Physiological Significance of 2-Buten-4-Olide (2-B4O), an Endogenous Satiety Substance Increased in the Fasted State

YUTAKA OOMURA,*[,1] SHUJI AOU,† IKUO MATSUMOTO,‡ AND TOSHIIE SAKATA§

*Department of Integrated Physiology, Faculty of Medicine, Kyushyu University, Fukuoka, Japan; †Department of Brain Science, Life Science Technology, Kyushyu Institute of Technology, Kita-Kyushyu, Japan; ‡Department of Physiology, Faculty of Medicine, Nagasaki University, Nagasaki; And §Department of Internal Medicine, Oita Medical University, Oita, Japan

A sugar acid, 2-B4O, has been found to increase from 3.5 to 13 μM in rat serum at 36 h after food deprivation. Injections of 2-B4O (2.5 μM) into the rat III cerebral ventricle (III ICV) suppress food intake and single neuronal activity in the lateral hypothalamic area (LHA). 2-B4O is effective even in 72 h food-deprived rats. 2-B4O hyperpolarizes glucose-sensitive neurons in the LHA via Na^+-K^+ pump activation, but depolarizes glucoreceptor neurons in the ventromedial nucleus (VMH) via closure of ATP-sensitive K channels. The plasma levels of glucose, corticosterone, and catecholamines, and the firing rate in both parvocellular neurons in the paraventricular nucleus (PVN) and sympathetic efferent nerves, all increase 2-B4O intravenous (iv) injection, indicating activation of the hypothalamo-pituitary-adrenal axis. A 2-B4O iv injection facilitates emotional and spatial learning and memory, and pretreatment with anti-acidic fibroblast growth factor (aFGF) antibody ICV eliminates these effects. aFGF is released from ependymal cells in the III cerebral ventricle in response to the glucose increase in CSF induced by 2-B4O iv injection. 2-B4O also suppresses the clinical symptoms of experimental allergic encephalomyelitis (EAE) in Lewis rats [induced by immunization with a myelin basic protein (MBP)], a model for human multiple sclerosis. After immunization with MBP, the delayed-type hypersensitivity response to MBP is also reduced in 2-B4O-treated rats. 2-B4O thus suppresses autoimmune responses. These results indicate that 2-B4O is not only a powerful satiety substance, but also effective as an activator of the hypothalamo-pituitary-adrenal axis and sympathetic efferent outflow, and as a memory facilitation and a modulator of immune functions. Exp Biol Med 228:1146–1155, 2003

Key words: 3,4-dihydroxybutanoic acid-γ-lacton; 2-buten-4-olide; satiety substance; learning and memory facilitation; sympathetic activity; corticotropin-releasing factor; immune modulation

Endogenous Sugar Acids

Plasma Concentration Changes During Food-Deprivation. A group of sugar acids has been found in the blood of rats. One of these, 2(s), 4(s), 5-trithydroxypentanoic acid γ-lactone (2,4,5-TP), induces feeding, while 2 others, 3(s), 4-dihydroxybutanoic acid γ-lactone (3,4-DB), and 2-buten-4-olide (2-B40), suppress feeding (1, 2). The actions of all 3 are mediated via glucose-sensitive neurons (GSNs) and glucoreceptor neurons (GRNs) in the hypothalamus. These 3 substances were selected from among 18 that were measured in the blood by gas mass spectrometry since while the levels of all 18 changed during food deprivation, the above 3 substances changed in ways that suggested they might affect feeding behavior. The changes in the concentrations of these 3 substances in the blood during a period of food deprivation are shown in Figure 1 (3). During such deprivation, the level of 2,4,5-TP increased from 220 μM to a peak of about 360 μM at 12 h, decreased slightly at around 24 h, then began rising again, and was still rising at 60 h. The 3,4-DB level increased from an initial level of about 120 μM to a peak of about 160 μM at 48 h after the start of deprivation. The 2-B4O level increased from an initial level of 3.5 μM up to 13.5 μM at 48 to 60 h with a similar time course to that of 3,4-DB.

Effects of Sugar Acids on Food Intake. In agreement with the time courses of the above changes, 2,4,5-TP increases and 3,4-DB depresses feeding, although the effects of both depended on the time of application during the day, and the effects of 3,4-DB also depend on the route of application (1). When 2,4,5-TP was injected III ICV into rats at 10:30 h, a dose of 2.5 μmol transiently induced feeding, but the total daily increase in food intake was not significant. Injection of the same dose at 1900 h increased food intake significantly until 2300 h, but not thereafter. Injection iv or ip at 1900 h increased food consumption dose-dependently, the range being from +17% at 100 μmol up to +39% at 500 μmol. Injection III ICV of 2.5 μmol 3,4-DB at 1630 h significantly decreased food consumption

[1] To whom requests for reprints should be addressed at Department of Integrated Physiology, Faculty of Medicine, Kyushyu University, 6-4-17, Kawamo, Takarazuka, Hyogo 665-0848, Japan. E-mail: y_oomura@hotmail.com

