to 3 skull screws. Rats had free access to rat chow the days before surgery and experiments. The jugular and carotid catheter lines were kept patent by flushing with 150 U/mL of heparin every 3 days. Seven days postsurgery, only rats with greater than 90% of their presurgery body weight were used for the experiments.

2.3. Experimental design

Rats were studied after an overnight fast and remained conscious and unrestrained during the experimental period. The morning of the study, extensions were placed on the exteriorized catheters for ease of access. Rats then had either a 5% OH or isotonic sodium chloride solution (or normal saline; SAL) infusion into the lateral or fourth cerebral ventricle of the brain or peripherally through the jugular catheter from time 0 to 120 minutes at a rate of 1 μL/min. This timing was chosen to expose the rats to OH before rather than during hypoglycemia because previous studies have demonstrated delayed effects of OH on counterregulatory responses rather than immediate effects during hypoglycemia [2,31]. From time 120 to 240 minutes, rats were exposed to a hyperinsulinemic (30 pmol/[kg min]) hypoglycemic clamp as described previously [16]. Thus, the 4 groups of rats studied were as follows: peripheral alcohol (POH) (n = 10), lateral ventricle alcohol (LVOH) (n = 12), left ventricle alcohol (4VOH) (n = 11), or lateral ventricle saline (n = 8). To prevent a fall in hematocrit, after each blood draw, red blood cells plus isotonic sodium chloride solution were reinfused through the carotid cannula. To measure glucose kinetics during the clamp, a primed (10 μCi) constant (0.2 μCi/min) infusion of high-pressure liquid chromatography–purified [3-^{3}H] glucose (Perkin Elmer Life Sciences, Boston, MA) was administered via a precalibrated infusion pump (Harvard Apparatus, South Natick, MA) at time 0 minute and continued through 240 minutes. During the experimental period, blood was drawn every 5 minutes for measurements of plasma glucose; every 10 minutes during the basal period and every 15 minutes during the experimental periods for 3-^{3}H-glucose; and at time 90, 120, 180, 210, and 240 minutes for counterregulatory hormones. Rats were then euthanized; and placement of intracerebroventricular (ICV) (by infusion of cresyl violet), carotid, and jugular cannulas was verified. Rates of glucose appearance, endogenenous glucose production (EGP), and glucose utilization were calculated as described previously [16].

2.4. Analytical methods

Plasma glucose was measured in duplicate by the glucose oxidase technique on a Beckman (Fullertpn, CA) glucose analyzer. Ethanol was measured by Analox Instruments (Lunenburg, MA) using an oxidase enzyme reaction. Catecholamines were determined by high-pressure liquid chromatography [17]; and corticosterone, insulin, and glucagon were all measured using radioimmunoassay techniques described previously [16].

2.5. Statistical analysis

Data are expressed as mean ± SE and were analyzed using standard, parametric, 2-way analysis of variance, with repeated measures where appropriate. A Tukey post hoc analysis was used to delineate statistical significance. A P value < .05 was accepted as statistical significance.

3. Results

3.1. Glucose and insulin

Plasma glucose (2.8 ± 0.1 mmol/L; Fig. 1) and insulin (1285 ± 103 pmol/L) levels were not significantly different between all 4 groups of rats during the hypoglycemic clamps. We attempted to measure plasma OH levels in these rats for all groups, but the results with the assay we used were below the limits of detection of the assay (0.1 mmol/L).

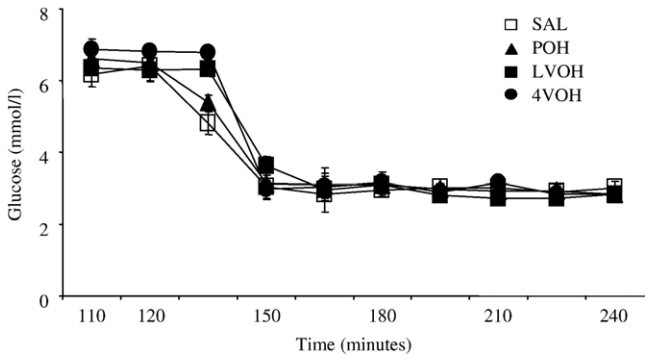

Fig. 1. Plasma glucose levels after infusion of 5% OH or SAL during hyperinsulinemic hypoglycemia (SAL, POH, LVOH and 4VOH).

