Effects of 2-buten-4-olide, an endogenous satiety substance, on plasma glucose, corticosterone, and catecholamines

ITSURO MATSUMOTO, YUTAKA OOMURA, HIITO NISHINO, SEIJI NEMOTO, SHUJI AOU, AND TADAOMI AIKAWA

Department of Physiology, Nagasaki University School of Medicine, Nagasaki 852; Institute of Bio-Active Science, Nippon Zoki Pharmaceutical, Yashiro, Hyogo 673-14; and Department of Higher Humoral Control, National Institute for Physiological Science, Okazaki 444, Japan

Matsumoto, Itsuro, Yutaka Oomura, Hitoo Nishino, Seiji Nemoto, Shuji Aou, and Tadaomi Aikawa. Effects of 2-buten-4-olide, an endogenous satiety substance, on plasma glucose, corticosterone, and catecholamines. Am. J. Physiol. 266 (Regulatory, Integrative Comp. Physiol. 35): R413-R418, 1994.—Effects of 2-buten-4-olide (2-B40), an endogenous satiety substance, on levels of plasma glucose, corticosterone, and catecholamines were examined in fed, conscious, and unrestrained rats. A vascular indwelling catheter was inserted into the right atrium of the animal from the jugular vein 1 wk before the experiment. Injection of 2-B40 and blood sampling were performed through the catheter in an unanesthetized condition. The levels of plasma glucose, corticosterone, epinephrine, and norepinephrine increased significantly for 2 h after the start of intravenous injection of 2-B40 in a dose dependent manner. The increase in glucose and catecholamines induced by 2-B40 injection were abolished by bilateral splanchnicotomy (SPX) but not by pretreatment with anti-corticotropin-releasing factor (CRF) antibody. The increase in corticosterone was abolished not by the SPX but by pretreatment with anti-CRF antibody. These findings suggest that 2-B40, endogenously produced during food deprivation, may facilitate sympathoadrenal and hypothalamicpituitary-adrenal functions through the central nervous system.

sugar acid; blood glucose; epinephrine; norepinephrine

RECENTLY, THREE GROUPS OF ENDGENOUS SUGAR ACIDS WERE FOUND IN THE BLOOD OF FED AND FOOD-DEPRIVED RATS. 1) THE ACID (2S, 4S)-2,4,5-trihydroxypentanoic acid γ-lactone (2,4,5-TP) INDUCED FEEDING (16, 22), INCREASED INSULIN SECRETION (21), AND ENHANCED VAGAL EFFERENT ACTIVITY (15). 2) THE ACID (3S)-3,4-dihydroxybutanoic acid γ-lactone (3,4-DB) SUPPRESSED FEEDING (22), POTENTIATED SYMPATHETIC EF FERENT ACTIVITY, AND INCREASED BLOOD LEVEL OF GLUCOSE AND FREE FATTY ACID (FFA) (17). 3) THE SUBSTANCE 2-buten-4-olide (2-B40), A DERIVATIVE OF 3,4-DB, IS PHYSIOLOGICALLY MORE EFFECTIVE THAN 3,4-DB WHEN APPLIED BY PERIPHERAL ROUTES (19). IT HAS BEEN CONFIRMED THAT THE ACTION OF THESE SUGAR ACIDS ON FOOD INTAKE IS MEDIATED THROUGH CHANGE IN THE ACTIVITY OF GLUCOSE-SENSITIVE NEURONS IN THE LATERAL HYPOTHALAMIC AREA (LHA), A FEEDING CENTER, AND OF GLUCORECEPTOR NEURONS IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS (VMH), A SATIETY CENTER (7, 17, 19, 20, 23). THEIR CONCENTRATION IN BLOOD CHANGED IN DIFFERENT WAYS DURING FOOD DEPRIVATION (18), SUGGESTING THAT THEY MAY AFFECT FOOD INTAKE BY MEDIATING HUNGER AND SATIETY SENSATIONS.