1535-3702/03/22810-1146$15.00

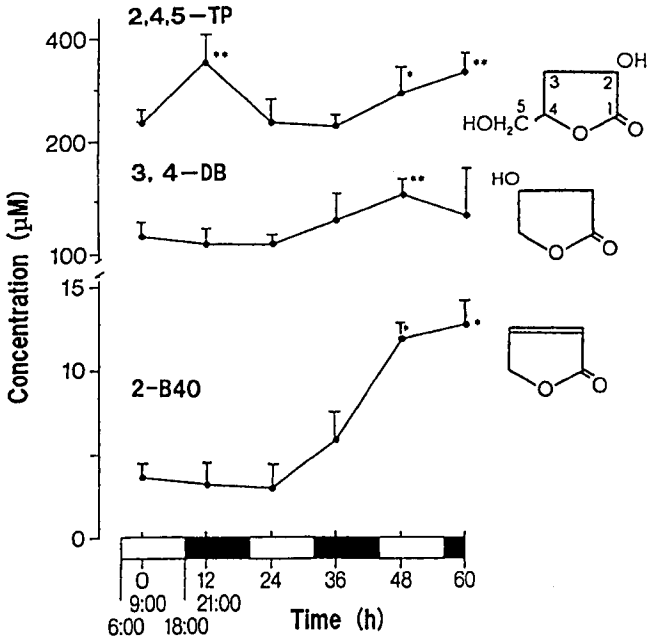

Figure 1. Changes in 2,4,5-TP, 3,4-DB, and 2-B4O levels after food deprivation. 2,4,5-TP increased initially at the normal feeding time and again after 2 days of deprivation, suggesting an induction of the drive to eat. 3,4-DB and 2-B4O increased during the period of severe deprivation, which might be accounted for by starvation-induced anorexia. Although the absolute level of 2-B4O was far below those of 3-,4-DB and 2,4,5-TP, its apparent sensitivity to deprivation was much greater. Abscissa, time: upper scale, hours of deprivation; lower scale, real time; black area, dark period. Ordinates, μM concentrations in rat plasma; note scale differences. Mean ± SEM (n = 10 rats). Significantly different from basal level, $^{*}P < 0.05$; $^{**}P < 0.01$ (Reproduced with permission from Ref. 3).

for 12 h, followed by recovery at 24 h. Motor activity was not affected, and there was no other overt evidence of toxicity. Similar results were observed in rats deprived of food for 72 h. Despite the effectiveness of III ICV injections, injection of 100 to 500 μmol 3,4-DB either iv or ip at 1900 h did not affect food consumption. The lack of effect of peripheral injections was attributed either to failure to cross the blood-brain barrier or to adhesion of 3,4-DB to blood vessels or other organ tissue. Massive 2.5- mmol doses injected into the carotid artery did suppress feeding. However, iv or ip injection at 1900 h of 1.25 or 2.5 mmol 3,4-DB encapsulated within liposome vesicles significantly depressed feeding in a dose-related manner (3). Probably 8% of the amount used was actually encapsulated (0.1 and 0.2 mmol), and 1% of this reached the brain (1 and 2 μmol). These amounts reaching the brain are comparable with the effective intracerebroventricular dose.

A physicochemical derivative of 3,4-DB is 2-B4O, and this has equal biological activity, but greater lipophilicity. Interestingly, 2-B4O was initially synthesized for testing, but it was later identified in and isolated from blood serum. The effects of III ICV injection of 1.0 μmol 2-B4O were found to be similar in nature and in magnitude to those of 2.5 μmol 3,4-DB in both the normal and 72-h food-deprived rat (2). When ip administered, 2-B40 significantly depressed feeding dose-dependently at 0.3 to 1.1 mmol/kg. Administration of 2-B4O intragastrically in doses of 0.5 to 3.3 mmol/kg also suppressed food intake dose-dependently. Thus, 2-B40 is biologically as active as 3,4-DB, but unlike 3,4-DB is effective by central, peripheral, or oral application (2).

If the volume of rat CSF is taken as about 300 μl, the concentrations of any of these sugar acids in CSF could be estimated as 4.2 to 8.3 mM after 1.2 to 2.5 μmol injection III ICV (if they are not metabolized). If CSF is produced at a rate of 0.5% of the total volume per minute, the concentration of sugar acids would be diluted to 22% of the initial concentration (i.e., 0.9–1.8 mM) at about 5 h after their injection. The concentration of ketone bodies in CSF is about 50% of that in the blood (1), and increases from 0.12 to 1.6 mM after 96-h food deprivation. This 1.6 mM value is similar to the 5 h concentration estimated for the sugar acids. Because diffusion from the cerebral ventricle to the brain parenchyma is about 1% after 20 min, the concentrations of 3,4-DB, 2-B40, and 2,4,5-TP available to act on the LHA and VMH at this time should be no more than 50 to 100 μM, and only 10 to 20 μM at 5 h after their injection.

Changes in Glucose and Insulin Concentrations in Plasma. The plasma levels of glucose and insulin are changed by III ICV injections of 3,4-DB and 2,4,5-TP at 2.5 μmol concentration (4). As shown in Figure 2A, blood glucose was increased by approximately 20% by 3,4-DB, at first transiently, and then persistently for up to 8 h after the injection. In contrast, plasma insulin was not significantly affected by 3,4-DB (Fig. 2B). Glucose was initially depressed by 20%, but later recovered after 2,4,5-TP injection, while the insulin level increased to 2.6 times control. The increase in the insulin level induced by 2,4,5-TP agrees with the increased vagal efferent activity and decreased splanchnic efferent activity to the pancreas that appear after a 2,4,5-TP-induced increase in neuronal activity in the LHA and decrease in that in the VMH. Blood glucose was increased for approximately 5 h to 1.5 times control by an ip injection of 30 μmol/kg 2-B4O. During that time, vagal efferent activity decreased and splanchnic efferent activity to the pancreas increased (4).

The changes induced in the plasma levels of glucose and insulin by 3,4-DB, 2-B4O, and 2,4,5-TP are consistent with the effect that each substance has on feeding behavior. The gastric acid secretion induced by 2-deoxy-D-glucose was suppressed by application of 3,4-DB or 2-B4O to the LHA, while secretion was elicited by 2,4,5-TP applied the same way (5).