3.2. Counterregulatory hormones

Norepinephrine levels at the basal period (ie, before the start of the hypoglycemic clamp after alcohol infusion) and the final 30 minutes of hypoglycemia were significantly lower in POH and 4VOH vs LVOH and SAL (final 30 minutes: 0.4 ± 0.1 and 0.3 ± 0.03 vs 1.4 ± 0.2 and 2.0 ± 0.4 nmol/L; $P < .05$; Fig. 2). Values were also significantly lower in LVOH compared with SAL ($P < .05$). Epinephrine responses were also significantly lower in POH and 4VOH vs both LVOH and SAL (4 ± 1 and 5 ± 1 vs 15 ± 2 and 21 ± 3 pmol/L; $P < .05$; Fig. 2) and in LVOH vs SAL ($P < .05$). Glucagon levels significantly increased from basal values of 66 ± 8 ng/L to 738 ± 148, 528 ± 91, 602 ± 159, and 678 ± 102 ng/L in SAL, POH, LVOH, and 4VOH, respectively; but there were no significant differences in the magnitude of this increase (Fig. 3). Similarly, basal and hypoglycemic increases in corticosterone were not significantly different between the groups (Fig. 3).

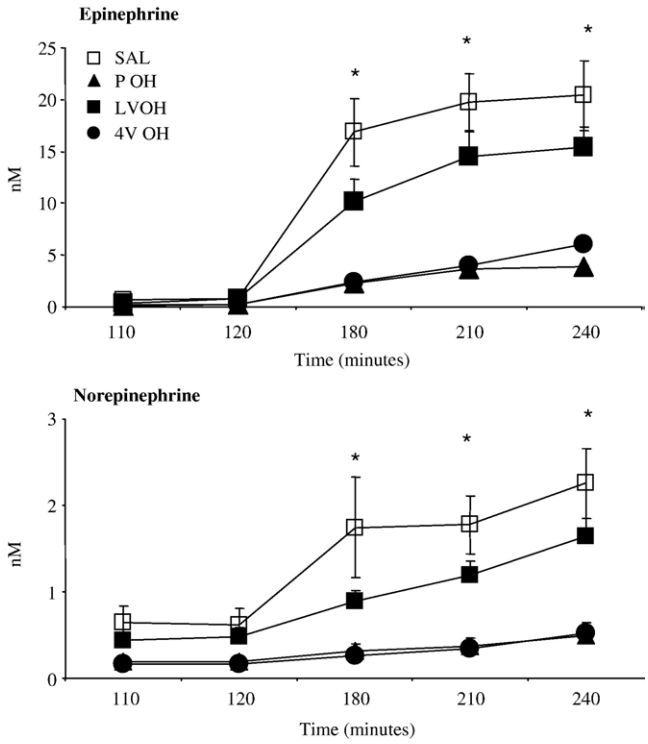

Fig. 2. Norepinephrine and epinephrine levels after infusion of 5% OH or SAL during hyperinsulinemic hypoglycemia (SAL, POH, LVOH and 4VOH). *$P < .05$ for SAL vs all other groups.