IT IS WELL KNOWN THAT THE PITUITARY-ADRENAI AXIS IS ACTIVATED (6, 12, 13) AFTER FOOD DEPRIVATION DESPITE A DECREASE IN OTHER HYPOPHYSIAL HORMONE SECRETIONS (5, 24, 27). FURTHERMORE, FASTING IN A COLD ENVIRONMENT OR FASTING HYPOGLYCEMIA INCREASES ADRENAL-MEDULLARY SECRETORY ACTIVITY, BUT NOT SYMPATHETIC ACTIVITY (29). AS ADRENOCORTICAL AND MEDULLARY HORMONES EXHIBIT PERMISSIVE ACTION ON ELEVATION OF BLOOD GLUCOSE, THE INCREASE IN THESE CATABOLIC HORMONES AFTER FOOD DEPRIVATION MAY FUNCTION IN THE PREVENTION OF HYPOGLYCEMIA DURING PROLONGED FASTING (3). THE DATA SUGGEST THAT THESE SUGAR ACIDS, PRODUCED ENDOGENOUSLY DURING FOOD DEPRIVATION, MAY AFFECT INCREASE IN CATABOLIC HORMONES THROUGH ACTIVATION OF THE CENTRAL NERVOUS SYSTEM, AND THESE GLUCOSE COUNTERREGULATORY FACTORS MAY PREVENT SERIOUS HYPOGLYCEMIA IN FOOD DEPRIVED ANIMALS. THE PURPOSE OF THE PRESENT STUDY WAS TO EXAMINE WHETHER OR NOT 2-B40 AFFECTS THE LEVEL OF BLOOD GLUCOSE, THE SECRETION OF ADRENOCORTICAL AND ADRENOGLUCOCORTICOIDAL HORMONES, AND THE ACTIVATION OF SYMPATHETIC OUTFLOW IN ORALLY FEEDED RATS.

MATERIALS AND METHODS

ANIMALS. Male Wistar rats (300-350 g) were housed individually in a 12:12-h light-dark cycle (lights on at 0600 h) and maintained at a temperature of 24 ± 1°C with free access to food and water. Before each experiment all animals were handled for 5 min daily for 6 successive days. After being anesthetized with sodium pentobarbital (50 mg/kg), each rat was implanted with a chronically indwelling silicone catheter (OD 1.0 mm) inserted into the right jugular vein at a point just outside the atrium and brought out subcutaneously at the back of the neck. The animals were allowed to recover from the operation for 1 wk before being used for experiments. Through the implanted silicone catheter, 0.4 ml blood samples were taken within 2 min. Blood cells of each sample were resuspended in the same volume of saline and replaced in the animal after the next sampling period. All experimental procedures involving blood sampling were performed from 0900 to 1400 h. Plasma was stored at −20°C with 0.2% EDTA, for not more than 2 mo, until assayed for catecholamines and corticosterone. In six rats, the bilateral splanchnic nerves were dissected below the diaphragm under pentobarbital sodium anesthesia 2 wk before the experiment.

ADMINISTRATION OF DRUGS. 2-B4O (Aldrich) was dissolved in 0.3 ml saline and administered from 0900 to 0930 h via the implanted catheter. Animals were allowed free access to food throughout the experiment. Lyophilized anti-corticotropin-releasing factor (CRF) antibody (Peptide Institute) was dissolved in saline and used within 3 days. Anti CRF antibody solution of 0.3 ml (containing 20 μl rabbit antiserum against human CRF) was administered to an animal 1 h before the start of an experiment.

MEASUREMENTS. Corticosterone in plasma was measured by a fluorometric method after purifying the plasma sample by thin-layer chromatography on silica gel. Plasma glucose was assayed by the glucose oxidase-para-aminophenol method. Plasma catecholamines were measured by the coulometric electrochemical determination method (9) using a high-sensitivity fluorometer with a glass electrode. Blood pressure and heart rate were measured with a water-coupled catheter transducer (Nihon Kohden BP-204S). The experiment was terminated 3 h after the start of drug administration by decapitation. Three to four rats were used per condition and all results are expressed as means ± SE. The Student's t-test was used for statistical analysis.

Plasma glucose, insulin, corticosterone, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode. Plasma glucose, insulin, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode. Plasma glucose, insulin, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode. Plasma glucose, insulin, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode. Plasma glucose, insulin, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode. Plasma glucose, insulin, and catecholamine levels were examined in the present study as indicators of the metabolic effects of the catabolic hormones on glucose and free fatty acid storage.