Channel Activity Changes in Glucose-Sensitive Neurons and Glucoreceptor Neurons by Sugar Acids. In anesthetized rats, electrophoretic applications of 3,4-DB and 2-B4O (using the multibarreled-electrode technique) suppressed, while application of 2,4,5-TP enhanced the activity of GSNs in the LHA (each, dose-dependently) (1). The effects of 3,4-DB, 2-B4O, and 2,4,5-TP on non-GSNs were negligible. The suppression of GSNs induced by

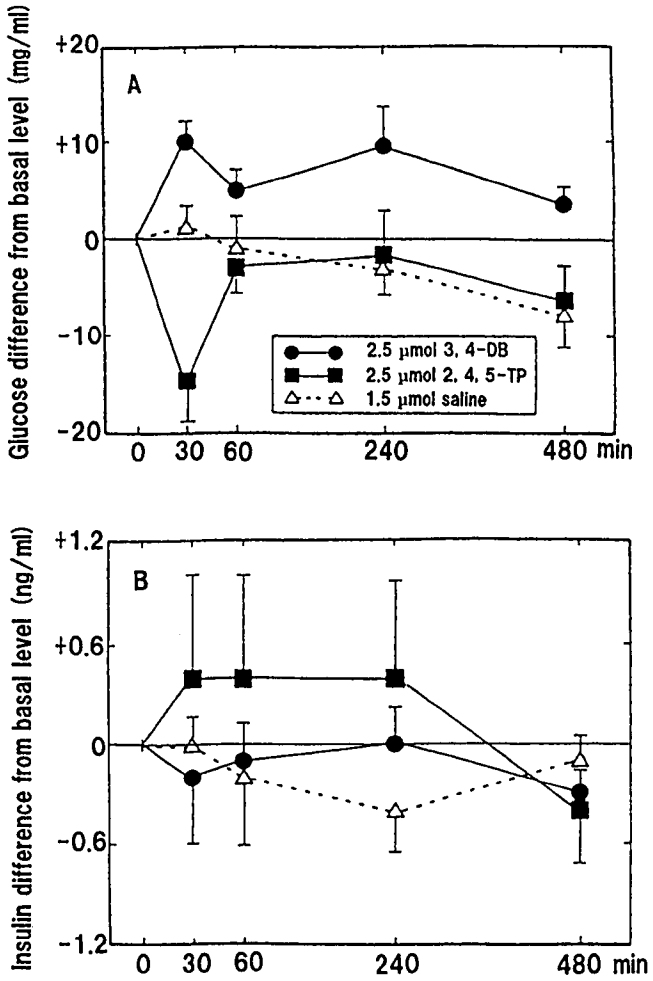

Figure 2. Time course of changes in (A) serum glucose and (B) insulin after infusion of 2.5 μmol 3,4-DB, 2.5 μmol 2,4,5-TP, or 1.5 μmol saline into the rat III cerebral ventricle at 1100 h. For (A) glucose or (B) insulin, values are mean differences ± SEM from corresponding basal value (measured for 2 days before infusion). Mean baseline values ± SEM in the 3,4-DB, 2,4,5-TP, and saline (control) groups were: for glucose, 118.3 ± 2.6 mg%, 116.3 ± 3.4 mg%, and 118.0 ± 3.9 mg%; for insuline, 2.1 ± 0.4 ng/ml, 1.7 ± 0.3 ng/ml, and 1.7 ± 0.1 ng/ml; n = 5 rats in each infusion set (Reproduced with permission from Ref. 4).

3,4-DB (and that induced by glucose) was reversibly blocked by ouabain. GRN activity in the VMH was dose-dependently inhibited by electrophoretic application of 2,4,5-TP, but facilitated by 3,4-DB and 2-B4O (1, 6, 7). No non-GRNs were affected by any of these 3 compounds, and no cortical neurons that responded to electrophoretic application of 3,4-DB, 2-B4O, or 2,4,5-TP were found. The effects of 3,4-DB, 2-B4O, and 2,4,5-TP on membrane potential and resistance have been investigated in rat hypothalamic slice preparations (7, 8).

Glucose-sensitive neurons were hyperpolarized by 3,4-DB, while membrane resistance remained constant, and the reversal potential for the 3,4-DB hyperpolarization could not be obtained. Moreover, depression of this hyperpolarization by ouabain indicated that the effect of 3,4-DB, like that of glucose, was due to Na^+-K^+-pump activation through the action of excessively produced ATP. GRNs were depolarized by both 3,4-DB and 2-B4O, while membrane resistance increased (Fig. 3A). The reversal potentials for the 3,4-DB- and 2-B4O-induced depolarizations were each about −90 mV, close to the K^+ equilibrium potential (Fig. 3B). Since this result was the same for glucose, the depolarization induced by glucose and those induced by 3,4-DB and 2-B4O are all caused by K_{ATP}-channel closure. These results also suggest that 3,4-DB and 2-B4O can be metabolized by the brain, like ketone bodies, if there is a shortage of glucose. Facilitation of GSN activity by 2,4,5-TP was due to depolarization, with a decrease in membrane resistance. The reversal potential for this depolarization was about + 40 mV, close to the Na^+ equilibrium potential, indicating Na^+ and Ga^{2+} channels opening. Inhibition of GRN activity by 2,4,5-TP was due to hyperpolarization, with a decreases in membrane resistance. The reversal potential for this hyperpolarization was about −90 mV, showing that 2,4,5-TP opened K_{ATP} channels (8).

