Increased Thirst and Plasma Arginine Vasopressin Levels during 2-Deoxy-D-glucose-induced Glucoprivation in Humans

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ABSTRACT Insulin-induced hypoglycemia by unknown mechanism(s) increases plasma arginine vasopressin (AVP) levels in humans. Mechanisms for increased AVP levels during central nervous system glucoprivation were investigated by administering 20-min i.v. infusions of 2-deoxy-D-glucose (50 mg/kg), a competitive inhibitor of glucose utilization, or normal saline (sham), to 24 normal volunteers. Some of the infusions were administered in combination with neuropharmacological blocking agents (placebo). The behavioral, physiological, metabolic, and hormonal correlates of 2-deoxy-D-glucose (2DG)-induced glucoprivation and AVP secretion were studied in a group (n = 5) pretreated for 1 wk with either mazindol (1 mg *per os* three times per day), a potent norepinephrine and dopamine-reuptake blocker, or placebo. A second group (n = 5) received either propranolol (3 mg/3 min followed by 80 μg/min) or normal saline infusion before and during 2DG administration. With 2DG alone, plasma AVP levels increased from 1.3±0.3 pg/ml at base line to a peak of 4.5±1.4 pg/ml at 60 min and remained elevated for 150 min. From 30 to 180 min after 2DG administration, the 2DG-infused volunteers increased their water intake in comparison with sham-infused volunteers. Marked increases in epinephrine and slight increases in norepinephrine were associated with increases in plasma glucose and renin activity and decreases in plasma potassium. Plasma sodium and osmolality increased transiently and mean arterial pressure (MAP) fell. These changes, however, were small and inconstant and could not account for the observed increases in thirst and AVP levels. Pretreatment with mazindol prevented the decrease in MAP and the increase in plasma renin activity (PRA) following 2DG infusions without modifying increased thirst, water intake, or AVP responses to glucoprivation. Pretreatment with propranolol effectively blocked β-adrenoreceptors as evidenced by increased MAP and plasma epinephrine, and abolition of the RPA increases during 2DG-induced glycoprivation, but did not suppress AVP and thirst responses. A cervical cord-sectioned patient lacking descending sympathetic outflow had a potentiated thirst response to 2DG-induced glucoprivation in the absence of increases in sodium, catecholamines, and PRA. Thus 2DG administration activates mechanisms for increased thirst and AVP which are unrelated to changes in peripheral catecholamines, MAP, PRA, and osmolality.

INTRODUCTION

Central nervous system (CNS)[1] glucoprivation induced by infusions of 2-deoxy-D-glucose (2DG), a competitive inhibitor of glucose transport (1) and phosphohexoisomerase activity (2), increases anterior pitui-

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[1] *Abbreviations used in this paper*: AVP, arginine vasopressin; CNS, central nervous system; 2DG, 2-deoxy-D-glucose; E, epinephrine; MAP, mean arterial pressure; NE, norepinephrine; PRA, plasma renin activity.

tary hormone secretion (3, 4), catecholamine-dependent renin release (5), and plasma free fatty acid and glucose levels through activation of descending sympathetic adrenomedullary pathways (6). Infusion of 2DG in humans induces eating and drinking, as well as a relative insulinopenia characteristic of the diabetic state (7).

Insulin-induced hypoglycemia increases water intake in rats (8) and plasma arginine vasopressin (AVP) in rats (9) and humans (10). Since insulin-induced hypoglycemia increases renin secretion (11), Fitzsimons (12) has suggested that the renin-angiotensin system may activate drinking during hypoglycemia. Stimuli that increase AVP secretion, such as increased osmolality (13) and angiotension II (14) levels and decreased blood volume and pressure (15) may also activate drinking.

We investigated the effects of glucoprivation on water intake and AVP levels by infusing healthy male volunteers with 2DG. Metabolic, hormonal, and physiological correlates of 2DG-induced glucoprivation were measured in healthy volunteers and in a cervical cord-sectioned patient to determine which, if any, might be related to any observed changes in thirst and AVP secretion. To eliminate two potential peripheral water balance control mechanisms (hypotension and hyperreninemia), mazindol (Sanorex, Sandoz Pharmaceuticals Inc., Hanover, N. J.), a blocker of norepinephrine (NE) and dopamine reuptake (16), or propranolol (Inderal, Ayerst Laboratories, New York), a β-adrenoreceptor antagonist, were administered before 2DG infusion.

METHODS

Normal-weight healthy male volunteers (n = 24) gave informed consent to receive 20-min i.v. infusions of either 2DG (50 mg/kg) or normal saline (sham) after a 12-h, overnight, food-deprivation period. The responses of seven subjects receiving 2DG infusions were compared to the responses of seven different volunteers receiving normal saline (sham) infusions. Because other experiments were done to analyze the effects of mazindol on the responses to 2DG (sham) infusions, these subjects also took placebo capsules for 1 wk before 2DG (sham) infusions. Of the seven subjects who received a 2DG infusion after 1 wk of placebo treatment, five also received another 2DG infusion after 1 wk of mazindol (1 mg TID po) pretreatment in a randomized, paired design. Five other subjects were pretreated with mazindol for 1 wk before receiving a sham infusion to determine the effects of mazindol alone on behavioral and hormonal responses.

In a second set of experiments, five subjects received two 2DG infusions randomly paired with propranolol (3 mg/3-min loading dose followed by 80 μg/min continuous infusion) or a normal saline (sham) infusion. The effects of intravenous propranolol alone were evaluated in the 30-min base-line period before 2DG infusion. Finally, one patient with transsection of the spinal cord between the 5th and 6th cervical vertebrae gave informed consent to receive a 2DG infusion.