Plasma glucose levels were measured by the glucose oxidase-para-aminophenol method. Plasma insulin and corticosterone were measured by radioimmunoassay. Plasma catecholamines were measured by electrochemical determination using a high-sensitivity fluorometer with a glass electrode.
performance liquid chromatographic analytic system (ESA, model 5100A, coulochem detector). In brief, 0.2 ml plasma was placed in a disposable column for column chromatography (Sepacol, Seikagaku Kogyo, Tokyo) with 80 mg alumina, 1 ml tris(hydroxymethyl)aminomethane (Tris) buffer solution (pH 8.6), 1 ml phosphate buffer solution (pH 7.0), and a known amount of 3,4-dihydroxybenzylamine, which served as an internal standard. Each sample was shaken for 20 min to allow amines to be adsorbed onto the alumina, and the supernatant was aspirated to near dryness. After 160 ~1 of 0.2 M perchloric acid were added to the alumina phase, the acidic eluate containing the desired catecholamines was centrifuged in the receiver tube, and 80 ~1 of the final acidic eluate were injected onto a C18 reverse-phase column. The mobile phase contained 0.02 M trichloroacetic acid, 0.075 M sodium dihydrogenphosphate, 0.05 % EDTA, and 5% methanol-10% acetonitrile-85% H2O (pH 3.1). The mobile phase was circulated at a flow rate of 1.2 ml/min at 40°C by a column stove. All components were eluted within 12 min. Minimal detectable concentration of desired catecholamines ranged from 3 to 5 pg/vial. We also calculated the integrated glucose and corticosterone responses induced by 2-B40 (area under the curve) in intact and treated animals. The integrated responses were the incremental values above basal levels of their respective concentrations over a period of 240 min after the 2-B40 injection.

Statistical analysis. Data were analyzed by one- or two-way analysis of variance (ANOVA) corrected for repeated measures by means of a computer software program for statistical analysis (Fisher, Tokyo University, Tokyo, Japan) using a personal computer (NEC, PC-9801). When there was significant overall effect by ANOVA, the significant differences from baseline within and between groups at each time point were tested by appropriate post hoc statistics using the same computer software system. Differences in the integrated corticosterone responses induced by 2-B40 injection between animals with intact splanchnic nerve and splanchnicotomized animals were analyzed by Student’s t test.

RESULTS

Plasma glucose concentration. Changes in plasma glucose concentration induced by intravenous administration of 2-B40 are shown in Fig. 1, A and B. Significant increases in glucose level induced by ≥1 μmol/kg 2-B40 were observed (Fig. 1A). The glucose level at 40 min and the integrated glucose response for 240 min after the injection increased dose dependently (Fig. 1B, semilog plot). The increase peaked 20–60 min after the 2-B40 administration. The significantly elevated glucose concentration induced by ≥30 μmol/kg 2-B40 had not yet returned to the basal level 4 h after administration.

Plasma corticosterone concentration. Changes in plasma corticosterone concentration induced by intravenous administration of 2-B40 are shown in Fig. 2, A and B. Significant increases in the corticosterone level induced by ≥1 μmol/kg 2 B40 were observed (Fig. 2A). The corticosterone level at 40 min was saturated by 3 μmol 2-B40, but the integrated corticosterone response for 240 min after the injection increased dose dependently (Fig. 2B, semilog plot). The peak occurred 60 min after the 2-B40 administration, after which the level gradually decreased to the basal level 180 min after administration.

Plasma catecholamine concentrations. Significant increases in epinephrine from 20 to 120 min and in norepinephrine from 20 to 60 min after the start of intravenous administration of 30 μmol/kg 2-B40 are shown in Figs. 3 and 4, respectively. The concentrations reached their peaks at 40 min and then gradually returned to the basal level.

Effect of splanchnicotomy on plasma catecholamines. Effects of bilateral splanchnicotomy (SPX) on increases in epinephrine and norepinephrine in response to administration of 30 μmol/kg 2-B40 are also shown in Figs. 3 and 4, respectively. The basal level of epinephrine was significantly depressed and the increase was completely suppressed by SPX. No significant changes in norepinephrine levels after the 2-B40 injection were observed in splanchnicotomized rats (time effect). Significant differences in norepinephrine levels after the injection were found between splanchnic nerve intact and splanchnic...
ADRENAL SECRETIONS BY A SATIETY SUBSTANCE IN RATS

Fig. 2. Effect of 2-B40 on plasma corticosterone concentration. A: changes in plasma corticosterone concentration before and after administration of saline or 1, 3, 30, or 300 μmol/kg 2-B40 iv in fed rats. B: dose-response curves of corticosterone level at 40 min (●) and integrated corticosterone response for 240 min (●) after administration of 2-B40. Values are means ± SE; nos. in parentheses are nos. of animals. Significantly different from saline control: *P < 0.05; **P < 0.01 (ANOVA and post hoc test).