Structural Characteristics of Endogenous Sugar Acids and Their Relation to Feeding Modulation

3,4-DB and 3,4,5-trihydroxypentanoic acid γ-lactone (3,4,5-TP) (2.5 μmol) decrease rat food intake following their III ICV application for at most 24 h (4). Infusion of 2,4-dihydroxybutanoic acid γ-lactone (2,4-DB) (1.25

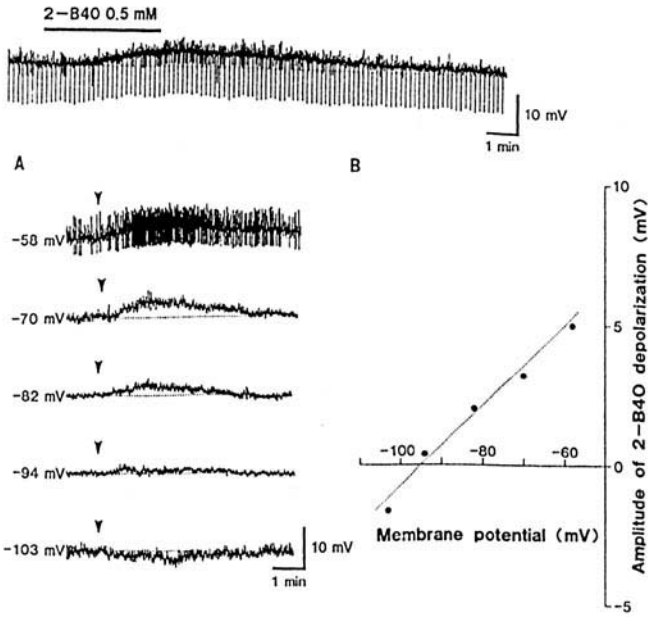

Figure 3. Effect of 2-B4O on rat VMH neurons. *In vitro* brain slice preparation. Upper: 2-B4O depolarization. Resting membrane potential, −55 mV. Bar above trace, application period. Downward deflection, electrotonic potential induced by constant-current hyperpolarizing pulses passed through recording electrode to measure input resistance. (A) Measurement of reversal potential for the depolarization induced by 2-B4O. Responses to 2-B4O recorded at various membrane potentials. Depolarization was reversed to hyperpolarization by passing current between -94 and -103 mV. Arrowhead: application of 2-B4O (0.5 mM) into perfusing medium. (B) Plots of values obtained in A. Reversal potential for 2-B4O depolarization was −95 mV (Reproduced with permission from Ref. 7).

μmol), 2,4,5-TP; (2.5 μmol), or an exogenous compound, 2,4,5,6-tetrahydroxyhexanoic acid γ-lactone (2, 4, 5, 6-TH) (2.5 μmol), all induced transient initial feeding that was not necessarily accompanied by periprandial drinking (4). Ambulation was concomitantly increased. Of these organic acids, 3,4-DB and 2,4,5-TP were the most potent in their effects on feeding. The only apparent structural difference between these two 4-butanolides is the attachment of a hydroxyl group on C-3, which seems to be related to satiation, or on C-2 of the lactone ring, which seems to be related to hunger (see Fig. 1). The attachment of a hydroxymethyl or dihydroxyethyl group on a residual fourth carbon seems to modulate the potency of a satiety or hunger substance, as in 2,4,5-TP, 3,4,5-TP, and 2,4,5,6-TH (Fig. 1) (4). Thus, it is probable that these structurally different, endogenous organic acid γ-lactones are important in feeding modulation because, albeit with different potencies, they all convey intrinsic satiety or hunger signals to neurons in the hypothalamus.

Effects of 2-B4O

Effects of 2-B4O on Plasma Glucose, Corticosterone, and Catecholamines, and on Activation of the Hypothalamo-Pituitary-Adrenal Axis. The effects of 2-B4O on the plasma levels of glucose, corticosterone, and catecholamines were examined in fed, conscious, unrestrained rats (9). The changes in the plasma glucose and corticosterone concentrations induced by iv administrations of 2-B4O are shown in Figures 4 and 5. The levels of both increased significantly and dose-dependently. The increase in glucose peaked at 20 to 60 min, while that of corticosterone peaked at 40 min. Significant increases in epinephrine from 20 to 120 min and in norepinephrine from 20 to 60 min after the start of iv administration of 30 μmol/kg 2-B4O are shown in Figure 6A. The concentrations reached their peaks at 40 min, then gradually returned to the basal level. As shown in Figure 6 (upper) the basal level of epinephrine was significantly depressed and the increase induced by 2-B4O was completely suppressed by splanchnicotomy (SPX). On the other hand, the basal level of norepinephrine was not altered by SPX, although the increase induced by 2-B4O was significantly attenuated. Although the basal plasma glucose concentration was not significantly affected, the increase in the plasma glucose concentration induced by 2-B4O was significantly attenuated by SPX. The basal level of plasma corticosterone was not significantly lowered by SPX, and 2-B4O was still able to induce a significant elevation in this parameter. Pretreatment with anti-corticotropin–releasing factor (CRF) antibody (20 μmol in 0.3 ml Krebs-Ringer solution, iv) did not significantly suppress the basal level of plasma corticosterone. The increase in plasma corticosterone induced by 2-B4O, however, was significantly attenuated by this antibody pretreatment (Fig. 6B). The glucose concentration was not affected by this pretreatment alone. These data on plasma glucose indicate that the increase in glucose induced by 2-B4O is mainly mediated by increased adrenomedullary secretion and increased sympathetic outflow to the visceral organs. As mentioned in the following section (on the changes in sympathetic efferent nerve activity induced by 2-B4O), efferent hepatic as well as pancreatic sympathetic activities are increased by iv 2-B4O, so both glucagon and liver glycogen may contribute to the increase in plasma glucose induced by 2-B4O, together with a release of adrenomedullary epinephrine. The above data on plasma corticosterone indicate that the activation of adrenocortical and medullary secretory responses induced by 2-B4O is mediated via the central nervous system. As shown in the next section, 2-B4O facilitated the neuronal activity of parvocellular neurons in the PVN. Thus, 2-B4O facilitates CRF release and activates the pituitary-adrenal axis. Anti-CRF antibody injected iv may immunoneutralize endogenous CRF in the portal vessels and/or in the pituitary gland, and may thereby suppress the secretory activity of corticotropic cells (10). The concentration of 2-B4O in plasma was found to increase from 3.5 to 13.5 μM at 48 to 80 h after the start of food deprivation in rats (3). In that study, we did not determine the concentration of 2-B4O in the plasma after its iv injection. Assuming the total plasma volume in the rat to be approximately 11 ml, the concentration of 2-B4O in the plasma could be estimated to be 28 μM after a single 1 μmol/kg 2-B4O injection (if not metabolized and not absorbed by red or white blood cells).