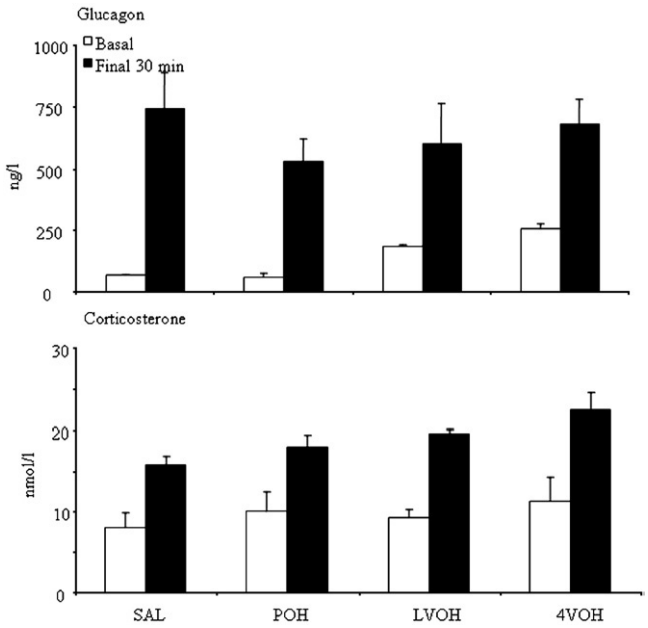

Fig. 3. Glucagon and corticosterone after ICV infusion of 5% OH or SAL during the basal period and the final 30 minutes of the hyperinsulinemic hypoglycemic clamp (SAL, POH, LVOH, and 4VOH).

3.3. Glucose kinetics

Specific activity, listed in Table 1, was stable during the basal period and the final 30 minutes of the hyperinsulinemic hypoglycemic clamps in all groups, with an average coefficient of variation of 3% ± 1% for both periods. During the final 30 minutes of hypoglycemia, EGP (39 ± 11, 31 ± 5, 52 ± 13, and 38 ± 5 μmol/[kg min]), glucose rate of disappearance (63 ± 16, 51 ± 2, 65 ± 10, and 58 ± 6 μmol/[kg min]), and glucose infusion rates (21 ± 7, 21 ± 4, 9 ± 4, and 19 ± 4 μmol/[kg min]) were not significantly

Table 1
Glucose specific activity (disintegrations per minute per millimole) at baseline and final 30 minutes during hyperinsulinemic hypoglycemia (2.9 ± 0.1 mmol) in conscious rats

Group	Time (min)					
	100	110	120	210	225	240
SAL	169 ± 57	168 ± 40	145 ± 20	103 ± 18	113 ± 19	111 ± 16
POH	168 ± 11	164 ± 11	172.12	131 ± 4	131 ± 8	122 ± 4
LVOH	120 ± 21	116 ± 20	110 ± 17	148 ± 51	141 ± 56	199 ± 99
4VOH	169 ± 19	174 ± 17	171 ± 16	168 ± 18	164 ± 20	170 ± 14

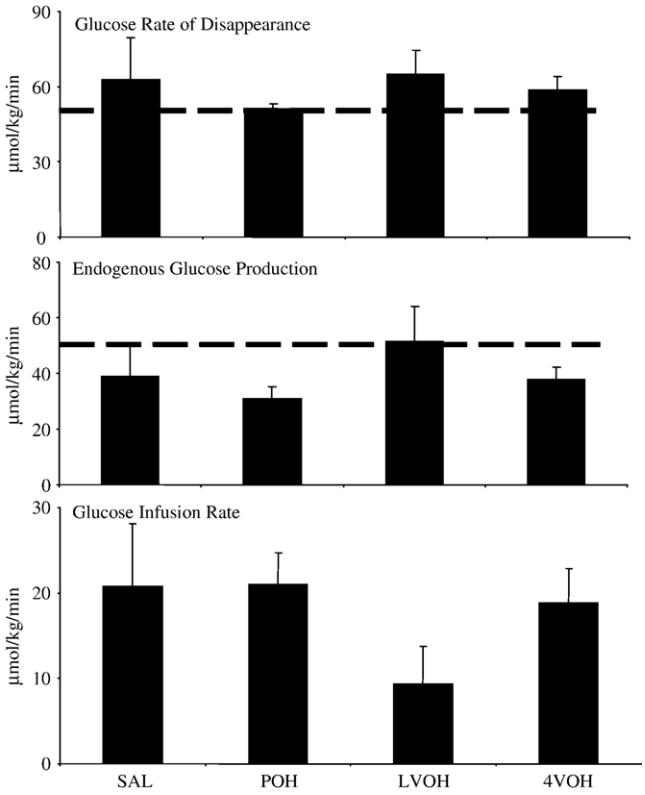

Fig. 4. Endogenous glucose production, glucose rate of disappearance, and glucose infusion rate after infusion of 5% OH or SAL during hyperinsulinemic hypoglycemia (SAL, POH, LVOH, and 4VOH). Dotted line refers to basal levels of glucose rate of disappearance and endogenous glucose production.

different between SAL, POH, LVOH, and 4VOH, respectively (Fig. 4).