Individuals were deprived of food overnight, but could drink tap water at room temperature (21°C) *ad lib.* before and during the experiment. The experimenter unobtrusively measured water ingested from a 250-ml opaque cup, at 30-min intervals, before and after the 2DG infusion. In patients preteated with mazindol or placebo, systolic and diastolic blood pressures were obtained by sphygmomanometry in the supine position. Mean arterial pressure (MAP) was calculated as the diastolic pressure at which the sounds of Korotkoff disappeared (17), plus one-third of the pulse pressure. In patients pretreated with propranolol or saline, systolic and diastolic pressures were measured with an Arteriosonde 1216 automatic blood pressure monitor which detects arterial wall motion by ultrasound (Roche Medical Electronics, Inc., Cranbury, N. J.). This instrument reliably detects the reduction in intensity of the sounds of Korotkoff as a measure of diastolic pressure (18) rather than the complete disappearance of sound. MAP was calculated accordingly.

At 30-min intervals the subjects gave ratings of thirst on a paper scale (7) consisting of a series of horizontal lines (30 cm long) anchored in the center by a vertical line labeled "Standard". The subjects placed a vertical mark on the appropriate horizontal line to represent greater (to the right) or lesser (to the left) degrees of thirst relative to the Standard at 30 min before 2DG infusion. Estimates of thirst changes from base line were obtained by measuring the distance in millimeters between the vertical mark and the fixed Standard line.

Supine subjects had an indwelling butterfly needle inserted in an antecubital vein for 1 h before 2DG infusion. Blood was collected at 30-min intervals in chilled heparinized tubes for plasma determinations of glucose by a glucose oxidase method on a Beckman Glucose Analyzer (Beckman Instruments, Cedar Grove, N. J.); osmolality by the freezing point depression method on a model 3L Advanced osmometer, (Advanced Instruments, Inc., Needham Heights, Mass.); sodium and potassium by flame photometry; and AVP by radioimmunoassay (13). Insulin (Amersham/Searle Corp., Arlington Heights, Ill.) and aldosterone (Diagnostic Products Corp., Los Angeles, Calif.) plasma levels were determined by radioimmunoassay. Blood samples for plasma renin activity (PRA) were collected at 60-min intervals after 2DG infusion in chilled tubes containing 5 mM EDTA. PRA was determined by the Beckton Dickinson, Orangeburg, N. Y.) radioimmunoassay kit for angiotensin I generated in a 3-h incubation at pH 7.4. Blood for catecholamine determinations was collected in chilled heparinized tubes containing enough reagents to produce a solution buffered to pH 5.5 of 4 mM reduced glutathione and 5 mM EGTA after addition of whole blood. NE and epinephrine (E) were measured by a modified single isotope derivative radioenzymatic assay (19, 20) in which standard curves are developed for each subject's samples using plasma from the same subject on the day of the experiment. The intra-assay coefficients of variation are 6.7 and 5.2% for E and NE at concentrations of 90 and 320 pg/ml, respectively. The inter-assay coefficients of variation are 9.3 and 9.4% for E and NE at concentrations of 39 and 250 pg/ml, respectively.

Plasma was separated and stored at −20°C. Glucose and osmolality determinations were performed immediately. Catecholamine samples were stored at −70°C and analyzed within 3 mo.

Means and standard errors of the mean were calculated and the data were analyzed for statistical significance by means of unpaired one-tailed Student's t tests comparing values from placebo-2DG-treated and mazindol-sham-infused subjects to values from placebo-sham-infused subjects. Paired one-tailed Student's t tests were performed on data collected in subjects acting as their own controls during mazindol-2DG and placebo-2DG treatments or propranolol-2DG and sham-2DG treatments. Since base-line data before 2DG infusion tended to differ across pretreatments, the data were converted to

percentage of base line to evaluate the stimulated responses for insulin and aldosterone. Marked deviations from a normal distribution necessitated the use of a nonparametric test of significance (Wilcoxon signed rank test) for aldosterone data after propranolol administration.

RESULTS

Side effects from 2DG infusions included a 0.9°C fall in tympanic membrane temperature, marked diaphoresis with increased feelings of warmth and tiredness, 10 to 15 beats/min pulse rate increase, augmented ratings of hunger, and increased food intake 3 h after 2DG infusion.

AVP levels increased significantly at 60, 90, and 150 min after 2DG infusion with a peak 3.8-fold (4.5±1.4 pg/ml after 2DG vs. 1.2±0.4 pg/ml after sham infusion) elevation occurring at 60 min, but did not change in sham-infused subjects (Fig. 1). Subjects drank more water ($P < 0.01$) during 2DG-induced glucoprivation (481±121 ml) than during sham infusion (84±16 ml) with the peak response occurring at 120 min (Fig. 1). Thirst ratings increased from −1.4±2.2 mm at 0 min to 21.4±11.1 mm at 60 min and remained elevated until 120 min in the placebo-2DG-treated subjects, but did not change in placebo-sham-infused subjects.

Plasma osmolality did not increase significantly in association with 2DG-induced drinking and AVP increases. At 60 min, plasma osmolality increased insignificantly from 283.3 to 284.8 mosmol/kg while AVP increased 3.2 pg/ml. Simultaneously, sodium levels transiently increased from 138.2 to 139.0 meq/liter ($P < 0.05$) and glucose levels rose from 82 to 138 mg/dl ($P < 0.01$), while potassium levels decreased from 3.8 to 3.3 meq/liter ($P < 0.01$). Sodium levels were not significantly elevated after 60 min, but AVP levels and water intake remained elevated until 150 and 180 min, respectively. Potassium levels declined from 3.8 to 3.2 meq/liter at 90 min and remained reduced while glucose levels progressively increased until a peak response 108% greater than base-line was attained at 150 min. Insulin levels increased from 10.4 μU/ml to 164, 192, and 310% of base line in association with 68, 93, and 105% increases in glucose at 60, 120, and 180 min, respectively. MAP calculated from blood pressures obtained by auscultation decreased from a base-line of 78-72 mm Hg at 60 min and remained reduced until 180 min. Plasma E levels increased from 17 to 949 pg/ml while plasma NE levels increased only slightly from 175 to 284 pg/ml at 60 min. E levels remained elevated throughout the post-2DG stimulation period, whereas NE levels were no longer significantly elevated at 180 min. PRA levels increased from 0.53 to 1.41 ng/ml per h 60 min after 2DG administration and remained increased until 180 min. At 60 and 90 min aldosterone levels increased, respectively, 79 and 69% above base-line levels of 64 pg/ml in association with increases in