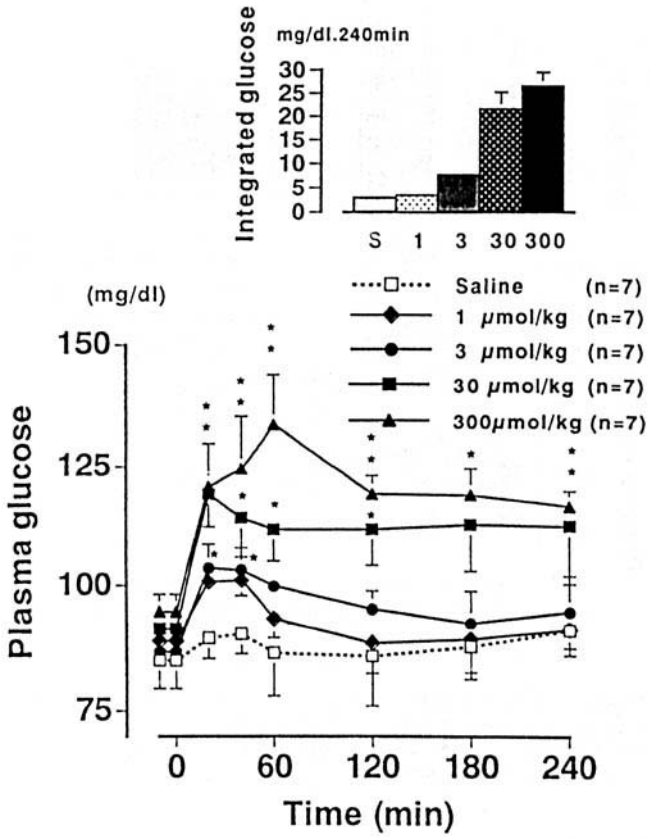

Figure 4. Effect of 2-B4O on plasma glucose concentration. Changes in plasma glucose concentration after administration of saline or 1, 3, 30, or 300 μmol/kg 2-B4O iv in fed rats. *n* = number of rats (Reproduced with permission from Ref. 9).

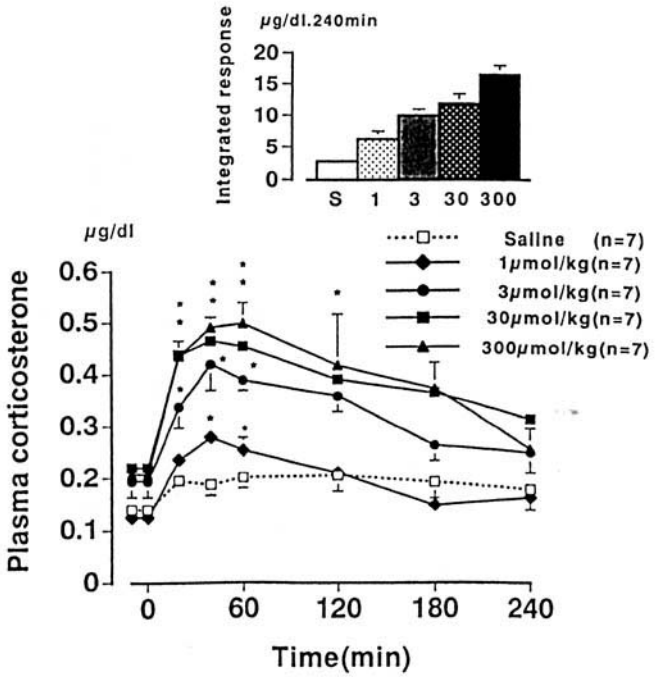

Figure 5. Effect of 2-B4O on plasma corticosterone concentration. Changes in plasma corticosterone concentration after administration of saline or 1, 3, 30, or 300 μmol/kg 2-B4O iv in fed rats. *n* = number of rats. (Reproduced with permission from Ref. 9).