4. Discussion

The results of this study demonstrate that prior peripheral and CNS infusion (both lateral and fourth ventricle) of OH blunts autonomic nervous system (ANS) counterregulatory (epinephrine and norepinephrine) responses to subsequent hypoglycemia in conscious unrestrained rats. Peripheral and fourth ventricle infusions of OH resulted in a similar magnitude of ANS counterregulatory blunting that was significantly greater than lateral ventricle infusions. This suggests that peripheral OH is sensed primarily by hindbrain regions to blunt ANS responses to hypoglycemia.

Because the flow of cerebrospinal fluid goes from the forebrain to the hindbrain, the fourth ventricle infusion of OH implies the direct effects of the alcohol on hindbrain regions. Neurons in this area of the brain have been found to be activated by OH infusion [18,19]. It is interesting that peripheral and fourth ventricle infusions provided greater blunting of ANS counterregulatory responses as compared with lateral ventricle (forebrain) infusions. In these current studies, the extent of the OH distribution within the brain after either peripheral or central administration was not determined; therefore, the extent of the influence of the CNS OH cannot be determined. However, immediate injection of radioactively labeled alcohol into the lateral ventricle has been found to be removed quickly from the brain [18] and does not spread locally beyond forebrain regions (ie, cortex and hypothalamus) [20]. In addition, peripheral administration of OH has been shown to have more widespread genomic activation of the brain compared with central administration [21]. These 2 studies may help place our results in context. First, they suggest that our infusions into either the lateral ventricle or the fourth ventricle were limited to the immediate areas surrounding the ventricle. Furthermore, it should also be noted that OH could not be detected in the systemic circulation during our fore- or hindbrain OH infusions. Second, because the effects of fourth ventricle and peripheral OH infusions on blunting ANS responses to hypoglycemia were so similar and bearing in mind that the hindbrain region has a limited blood brain barrier, it is highly suggestive (although not conclusive) that the hindbrain region is the major site of CNS sensing of alcohol.

Although peripheral and CNS delivery of OH decreased catecholamine responses to hypoglycemia, we did not observe metabolic consequences of the blunted catecholamines. Although epinephrine stimulates EGP and a drop in epinephrine would be expected to lead to a drop in EGP, glucagon levels were not blunted. Because of the redundancy in counterregulatory responses, glucagon, which also plays a key role in liver glucose output by increasing glycogenolysis and gluconeogenesis, could have compensated for the reduced epinephrine by maintaining EGP. The central mechanism responsible for our observations remains to be studied; however, OH is known to activate γ-aminobutyric acid type A and inhibit *N*-methyl-D-aspartate receptors, receptors known to play a role in reducing sympathetic nerve activity [19,22].

We analyzed the glucose kinetics during the final 30 minutes of the hypoglycemic clamp when specific activity was stable. However, it is interesting to note that the glucose levels tended to be higher at time 135 minutes (15 minutes after the start of insulin) in the groups where OH was infused centrally vs the POH and SAL groups. It is unknown why this occurred; however, there are several recent reports illustrating that CNS infusions of various hormones and nutrients cause changes in peripheral glucose homeostasis via changes in vagal efferent output [23-27]. Thus, it is possible that the ICV infusions of OH caused insulin resistance at either the liver or skeletal muscle that offset differences in counterregulatory action.

Epinephrine is the critical counterregulatory hormone in longer-duration T1DM because of the loss of glucagon responses to hypoglycemia [28]. Loss of both epinephrine and glucagon counterregulatory responses increases the risk of severe hypoglycemia 25-fold [29]. Therefore, our present results demonstrating a blunting of epinephrine and norepinephrine responses during hypoglycemia after OH may provide some insight into the mechanisms responsible