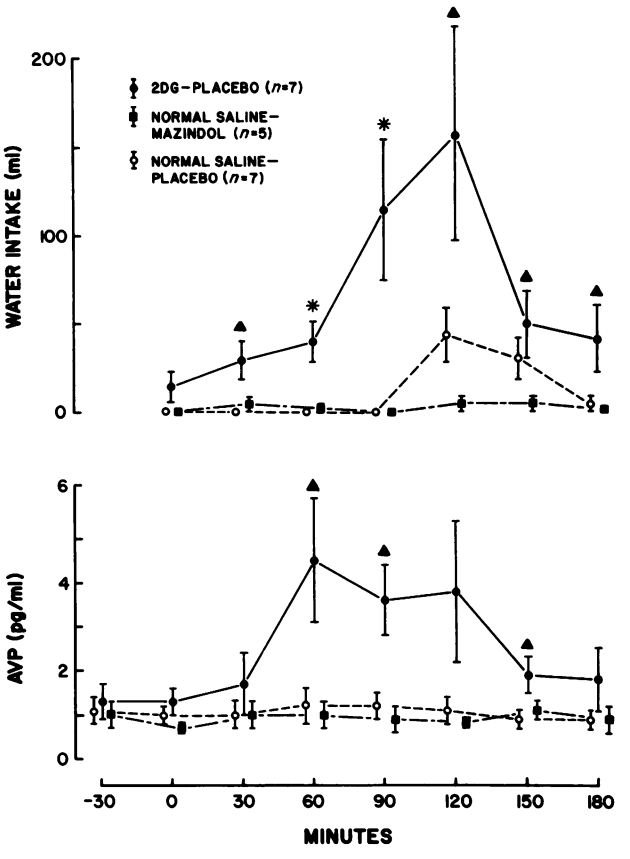

FIGURE 1 Effects of 50 mg/kg 2DG infused from 0 to 20 min on mean±SEM AVP and water intake in seven subjects taking one placebo capsule TID for 1 wk are compared with the effects of normal saline infusion in seven other subjects also taking placebo capsules. The effects of mazindol (1 mg TID for 1 wk) on mean±SEM AVP and water intake in five subjects receiving normal saline infusions are compared with the effects of normal saline-placebo treatment in seven subjects. Values for P (unpaired Student's t test) indicating differences in means at each time point are given.

PRA, decreases in potassium, and no change in sodium. In sham-infused subjects ($n = 7$), plasma osmolality, sodium, glucose, potassium, catecholamines, PRA, and MAP did not change.

Mazindol administration in five volunteers did not affect baseline thirst and AVP values or overall thirst, water intake, and AVP response to 2DG-induced glucoprivation (Fig. 2), but did prevent 2DG-induced hypothermia. Total water intake of 562±97 ml after 2DG infusion during mazindol pretreatment was not different from 583±138 ml during placebo pretreatment. Compared to basal levels, plasma AVP levels increased 60 and 90 min after 2DG in both placebo- and mazindol-treated subjects.

Pretreatment with mazindol suppressed plasma osmolality responses to 2DG at 30, 60, and 150 min

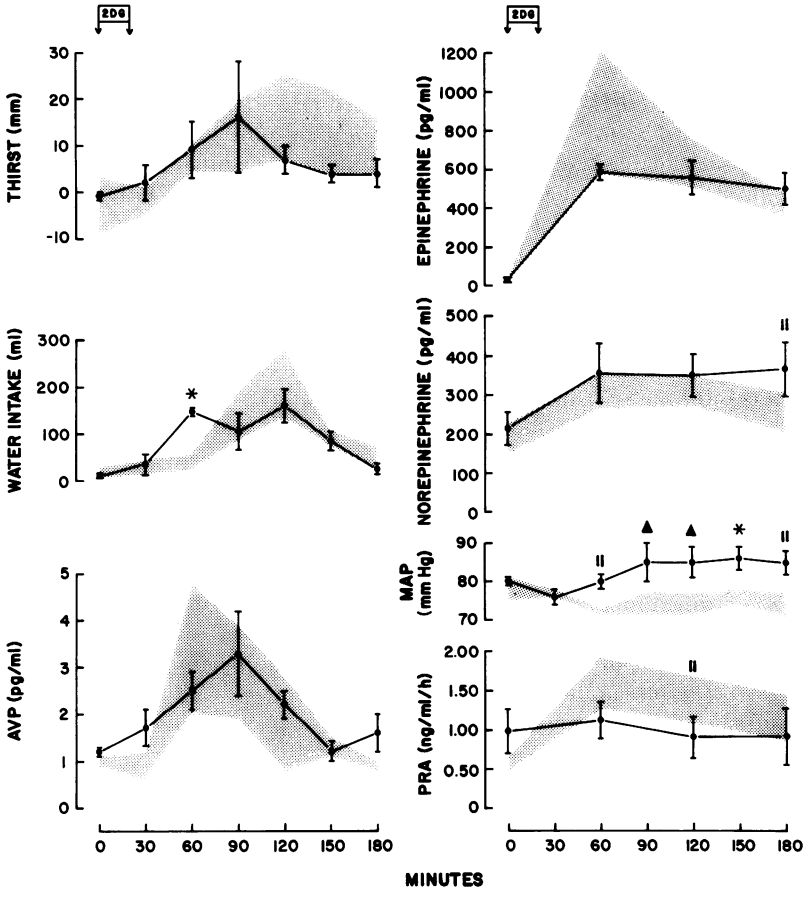

FIGURE 2 Effect of 50 mg/kg 2DG infused for 20 min on mean±SEM plasma AVP, thirst ratings, water intake, plasma E and NE, MAP, and PRA at the end of 1 wk of placebo (shaded area) or mazindol (1 mg TID po) treatment (solid circles) in five normal male volunteers. Values for P indicating differences in means between placebo and mazindol therapy are given.