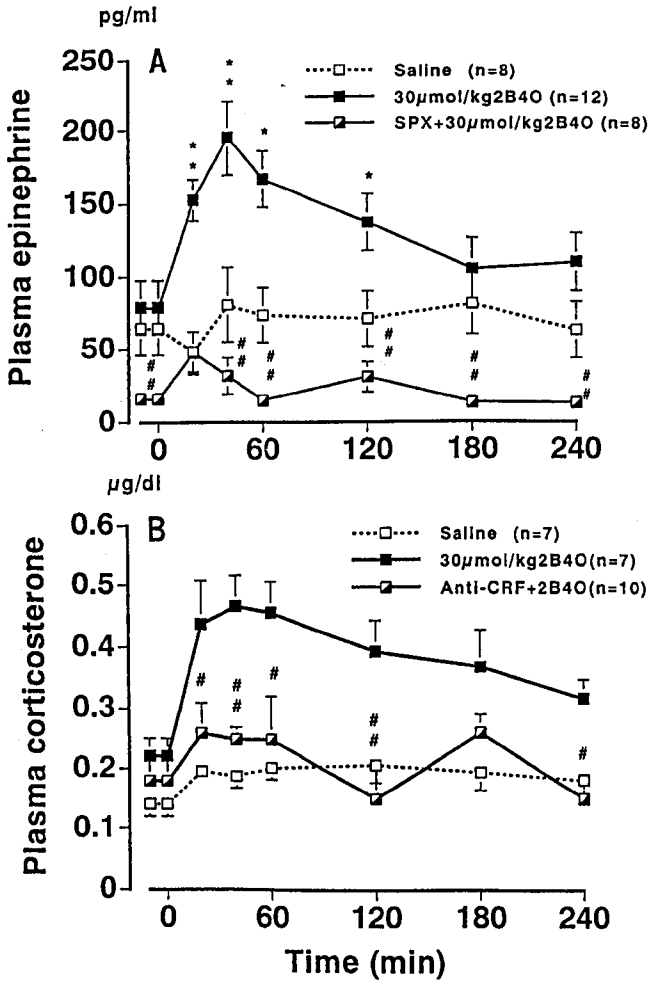

Figure 6. Changes in plasma epinephrine concentration after administration of 30 μmol/kg 2-B4O iv in fed rats. (A) Values are means ± SEM. n = number of animals. SPX, bilateral splanchnicotomy below diaphragm. Significantly different from saline control: $^{*}P < 0.05$; $^{**}P < 0.01$. Significantly different from splanchnic nerve-intact rats: $^{\#}P < 0.05$; $^{\#\#}P < 0.01$. (B) Changes in plasma corticosterone concentration after administration of 30 μmol/kg 2-B4O iv in fed rats with or without pretreatment with anti-corticotropin-releasing factor (CRF) antibody. Values are means ± SEM, *n* = number of animals. Significantly different from rats injected with 2-B4O without anti-CRF; $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ (Reproduced with permission from Ref. 9).

There is no information concerning the concentration of 2-B4O in the brain or CSF after its iv injection. However, it has been reported that the concentration of ketone bodies in CSF is approximately 50% of that in the blood (11). Thus, the concentration of 2-B4O in CSF could be estimated as approximately 14 μM. On this basis, the minimal effective dose of 2-B4O used in the experiments described above (Figs. 4–6) was comparable with the increased concentration of 2-B4O in the plasma at 48 to 80 h after the beginning of food deprivation.

Facilitation of Efferent Sympathetic Outflow. It is well known that CRF is produced in parvocellular neurons in the PVN, that it acts within the brain to stimulate the activity of the efferent sympathetic nervous system, and that electrical stimulation of PVN neurons in rats increases sympathetic outflow (12). As exemplified in Figure 7, 2-B4O dose-relatedly facilitated the activity of 30% of parvocellular PVN neurons *in vitro* slice preparations.

Injection of 2-B4O i.v. at 0.1 to 1.0 mM (in a volume of 0.2 ml) dose-relatedly increased the firing rates of fibers in the pancreatic, hepatic, splenic, and adrenal sympathetic efferent nerves in anesthetized rats. One example is shown in Figure 8. Quite similar activation was observed in the sympathetic efferent discharge to brown adipose tissue.

Improvement in Emotional and Spatial Learning and Memory Performance Induced by 2-B4O

Enhancement of Passive Shock-Avoidance Learning and Memory. The effects of 2-B4O on passive avoidance learning across 2 consecutive days are presented in Figure 9 (left). Injection (ip) of 2-B4O at 20 μmol/kg (or Krebs-Ringer solution) was given 2 h before training, and longer latencies indicate better performance. On day 1, all male DDY mice performed similarly and displayed short latencies (acquisition). On day 2, an obvious and significant increase in 24-h retention occurred in the 2-B4O group compared with the normal Krebs-Ringer group. This change in performance cannot be attributed to variations in shock threshold or level of activity, since locomotor activity [measured by an Animex-Auto counter; Fig. 9 (right)] and pain threshold (measured by the hot-plate test) were unaffected by 2-B4O injection.

Enhancement of Performance in the Water-Maze Task. In this experiment, 2-B4O at 20 to 50 μmol/kg was ip injected at 2 h before mice began the water-maze

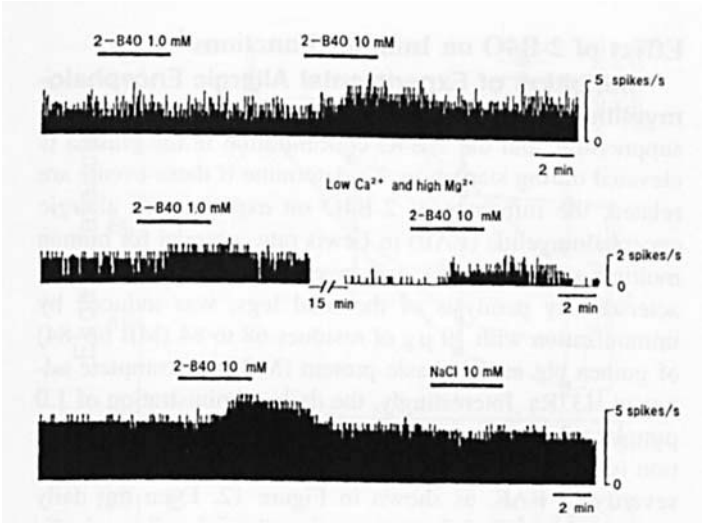

Figure 7. Facilitatory effect of bath-applied 2-B4O on rat single parvocellular neurons in the PVN. Brain slice preparation, traces recorded in the order upper to lower. Bars above traces, application periods. Middle, showing persistence of 2-B4O effect during elimination of synaptic inputs by perfusion with low Ca^{2+} (Ca^{2+} free), high Mg^{2+} (12 mM) solution. NaCl, no effect.