for 2 prospective studies that found that a fifth of all severe hypoglycemic episodes in patients with diabetes necessitating medical assistance were related to alcohol use [3,4]. In human studies, although contrasting data exist [30-35], are experimental data that have shown that alcohol ingestion has the potential to either exacerbate hypoglycemia [6-9,12,36] even when administered to levels of intoxication [12]. Two studies have found that alcohol ingestion reduced glucagon, cortisol, and growth hormone responses to hypoglycemia but, in contrast to our study, found normal catecholamine responses to hypoglycemia [10,11]. On the other hand, Kalacczynski et al [11] have demonstrated that epinephrine responses are blunted by alcohol during hypoglycemia. Only one of these studies found that alcohol both impaired recovery from hypoglycemia and reduced counterregulatory neuroendocrine responses [10]. The discrepancy in findings may be due to the timing of OH administration. The study by Avogaro et al [10] stopped administration of alcohol before hypoglycemia, whereas other studies [11] continuously administered alcohol throughout hypoglycemia. Somewhat consistent with this are data suggesting that evening alcohol ingestion predisposes patients with type 1 diabetes mellitus to hypoglycemia either later in the night or the next morning [8,37]. Thus, the detrimental effects of alcohol on hypoglycemic counterregulation may be prolonged.

The areas of the brain affected by a lateral ventricle infusion of OH would include areas of the hippocampus and the hypothalamus. Similar to hypoglycemia [38], the paraventricular nucleus of the hypothalamus is genomically activated by both OH ingestion [39] and lateral ventricle administration of OH [21]. The paraventricular nucleus contains corticotropin-releasing hormone neurons that activate the hypothalamic pituitary adrenal axis. However, we were unable to detect significant differences in corticosterone levels during central or peripheral OH infusions. A chemical lesion of the paraventricular nucleus has been found to blunt adrenocorticotropic hormone (and catecholamine) but not corticosterone responses to hypoglycemia [40]. The authors postulated that the adrenocorticotropic hormone response, despite being blunted, was enough of a signal to stimulate corticosterone secretion. We have found similar results in humans after episodes of antecedent hypoglycemia [41].

It is not easy to compare the dose of alcohol used in this study with previous experiments in humans. The total dose of alcohol used in this study was only 4.7 mg, which is a trivial amount compared with the content of a typical alcoholic beverage (7-17 g of alcohol). However, orally administered alcohol is absorbed quickly from the stomach and small intestine and distributed amongst whole-body water, with only a fraction reaching the CNS. Comparison with previous studies in rats where alcohol was administered peripherally indicates that the CNS content in the present study may be up to 20-fold higher than those in earlier studies [42,43]. This may also explain why some studies have not previously observed the effects of alcohol on the sympathetic nervous system.

In conclusion, our results demonstrate that administration of 5% OH blunted epinephrine and norepinephrine responses to subsequent hypoglycemia in conscious unrestrained rats. Although these results do not allow us to make conclusions on the specific area of the brain for OH action, these results do suggest that alcohol-induced susceptibility to hypoglycemia may be due partially to direct forebrain but primarily to hindbrain sensing and central inhibition of autonomic counterregulation.

Acknowledgments

We thank the Eric Allen, Angelina Penaloza, Pam Venson, and Wanda Snead for their expert technical assistance. This work was supported by a research grant from the Juvenile Diabetes Foundation International and Diabetes Research and Training Grant (5P60-AM-20593) and a National Institutes of Health grant (NHLBI;HL;5PO1 HL056693-07).

References

[1] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.

[2] The Diabetes Control and Complications Trial Research Group. Hypoglycaemia in the diabetes control and complication trial. Diabetes 1997;46:271-86.

[3] Nilsson A, Tideholm B, Kalen J, Katzman P. Incidence of severe hypoglycemia and its causes in insulin-treated diabetics. Acta Med Scand 1988;224:257-62.

[4] Potter J, Clarke P, Gale EA, Dave SH, Tattersall RB. Insulin-induced hypoglycaemia in an accident and emergency department: the tip of an iceberg? Br Med J (Clin Res Ed) 1982;285:1180-2.

[5] Kerr D, Macdonald IA, Heller SR, Tattersall RB. Alcohol causes hypoglycaemic unawareness in healthy volunteers and patients with type 1 (insulin-dependent) diabetes. Diabetologia 1990;33:216-21.

[6] Joffe BI, Shires R, Lamprey JM, Baker SG, Seftel HC. Effect of drinking bottled beer on plasma insulin and glucose responses in normal subjects. S Afr Med J 1982;62:95-7.