(Table I). Mazindol pretreatment did not suppress water intake and AVP increases following 2DG (Fig. 2) even though plasma osmolalities remained virtually unchanged from base-line (Table I). Sodium levels were lower at 120 and 150 min after 2DG infusion in mazindol-pretreated subjects compared to placebo-pretreated subjects (Table I). Water intakes and AVP levels, however, showed no corresponding differences at those time points (Fig. 2). Potassium levels (Table I) did not differ between placebo- and mazindol-treated group. Mazindol also failed to alter the glucose and insulin responses to 2DG (Table I).

Mazindol pretreatment prevented any slight fall in MAP following 2DG infusions (Fig. 2). MAP were higher 60 to 180 min after 2DG infusion during mazindol therapy compared to placebo treatment (Fig. 2). Mazindol pretreatment did not alter the plasma E response to 2DG (Fig. 2). NE levels increased slightly but not significantly during both placebo and mazindol treatment periods. However, during mazindol treatment, NE levels (Fig. 2) at 180 min (364±69 pg/ml) were greater ($P < 0.05$) than those during placebo therapy (258±51 pg/ml). During placebo treatment PRA increased ($P < 0.05$) 195%, 155%, and 114% above baseline at 60, 120, and 180 min, respectively, after 2DG infusion. During mazindol therapy 2DG infusion transiently increased PRA only 16% ($P < 0.05$) at 60 min. Furthermore, mazindol therapy suppressed both PRA (Fig. 2) and aldosterone (Table I) responses to 2DG infusion.

Mazindol administration to five sham-infused subjects did not change water intake or plasma AVP levels for 180 min after the infusions (Fig. 1). Furthermore, plasma osmolality, sodium, potassium, glucose, catecholamine, and PRA levels and MAP in sham-infused subjects (data not shown) were unaltered by mazindol pretreatment.

Propranolol infusions did not alter the thirst, water intake, and AVP response to 2DG-induced glucoprivation (Fig. 3). Consistent with its lack of effect on

TABLE I

Effect of 2DG on Plasma Concentrations in Five Normal Males Receiving Placebo or Mazindol

	Substance	Time (min)						
		0	30	60	90	120	150	180
Osmolality, *mosmol/kg*	P*	281.1±1.7	283.8±1.1	284.6±1.7	283.0±1.7	283.5±2.6	283.3±2.4	284.1±3.0
Osmolality, *mosmol/kg*	M*	278.9±2.2	278.5±2.8‡	278.4±3.2‡	279.7±4.0	279.1±3.7	278.8±3.9‡	279.3±3.8
Sodium, *meq/liter*	P	138.2±1.0	138.2±1.4	138.9±0.9	139.1±0.9	139.2±1.2	138.0±0.9	137.3±0.8
Sodium, *meq/liter*	M	137.0±0.4	137.0±1.0	137.9±0.5	137.6±0.5	135.4±1.0‡	135.1±0.9‡	135.5±0.8
Potassium, *meq/liter*	P	3.8±0.1	3.8±0.1	3.4±0.1	3.2±0.1§	3.2±0.1	3.2±0.1‖	3.3±0.1‖
Potassium, *meq/liter*	M	3.9±0.1	3.6±0.1‖	3.1±0.2	3.1±0.2	3.0±0.1‖	3.1±0.1‖	3.3±0.1‖
Aldosterone, *% of control*	P	100	94±12	188±32	186±46§	156±41	99±24	77±17
Aldosterone, *% of control*	M	100	87±11	126±34‡	90±12‡	62±9‡	57±8	41±11
Glucose, *pg/ml*	P	82±2	94±5	127±13	145±16	147±14	161±19	160±18
Glucose, *pg/ml*	M	78±4	100±4	140±10	158±14	166±16	174±15	182±20
Insulin, *μU/ml*	P	10.4±2.6	—	16.7±4.0	—	20.0±2.7§	—	33.2±9.3§
Insulin, *μU/ml*	M	10.8±1.8	—	14.5±1.5§	—	18.0±1.6§	—	27.8±4.0§

50 mg/kg 2DG infused for 20 min in patients receiving placebo or mazindol (1 mg *per os* three times a day for 1 wk). Values given are mean±SEM.

* P, placebo; M, mazindol.

‡ $P < 0.05$ by paired Student's t test with values during placebo treatment.

§ $P < 0.05$ paired Students t test with values at 0 min.

‖ $P < 0.01$ by paired Student's t test with values at 0 min.

the hyperglycemic response to 2DG, propranolol also failed to prevent small increases in osmolality (Table II). No significant increases in sodium levels were observed after 2DG infusion, and there were no differences in sodium levels between propranolol- and sham-infused groups (Table II). In association with 2DG-induced hyperglycemia, potassium levels decreased from 4.3±0.3 to 3.6±0.2 meq/liter at 90 min and remained depressed until 180 min (Table II). Propranolol infusion not only prevented the development of hypokalemia, but, in fact, raised potassium levels (Table II) between 60 and 180 min after 2DG infusion in association with increased catecholamine and blood pressure levels (Fig. 3). The peak potassium value of 4.6±0.1 meq/liter at 150 min was greater than both the base-line potassium of 4.0±0.1 meq/liter ($P < 0.01$) and the potassium value of 3.4±0.1 meq/liter ($P < 0.01$) observed 150 min after 2DG infusion alone. Propranolol infusion did not reduce the hyperglycemic response to 2DG until 180 min (Table II). In contrast, propranol pretreatment completely abolished the hyperinsulinemic response to 2DG-induced hyperglycemia (Table II).