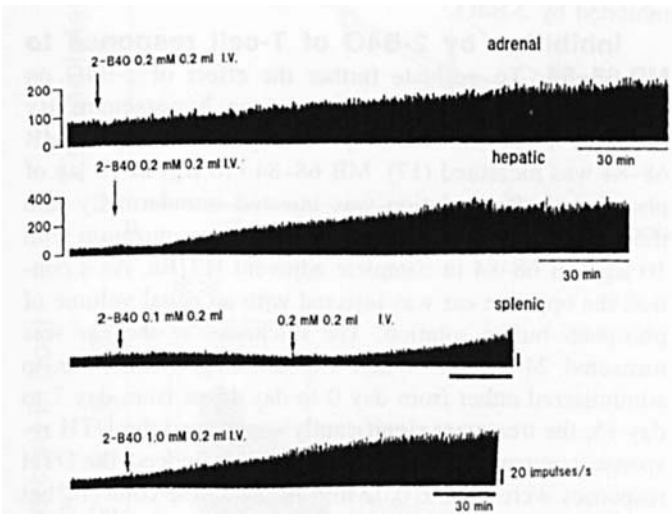

Figure 8. Effect of iv administration of 2-B4O on efferent firing rate in a sympathetic branch of the adrenal (upper), hepatic (middle), or splenic (lower) nerve. All from different nerves. Ordinates in upper two, impulses/5 s.

task. Four trials with an interval of 10 min were considered to make up one block, and blocks were on consecutive days. As shown in Figure 10A, both the 2-B4O–treated and control mice showed a progressive decrease in latency (i.e., the time needed to find the platform) as they went through blocks 1, 2, 3, and 4, but the 2-B4O-treated mice found the platform more quickly than the control mice did in blocks 2, 3, and 4.

A facilitating effect of 2-B4O was also evidenced by improved performance during a memory-probe trial carried out 10 min after the last trial in block 4 with the platform removed. The 2-B4O–treated mice spent a significantly longer time than the control mice in the goal quadrant in which the platform had previously been located (Fig. 10B). These results indicated that the 2-B4O–treated mice had a better spatial awareness than the controls (13).

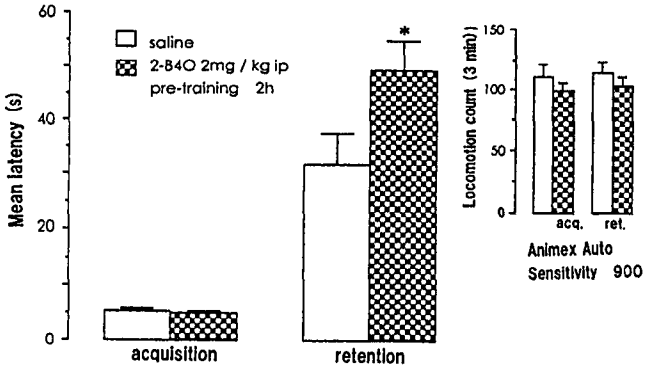

Figure 9. Effect of 30 μmol/kg 2-B4O ip injection on the performance of passive avoidance task by mice. $n = 20$ mice. Left, 2-B4O or control saline, applied 2 h before the acquisition trial. The retention trial was carried out 24 h after the acquisition trial. Ordinate, latency (mean ± SEM) for the acquisition and retention trials. $^*P < 0.05$. Right, locomotor activity was counted in a 3-min period (mean ± SEM) 2 h after the 2-B4O injection. There were no significant differences between the 2 groups.

Significant Blockade of the Effect of 2-B4O by Pretreatment with Anti-aFGF Antibody. An iv injection of 2-B4O increases the plasma glucose, epinephrine, norepinephrine, and corticosterone concentrations (9). An increase in the glucose level in the CSF while eating or following an ip injection of glucose induces release of acidic fibroblast growth factor (aFGF) from the ependymal cells in the III cerebral ventricle wall into the CSF, as well as into the brain parenchyma (14). The released aFGF facilitates the performance of emotional and spatial learning and memory (15), while also facilitating long-term potentiation via its effect on hippocampal CA 1 neurons (16). These observations suggest that 2-B4O may facilitate learning and improve memory through the glucose–aFGF mechanism. To examine this idea, the effect of 2-B4O on the performance of DDY mice in the spatial water-maze task was tested with or without pretreatment with anti-aFGF antibody (13).

The anti-aFGF antibody was icv injected 30 min before the 2-B4O (50 μmol/kg.ip). A repeated-measures ANOVA showed that the mice in all 4 groups progressively improved their ability to locate the platform over the 4 days of training (Fig. 11A). In the group given both an icv infusion of anti-aFGF antibody (at 600 ng/mouse, but not at 300 ng/mouse) and an ip injection of 2-B4O, the escape latency was similar to that in the control group (Fig. 11A). In terms of overall mean latency, the 600 ng/mouse group was not different from the controls ($P > 0.98$), but for the 600 ng/mouse group versus the 2-B4O group, the latency was different from controls ($P < 0.05$).