[7] Lange J, Arends J, Willms B. Alcohol-induced hypoglycemia in type I diabetic patients. Med Klin (Munich) 1991;86:551-4.

[8] Turner BC, Jenkins E, Kerr D, Sherwin RS, Cavan DA. The effect of evening alcohol consumption on next-morning glucose control in type 1 diabetes. Diabetes Care 2001;24:1888-93.

[9] Richardson T, Weiss M, Thomas P, Kerr D. Day after the night before: influence of evening alcohol on risk of hypoglycemia in patients with type 1 diabetes. Diabetes Care 2005;28:1801-2.

[10] Avogaro A, Beltramello P, Gnudi L, Maran A, Valerio A, Miola M, et al. Alcohol intake impairs glucose counterregulation during acute insulin-induced hypoglycemia in IDDM patients. Evidence for a critical role of free fatty acids. Diabetes 1993;42:1626-34.

[11] Kolaczynski JW, Ylikahri R, Harkonen M, Koivisto VA. The acute effect of ethanol on counterregulatory response and recovery from insulin-induced hypoglycemia. J Clin Endocrinol Metab 1988;67:384-8.

[12] Lecavalier L, Bolli GB, Cryer PE, Gerich JE. Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. Am J Physiol 1989;256:E844-51.

[13] Shirao I, Tsuda A, Ida Y, Tsujimaru S, Satoh H, Oguchi M, et al. Effect of acute ethanol administration on noradrenaline metabolism in brain