Although propranolol infusions did not alter the increased AVP, thirst, and water intake responses observed after 2DG infusions (Fig. 3), unopposed α-adrenoreceptor stimulation during β-adrenoreceptor blockade increased MAP from 82±5 mm Hg at base line to 100±5 mm Hg at 180 min. MAP did not change in 2DG-sham-infused subjects having blood pressures monitored automatically (Fig. 3). Plamsa E levels increased more than twice as much after propranolol and 2DG infusions than after 2DG alone (Fig. 3). Plasma NE increased after 2DG in both sham- and propranolol-treated subjects (Fig. 3). In the absence of propranolol, PRA increased from 0.77±0.11 ng/ml per h at 0 min to a peak of 3.66±1.19 ng/ml per h ($P < 0.05$) at 120 min following 2DG (Fig. 3). β-adrenoreceptor blockade completely suppressed the PRA response to 2DG without diminishing thirst and AVP responses (Fig. 3). In association with increases in potassium and MAP, aldosterone levels increased markedly while PRA levels did not change during β-adrenoreceptor blockade after 2DG infusions (Table II). Propranolol administration before 2DG infusions did not change basal AVP, E, and NE levels, MAP, thirst and water intake (Fig. 3), osmolality, sodium, potassium, and glucose levels (Table II), but did slightly reduce PRA (Fig. 3).

Thirst ratings in a cervical cord-sectioned patient increased from 60 to 150 min after 2DG infusion (Fig. 4). During the same time period water intakes were three to eight times greater in the cervical cord-sectioned patient receiving 2DG. The cervical cord-sectioned patient drank a total of 2,450 ml while normal volunteers drank only 548±118 ml after 2DG infusion. This marked increase in water intake in the cervical cord-sectioned patient occurred in the absence of significant increases in catecholamines, PRA, glucose, and sodium concentration (Fig. 4). Sodium levels decreased from 90 to 180 min in association with increased water intake.

DISCUSSION

Administration of 2DG to normal volunteers induces a state of glucoprivation in the CNS, as evidenced by

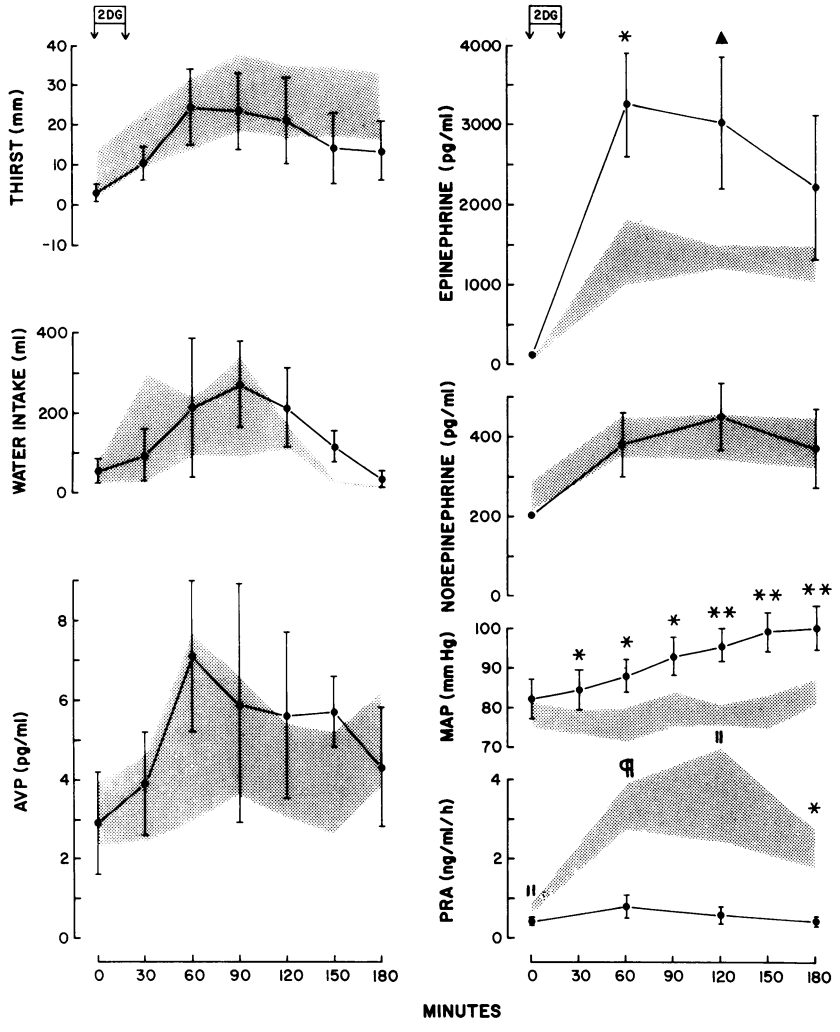

FIGURE 3 Effect of 50 mg/kg 2DG infused for 20 min on mean±SEM plasma AVP, thirst ratings, water intake, plasma E and NE, MAP, and PRA after normal saline (shaded area) or propranolol (3 mg/3 min followed by 80 μg/min) infusions (●) in five normal male volunteers. Values for *P* indicating differences in means between normal saline and propranolol treatments are given.