Fig. 3. Changes in plasma epinephrine concentration before and after administration of 30 μmol/kg 2-B40 iv in fed rats. Values are means ± SE; nos. in parentheses are nos. of animals. SPX, bilateral splanchnicotomy below diaphragm. Significantly different from saline control: *P < 0.05; **P < 0.01; #P < 0.05; ##P < 0.01 (ANOVA and post hoc test).

Fig. 4. Changes in plasma norepinephrine concentration before and after administration of 30 μmol/kg 2-B40 iv in fed rats. Values are means ± SE; nos. in parentheses are nos. of animals. Significantly different from saline control: *P < 0.05; **P < 0.01. Significantly different from splanchnic nerve-intact rats: *P < 0.05; **P < 0.01 (ANOVA and post hoc test).

Fig. 5. Effect of SPX on increase in plasma glucose concentration induced by injection of 30 μmol/kg 2-B40 iv. Values are means ± SE; nos. in parentheses are nos. of animals. Significantly different from splanchnic nerve-intact animals: *P < 0.05; **P < 0.01 (ANOVA and post hoc test).
plasma corticosterone level induced by the injection between splanchnic nerve intact and splanchnicotomyed rats were observed (group effect) (Fig. 6, left). However, no significant difference in the integrated corticosterone response induced by the injection was observed between the two groups (Fig. 6, right).

**Effect of anti-CRF antibody.** The effects of pretreatment with anti-CRF antibody on increases in the plasma corticosterone and glucose concentrations in response to 30 μmol/kg 2-B40 injection are shown in Figs. 7 and 8, respectively. In rats pretreated with anti CRF antibody, the basal level of plasma corticosterone was not significantly suppressed compared with rats without anti-CRF antibody pretreatment. The increase in plasma corticosterone was significantly attenuated by pretreatment with anti-CRF antibody. However, there was no significant change in the basal level or elevated level of plasma glucose concentration induced by 2-B40 after pretreatment with anti-CRF antibody. The increases in plasma concentration of epinephrine and norepinephrine were also unaffected by this pretreatment (data not shown).

**DISCUSSION**

It is well known that starvation results in significant increases in plasma level of ACTH (6) and corticosterone (6, 12, 13) and that fasting at cold temperature or fasting hypoglycemia increases adrenal medullary secretion (29). In the present experiment, the administration of 1-300 μmol/kg 2-B40 dose dependently increased the plasma levels of glucose (Fig. 1) and corticosterone (Fig. 2) in normally fed animals. In addition, administration of 30 μmol/kg 2-B40 increased the plasma level of epinephrine and norepinephrine (Figs. 3 and 4).

Niijima et al. (28) observed that intravenous injection of 2-B40 from 0.02 to 0.2 μmol dose dependently increased firing rates in the pancreatic, hepatic, splenic, and adrenal sympathetic efferent nerves in anesthetized rats. In the present experiments, the basal level of epinephrine was significantly decreased, and the increase in response to 2-B40 was completely abolished by SPX (Fig. 3). On the other hand, the basal level of plasma norepinephrine was not altered by SPX, although the increase caused by 2-B40 was significantly attenuated (Fig. 4). The increase in plasma glucose concentration in response to 2-B40 was completely abolished by SPX but not by pretreatment with anti-CRF antibody. These data suggest that the increase in glucose induced by 2-B40 is mainly caused through both adrenomedullary secretion and sympathetic outflow into visceral organs. Boyle et al. (3) studied the prevention of hypoglycemia during fasting and concluded the following. 1) Glucagon had a primary counterregulatory function in the prevention of hypoglycemia during fasting. 2) Catecholamines were not normally critical but compensated and became critical to the prevention of hypoglycemia during fasting. 3) The relevant catecholamine was most probably adrenomedullary epinephrine. Although
we did not monitor change in plasma glucagon concentration, 2-B40 increased firing of the hepatic and pancreatic sympathetic efferents, so glucagon as well as liver glycogen may also contribute to the increase in plasma glucose induced by 2-B40 together with adrenomedullary epinephrine. Furthermore, the increase in plasma corticosterone induced by 2-B40 may act to maintain the catabolic state in food-deprived animals (8, 13).