regions of stressed and nonstressed rats. Pharmacol Biochem Behav 1988;30:769-73.
[14] Roberto M, Schweitzer P, Madamba SG, Stouffer DG, Parsons LH, Siggins GR. Acute and chronic ethanol alter glutamatergic transmission in rat central amygdala: an in vitro and in vivo analysis. J Neurosci 2004;24:1594-603.
[15] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. Orlando (Fla): Academic Press; 1986.
[16] Sandoval DA, Ping L, Neill RA, Gong B, Walsh K, Davis SN. Brain region–dependent effects of dexamethasone on counterregulatory responses to hypoglycemia in conscious rats. Am J Physiol Regul Integr Comp Physiol 2005;288:R413-9.
[17] Causon R, Caruthers M, Rodnight R. Assay of plasma catecholamines by liquid chromatography with electrical detection. Ann Biochem 1982;116:223-6.
[18] Li G, Wang X, Abdel-Rahman AA. Brainstem norepinephrine neurons mediate ethanol-evoked pressor response but not baroreflex dysfunction. Alcohol Clin Exp Res 2005;29:639-47.
[19] Varga K, Gantenberg NS, Kunos G. Endogenous gamma-aminobutyric acid (GABA) mediates ethanol inhibition of vagally mediated reflex bradycardia elicited from aortic baroreceptors. J Pharmacol Exp Ther 1994;268:1057-62.
[20] Friedman HJ, Lester D. Intraventricular ethanol and ethanol intake: a behavioral and radiographic study. Pharmacol Biochem Behav 1975;3: 393-401.
[21] Crankshaw DL, Briggs JE, Olszewski PK, Shi Q, Grace MK, Billington CJ, et al. Effects of intracerebroventricular ethanol on ingestive behavior and induction of c-Fos immunoreactivity in selected brain regions. Physiol Behav 2003;79:113-20.
[22] Abe K, Niikura Y, Misawa M. GABAA receptor-mediated inhibition by ethanol of long-term potentiation in the basolateral amygdala-dentate gyrus pathway in vivo. Neuroscience 2004;125:113-7.
[23] Liu L, Karkanias GB, Morales JC, Hawkins M, Barzilai N, Wang J, et al. Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes. J Biol Chem 1998;273:31160-7.
[24] Obici S, Feng Z, Tan J, Liu L, Karkanias G, Rossetti L. Central melanocortin receptors regulate insulin action. J Clin Invest 2001;108: 1079-85.
[25] Obici S, Feng Z, Morgan K, Stein D, Karkanias G, Rossetti L. Central administration of oleic acid inhibits glucose production and food intake. Diabetes 2002;51:271-5.
[26] Lam TK, Gutierrez-Juarez R, Pocai A, Rossetti L. Regulation of blood glucose by hypothalamic pyruvate metabolism. Science 2005;309: 943-7.
[27] Minokoshi Y, Haque MS, Shimazu T. Microinjection of leptin into the ventromedial hypothalamus increases glucose uptake in peripheral tissues in rats. Diabetes 1999;48:287-91.
[28] Gerich JE, Langlois M, Noacco C, Karam J, Forsham PH. Lack of a glucagon response to hypoglycemia in diabetes: evidence for an intrinsic pancreatic alpha-cell defect. Science 1973;182:171-3.
[29] White NH, Skor DA, Cryer PE, Levandoski LA, Bier DM, Santiago JV. Identification of type I diabetic patients at increased risk for hypoglycemia during intensive therapy. N Engl J Med 1983;308: 485-91.
[30] Wettergren A, Schjoldager B, Mortensen PE, Myhre J, Christiansen J, Holst JJ. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig Dis Sci 1993;38: 665-73.
[31] Christiansen C, Thomsen C, Rasmussen O, Hansen C, Hermansen K. The acute impact of ethanol on glucose, insulin, triacylglycerol, and free fatty acid responses and insulin sensitivity in type 2 diabetes. Br J Nutr 1996;76:669-75.
[32] Wettergren A, Petersen H, Orskov C, Christiansen J, Sheikh SP, Holst JJ. Glucagon-like peptide-1 7-36 amide and peptide YY from the L-cell of the ileal mucosa are potent inhibitors of vagally induced gastric acid secretion in man. Scand J Gastroenterol 1994;29:501-5.
[33] Sipols AJ, Brief DJ, Ginter KL, Saghafi S, Woods SC. Neuropeptide Y paradoxically increases food intake yet causes conditioned flavor aversions. Physiol Behav 1992;51:1257-60.
[34] Hagan MM, Havel PJ, Seeley RJ, Woods SC, Ekhator NN, Baker DG, et al. Cerebrospinal fluid and plasma leptin measurement: covariability with dopamine and cortisol in fasting humans. J Clin Endocrinol Metab 1999;84:3579-85.
[35] Rasmussen BM, Orskov L, Schmitz O, Hermansen K. Alcohol and glucose counterregulation during acute insulin-induced hypoglycemia in type 2 diabetic subjects. Metabolism 2001;50:451-7.
[36] Koivisto VA, Tulokas S, Toivonen M, Haapa E, Pelkonen R. Alcohol with a meal has no adverse effects on postprandial glucose homeostasis in diabetic patients. Diabetes Care 1993;16:1612-4.
[37] Aftab D, Guy D, Sandoval MA, Richardson D, Tate SN. Effects of glycemic control on target organ responses to epinephrine in type 1 diabetes. Am J Physiol Endocrinol Metab 2005;289:E25-65.
[38] Evans SB, Wilkinson CW, Bentson K, Gronbeck P, Zavosh A, Figlewicz DP. PVN activation is suppressed by repeated hypoglycemia but not antecedent corticosterone in the rat. Am J Physiol Regul Integr Comp Physiol 2001;281:R1426-36.
[39] Herring BE, Mayfield RD, Camp MC, Alcantara AA. Ethanol-induced Fos immunoreactivity in the extended amygdala and hypothalamus of the rat brain: focus on cholinergic interneurons of the nucleus accumbens. Alcohol Clin Exp Res 2004;28:588-97.
[40] Evans SB, Wilkinson CW, Gronbeck P, Bennett JL, Taborsky Jr GJ, Figlewicz DP. Inactivation of the PVN during hypoglycemia partially simulates hypoglycemia-associated autonomic failure. Am J Physiol Regul Integr Comp Physiol 2003;284:R57-65.
[41] Davis SN, Shavers C, Costa F, Mosqueda-Garcia R. Role of cortisol in the pathogenesis of deficient counterregulation after antecedent hypoglycemia in normal man. J Clin Invest 1996;98:680-91.
[42] Lai YL, Smith PM, Lamm WJ, Hildebrandt J. Sampling and analysis of cerebrospinal fluid for chronic studies in awake rats. J Appl Physiol 1983;54:1754-7.
[43] Yim HJ, Gonzales RA. Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol 2000;22: 107-15.