(*a*) hypothalamic-pituitary responses including increased levels of prolactin, growth hormone, and corticol (3, 4); (*b*) hypothermia (7, 21); and *c* increased food and water intake (7). Cervical cord-sectioned patients have a similar but potentiated hormonal and behavioral response to 2DG (3, 4, 22). These potentiated responses may be related to a lack of descending sympathetic outflow and deficient glucocounter-regulation associated with markedly reduced catecholamine secretion (3).

The present study shows that 2DG-induced CNS glucoprivation activates mechanisms for water conservation. Potential factors including hyperosmolality (23), hypotension (15), β-adrenoreceptor stimulation (24), and hyperreninemia (14) for stimulating thirst and AVP release during glucoprivation were systematically studied in a cervical cord-sectioned patient and by administering neuropharmacological blocking agents to normal volunteers to eliminate various peripheral mechanisms.

Several considerations suggest that the dipsogenic and vasopressinemic effects of 2DG were not due to osmotic stimulation. Although osmolality increased slightly (1.5 mosmol/kg) at 60 min following 2DG infusion, this increase was neither significant nor sufficiently large to account for a 3.2 pg/ml increase in AVP (15). Moreover, it could be explained entirely by a 56 mg/dl increase in glucose, which by itself is not an effective osmotic stimulus for increasing AVP under conditions of euhydration (23). Small increases (0.8

TABLE II

Effect of 2DG on Plasma Concentrations in Five Normal Males Receiving Propranolol or Sham Infusions

		Time (min)						
		0	30	60	90	120	150	180
Osmolality, *mosmol/kg*	NS	282.5±1.2	282.5±2.1	285.3±1.8	284.4±1.8*	285.1±2.0‡	285.4±1.5*	287.2±2.3*
Osmolality, *mosmol/kg*	PR	282.5±1.9	285.8±1.3*	287.6±1.5‡	285.5±1.7	283.6±2.7	283.7±2.9	283.7±2.1
Sodium, *meq/liter*	NS	151.4±1.0	149.8±1.9	149.4±1.5	149.8±1.3	148.2±1.0*	148.2±2.5	150.6±2.0
Sodium, *meq/liter*	PR	149.6±0.4	150.0±1.1	150.0±2.0	148.8±0.8	147.4±1.9	148.4±1.3	150.4±2.1
Potassium, *meq/liter*	NS	4.3±0.3	4.5±0.5	3.9±0.2	3.6±0.2*	3.4±0.1*	3.4±0.1‡	3.5±0.1*
Potassium, *meq/liter*	PR	4.0±0.1	4.3±0.2	4.4±0.1*§	4.3±0.2*	4.4±0.1‡§	4.6±0.1‡§	4.5±0.1‡§
Aldosterone, *pg/ml*	NS	49±7	51±4	128±21‖	123±22‖	111±24‖	80±18	60±12
Aldosterone, *pg/ml*	PR	54±12	103±52	383±178‖¶	538±289‖¶	550±293‖¶	583±349‖¶	596±407
Glucose, *mg/dl*	NS	94±2	102±5‡	151±13‡	172±17‡	181±21‡	189±24‡	189±25‡
Glucose, *mg/dl*	PR	91±5	110±6‡	156±11‡	170±12‡	174±11‡	166±13‡	156±14‡§
Insulin, *% of control*	NS	100	—	158±24	—	203±34	—	337±99*
Insulin, *% of control*	PR	100	—	101±25	—	92±8§	—	134±18*§

50 mg/kg 2DG infused for 20 min in patients receiving propranolol or sham infusions. Values given are mean±SEM. PR, propranolol (3 mg/3 min at −30 min followed by 80 μg/min); NS, sham infusion of normal saline.

* $P < 0.05$ by paired Student's t test with values at 0 min.

‡ $P < 0.01$ by paired Student's t test with values at 0 min.

§ $P < 0.05$, by paired Student's t test with values during sham infusion.

‖ $P < 0.05$ by Wilcoxon signed rank test with values at 0 min.

¶ $P < 0.05$ by Wilcoxon signed rank test with values during sham infusion.

meq/liter) in sodium levels were observed after 2DG in one experiment but were not evident in all cases (Table II) and, in any event, were not large enough to account for a 3.2 pg/ml increase in AVP. Finally, a cervical cord-sectioned patient markedly increased water intake after 2DG in the absence of any sodium or glucose elevations (Fig. 4). Thus thirst, water intake, and AVP levels increased in the absence of elevated effective osmolality and cation levels which might otherwise stimulate behavioral and physiological mechanisms for increasing hydration.

With marked increases in water intake and AVP levels after 2DG infusion, decreases in plasma osmolality and sodium levels might be expected but were not observed except in the cervical cord-sectioned patient, who had no increase in glucose levels and who drank 2,450 ml of water. Thus the absence of decreases in osmolality may be due to contributions to measured plasma osmolality levels from increased glucose levels and to shifts of sodium from intracellular to extracellular space associated with movement of potassium into cells. Alternatively, increased renal conservation of sodium through activation of the renin-aldosterone system (Table II) and decreased filtered load or altered renal tubular reabsorption of sodium in association with a peak 33% reduction in renal plasma flow as measured by *p*-aminohippurate clearance (25) could explain the absence of significant decrements in sodium levels after water ingestion. Since both PRA (Fig. 2) and aldosterone increases (Table I) after 2DG were suppressed by mazindol administration, it is not surprising that osmolality and sodium levels were correspondingly somewhat lower after 2DG in subjects pretreated with mazindol (Table I). In contrast, although propranolol administration blocked PRA responses to 2DG (Fig. 3), plasma aldosterone levels increased markedly during β-adrenoreceptor blockade (Table II). Thus, no reduction in osmolality or sodium levels during β-adrenoreceptor blockade would be expected, nor was it seen (Table II).