In the present experiment, the increases in the plasma level of corticosterone in response to 2-B40 were not abolished by SPX (Fig. 6) but they were attenuated by pretreatment with anti-CRF antibody (Fig. 7). These data suggest that activation of adrenocortical and medullary secretory responses induced by 2-B40 is caused via the central nervous system. Oomura et al. showed that 2-B40 and 3,4-DB activate neuronal activity in the paraventricular nucleus (PVN) (2) and acts within the brain to stimulate the activity of the sympathetic nervous system (4). Electrical stimulation of PVN neurons in rats increased sympathetic outflow (11). Although it remains uncertain how 2-B40 activates sympathoadrenomedullary and hypothalamic-pituitary-adrenocortical functions, we recently observed that microelectrophoretic application of 2-B40 to parvocellular neurons in the PVN (28) activates sympathoadrenomedullary and hypothalamic-pituitary-adrenocortical functions, we recently observed that microelectrophoretic application of 2-B40 to parvocellular neurons in the PVN. Furthermore it is likely that anti-CRF antibody, intravenously injected, may immunoneutralize endogenous CRF in the portal vessels and/or in the pituitary gland and then may suppress the secretory activity of corticotropic cells (15).

It has been shown that CRF is closely associated with anorexia or suppression of food intake (25). Intracerebroventricular injection of CRF depressed food intake in rats (1, 14). In patients with anorexia nervosa, the level of CRF in cerebrospinal fluid is significantly higher than that in normal subjects (10). However, it is still unclear whether the depression of food intake induced by exogenous or endogenous 2-B40 is due partly to any effect of CRF in the central nervous system.

Oomura (18) showed that the concentration of 2-B40 in plasma increased from 3.5 to 13.5 μM 48–60 h after the start of food deprivation in rats. We did not determine the concentration of 2-B40 in plasma after an intravenous injection of 2-B40. Assuming the total plasma volume in a rat to be ~11 ml, the concentration of 2-B40 in plasma could be estimated at 28 μM after 1 μmol/kg 2-B40 injection if not metabolized and not absorbed by red or white blood cells. There is no information concerning the concentration of 2-B40 in the brain or cerebrospinal fluid after the intravenous injection of 2-B40. However, it has been reported that the concentration of ketone bodies in cerebrospinal fluid was ~50% of that in the blood (26). Thus the concentration of 2-B40 in cerebrospinal fluid could be estimated as ~14 μM. Therefore, the minimal effective dose of 2-B40 used in this experiment was comparable to the increased concentration of 2-B40 in plasma 48–60 h after beginning food deprivation.

The tissue that is producing the endogenous sugar acids is at present unclear, although 2-B40 and 3,4-DB are derived from glutamate and 3,4,5-TP from glucose (22). The 2-B40 in blood might be a result of continuous carbohydrate and/or lipid metabolism during a catabolic state. It seems to be paradoxical that 2-B40 activates the counterregulatory action during food deprivation while at the same time suppresses food intake. The concentration of 2-B40 increases progressively from 36 h and elevates to the peak at 48–60 h, but 2,3,5 TP reaches the peak at 12 h after the deprivation (18). This fact might explain the poststarvation anorexia noted in rats and humans. These data suggest that some sugar acids, and at least in part 2-B40, that are produced endogenously may participate in maintenance of the catabolic state during food deprivation through facilitation of the visceral sympathetic outflow, and adrenomedullary and adrenocortical functions, and might also result in the weakened hunger sensation for adaptation to food deprivation via the central nervous system.

We thank Professor A. Simpson (Showa University) for help in preparation of the manuscript.

This work was supported partly by Grants-in-Aid for Scientific Research 60440997 and 61870102 (Y. Oomura) from the Ministry of Education, Science, and Culture of Japan.

Address for reprint requests: I. Matsumoto, Dept. of Physiology, Nagasaki Univ. School of Medicine, Nagasaki 852, Japan.

Received 23 July 1992; accepted in final form 29 June 1993.

REFERENCES