In contrast to the hypokalemia produced by 2DG administration alone (Table II), hyperkalemia was observed after 2DG in propranolol-treated subjects. This may be due to blockade of a β-adrenoreceptor mechanism for extrarenal disposal of potassium (26). Increases in potassium levels in spite of hyperglycemia are unexplained but may be related to unopposed α-adrenoreceptor-mediated effects on potassium homeostasis since, during E infusions, β-adrenoreceptor blockade with propranolol permits development of hyperkalemia which can be blocked by administering α-adrenoreceptor antagonists such as phentolamine (27).

Potentiation of the aldosterone response to 2DG administration during β-adrenoreceptor blockade (Table II) could be a result of hyperkalemia (28) or decreased metabolic clearance of aldosterone (29), but would be expected to decrease, not increase, plasma potassium levels. The absence of an insulin response to 2DG during propranolol infusions might preclude the development of hypokalemia (30), but does not by itself explain the development of hyperkalemia. Further-

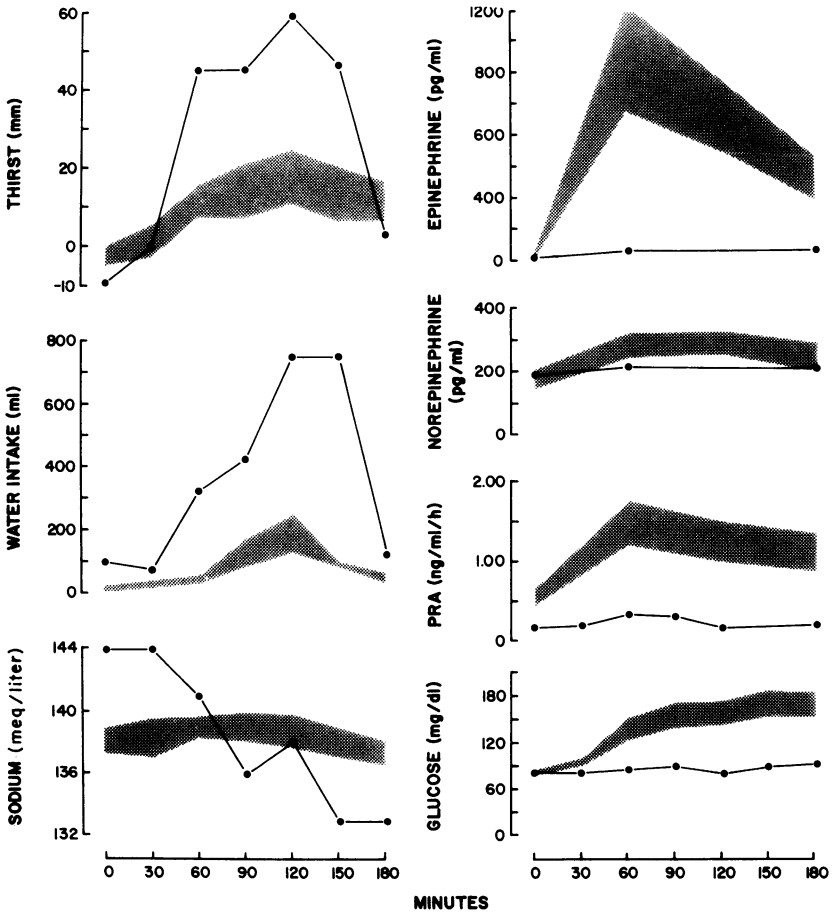

FIGURE 4 Effect of 50 mg/kg 2DG infused from 0 to 20 min on mean±SEM thirst ratings, water intake, plasma sodium, E, NE, PRA, and plasma glucose in a cervical cord-sectioned patient (●) and five normal subjects (shaded area).

more, other studies have indicated that small changes in insulin levels may not be sufficient to account for increased intracellular potassium uptake during hyperepinephrinemia or decreased intracellular potassium uptake found during β-adrenoreceptor blockade (26). Thus, the findings of the present studies are consistent with an α-adrenoreceptor-mediated mechanism for producing hyperkalemia (27), or blockade of a β-adrenoreceptor-mediated mechanism for diminishing potassium levels (26).

Peak AVP response occurred at 60 min while peak water intake occurred at 120 min after 2DG infusion, indicating that AVP secretion did not correlate well with water intake. Such a dissociation between AVP release and initiation of water drinking has been shown previously in dogs receiving hypertonic saline infusion (31). This temporal dissociation in response is unexplained but may indicate different mechanisms of control or different rates of response to a common control mechanism.

Nonosmotic factors including decreases in MAP (12, 15), changes in peripheral catecholamines (12, 24), and hyperreninemia (12, 14) did not provide the dipsogenic and vasopressinemic stimuli during 2DG-induced glucoprivation. The small (0–7.2%) decreases in MAP recorded in one of the present studies did not occur in others (Fig. 3) and, in any event, was insufficient to increase AVP levels 3–4 pg/ml (15). Moreover, mazindol administration eliminated any fall in MAP without diminishing the thirst and AVP responses to 2DG (Fig. 2). Finally, propranolol administration not only prevented any decrease after 2DG, but actually increased MAP 20.7%, without reducing thirst and AVP responses (Fig. 3). Therefore, it is unlikely that hypotension plays any role in the AVP and thirst response to 2DG-induced glucoprivation.

Increases in MAP with unopposed α-adrenoreceptor stimulation during β-adrenoreceptor blockade were expected (32), but they did not suppress AVP levels or drinking. The lack of AVP suppression in this circum-

stance is not in accord with previous demonstrations that NE infusions reduce antidiuretic hormone activity (24). The reason for this inconsistency is not apparent but may be related to differences in methods for producing α-adrenoreceptor stimulation, i.e., endogenous secretion of NE in this study versus peripheral administration of NE by others (24). Alternatively, 2DG-induced glucoprivation might inactivate neural pathways by which NE suppresses thirst or AVP secretion.

Activation of descending sympathetic pathways after 2DG-induced glucoprivation increases plasma levels of E, and to a lesser degree, NE. The increase in plasma E is associated with increases in thirst and AVP levels. Infusion of the β-adrenoreceptor agonist, isoproterenol, increases antidiuretic hormone response, while infusion of the α-adrenoreceptor agonist, NE, reduces antidiuretic hormone activity (24). Although it is possible that catecholamines affect AVP release and thirst through activation of a peripheral baroreceptor mechanism (24), or by stimulation of a CNS mechanoreceptor (33) in association with blood pressure changes, increases in E and NE are unlikely to have produced the AVP and thirst changes seen during 2DG-induced glucoprivation for the following reasons. First, if MAP decreases mediate the AVP and thirst response to β-adrenoreceptor stimulation, then the AVP and thirst response to 2DG must be mediated by a non-baroreceptor-mediated mechanism, since, as was noted, hypotension did not play a role in the observed responses. Second, E stimulation of β-adrenoreceptors is not the reason for increased AVP and thirst after 2DG, since β-adrenoreceptor blockade with propranolol did not supress the AVP and thirst response to glucoprivation (Fig. 3). The effectiveness of β-adrenoreceptor blockade with propranolol was established by finding a doubling of the hyperepinephrinemia normally seen after 2DG administration, an increase in MAP, and elimination of the renin response (Fig. 3). Potentiation of 2DG-induced hyperepinephrinemia by β-adrenoreceptor blockade may be related to a reduction in metabolic clearance of E, unopposed stimulation of α-adrenoreceptor-mediated neurotransmitter mechanisms, or blockade of critical β-adrenoreceptor negative feedback neurotransmitter systems in the CNS at the adrenal medulla. Raised E levels with β-adrenoreceptor blockade during insulin-induced glucopenia have been reported by others (32). Also, since propranolol passes the blood-brain barrier, β-adrenoreceptors located in the CNS are unlikely to be involved in the dipsogenic and vasopressinemic response to 2DG. Third, marked increases in thirst and water intake were produced by 2DG administration to a cervical cord-sectioned patient, who had no evidence of increased plasma E levels after 2DG (Fig. 4).

Finally, peripheral NE increases (Fig. 3) might be expected to decrease AVP levels (24), not increase them. Thus, increased peripheral E and NE levels and β-adrenoreceptor stimulation do not account for the dipsogenic and vasopressinemic response to glucoprivation. Our study, however, does not exclude the possibility that central noradrenergic neurotransmitter pathways are involved in the dipsogenic (34) and vasopressinemic response to 2DG.

Increases in AVP have been reported in association with stimulation of emetic centers (35). No nausea or vomiting was present in volunteers receiving 2DG and therefore is not likely to explain the thirst and AVP response to 2DG.

Hyperreninemia occurred in most volunteers receiving 2DG (Figs. 2 and 3). Since angiotensin II infusions are known to increase AVP in man (14) and thirst in rats (12), it is possible that 2DG-induced glucoprivation induces thirst and AVP secretion by activation of the peripheral renin-angiotensin system. This possibility is excluded by the following: First, both mazindol (Fig. 2) and propranolol (Fig. 3) administration suppressed PRA response to 2DG without diminishing the thirst and AVP responses. Second, 2DG failed to increase PRA levels in a cord-sectioned patient who nonetheless drank enormous quantities of water (Fig. 4). Finally, these observations are consistent with those of others who have reported no correlation betwen PRA and AVP levels in man during various states of hydration and volume depletion (36), and no diminution of the AVP response to orthostasis during propranolol blockade which, nevertheless, eliminated the PRA response to orthostasis (37). Since angiotensin II may act as a neurotransmitter in some hypothalamic areas it is still possible that central angiotensin II mediates the dipsogenic and vasopressinemic response to 2DG.

Although there is little evidence to suggest that hypersulinemia plays a direct role in the release of AVP and the stimulation of thirst, it is possible. Earlier studies have indicated that insulin levels do not change (3), or increase only slighly (21), relative to the hyperglycemia observed after 2DG infusions. In the present study the gradual rise in insulin levels was less than what might be expected for the level of hyperglycemia (38). This relative insulinopenia may be related to activation of catecholaminergic mechanisms controlling insulin secretion (39) and can be converted into an absolute insulinopenia by administering the β-blocking agent, propranolol (Table II). AVP levels and thirst, nonetheless, increased in the absence of an insulin response to 2DG during β-adrenoreceptor blockade. Thus, changes in plasma insulin do not mediate the effects of 2DG on thirst and AVP.

While hypovolemia may stimulate thirst (12) and vasopressin secretion (15), it is unlikely that this played a role in the thirst and AVP response to 2DG since normal saline was infused to replace the volume of

blood withdrawn and because blood volume decreases <5% as indicated by increases in hematocrit of <2% after 2DG infusions (40), whereas >10% fall in blood volume is necessary to stimulate AVP secretion in man (15).

Finally, increases in AVP levels may be the result of decreased metabolic clearance. A 20–30% decrease in renal plasma flow (25) could account for some AVP increases, but this effect on AVP levels would most likely be more prolonged than the one we observed. Furthermore, mazindol and propranolol administration eliminated both the MAP fall and secondary PRA increases after 2DG infusions, without affecting AVP and thirst responses. Thus, changes in renal clearance are unlikely to account for the increased AVP response to 2DG infusions. This conclusion is strengthened by the fact that thirst, a centrally determined event presumably occurring in parallel with activation of AVP release mechanisms, increased under all 2DG treatment conditions studied.

The findings from this investigation, in which AVP and thirst responses have been dissociated from peripheral mechanisms of control, and in related studies in which glucoprivation has been induced in cervical cord-sectioned patients (22), suggest that thirst and hunger, water and food intake, and AVP secretion, are under the control of CNS pathways whose functions are sensitive to changes in glucose metabolism.

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