To exclude any possible effects of anti-aFGF antibody alone or pre-immune IgG, mice were examined after icv injection of the same volume of anti-aFGF antibody [either at the lower (300 ng/mouse) or higher (600 ng/mouse) doses] or IgG, of followed by an ip injection of saline (Fig. 11B). All groups progressively improved their ability to locate the platform over the 4 blocks, and no drug effect was

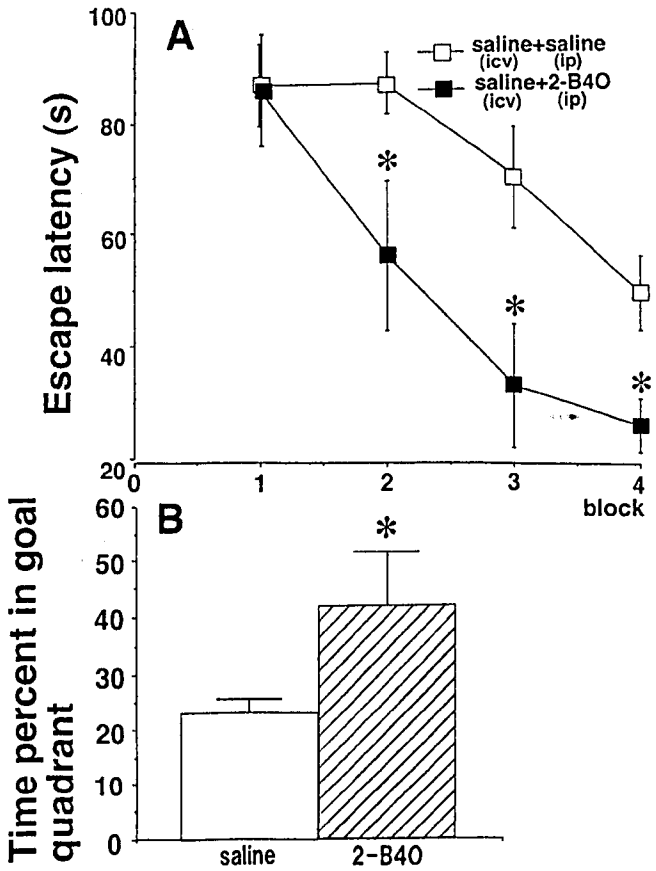

Figure 10. Effect of ip injection of 50 μmol/kg 2-B4O on the performance of water-maze task in mice. (A) Latency to escape onto the platform is shown for each block of trials as the mean ± SEM. 2-B4O-treated mice (filled square, $n = 5$) found the platform more quickly than did control mice (open square, $n = 10$) in blocks 2, 3, and 4. $^*P < 0.05$. (B) Probe test carried out 10 min after the fourth trial in the fourth block. When the platform was absent, the 2-B4O-treated mice ($n = 5$, hatched bar) stayed significantly longer in the platform quadrant (shown as a percentage) than did control mice ($n = 10$, open bar). $^*P < 0.05$ (Reproduced with permission from Ref. 13).

observed, the escape latencies all being similar to those in the saline control group. The swimming velocities were not significantly different among the groups, so the different latencies in training trials were not due to differences in swimming velocity.

Similar results were obtained in a probe test measuring the duration that mice spent in the goal quadrant. Again, the pre-icv administration of anti-aFGF antibody blocked the facilitating effect of 2-B4O, the mice pretreated with anti-aFGF antibody at a dose of 600 ng/mouse (but not at 300 ng/mouse) staying in the goal quadrant about the same length of time as the control mice (Fig. 11C).

Thus, 2-B4O was found to facilitate spatial performance in DDY mice, and pretreatment with anti-aFGF antibody completely abolished the facilitating effect of 2-B4O, although the antibody alone had no effect on memory. These data support our hypothesis that 2-B4O has a spatial memory-facilitating effect that is mediated by aFGF.

Effect of 2-B4O on Immune Functions

Inhibition of Experimental Allergic Encephalomyelitis. Starvation is well known to indicate immune suppression, and the 2-B4O concentration in the plasma is elevated during starvation. To determine if these events are related, the influence of 2-B4O on experimental allergic encephalomyelitis (EAE) in Lewis rats, a model for human multiple sclerosis (MS), was investigated (17). EAE, characterized by paralysis of the hind legs, was induced by immunization with 10 μg of residues 68 to 84 (MB 68–84) of guinea pig myelin basic protein (MBP) in complete adjuvant H37Ra. Interestingly, the daily administration of 1.0 mmol/kg 2-B4O ip from the day of MB 48–84 immunization (day 0) to day 20 dramatically suppressed the clinical severity of EAE, as shown in Figure 12. Even the daily administration of 2-B4O from day 0 to day 7 markedly reduced the clinical symptoms of EAE. Histological examination confirmed the clinical findings inasmuch as infiltrations by inflammatory mononuclear cells into the spinal cord, leptomeninges, and choroid plexus were significantly inhibited by 2-B4O.

Inhibition by 2-B4O of T-cell response to MB 68–84. To evaluate further the effect of 2-B4O on immune responses, the delayed-type hypersensitivity (DTH) a T-cell immune response, against 10 μg of MB 68–84 was measured (17). MB 68–84 (10 μg) in 10 μg of phosphate-buffer solution was injected intradermally into the right ear of Lewis rats 15 days after immunization with 10 μg MB 68–84 in complete adjuvant H37Ra. As a control, the opposite ear was injected with an equal volume of phosphate-buffer solution. The thickness of the ear was measured 24 h later. When 1.0 mmol/kg 2-B4O was ip administered either from day 0 to day 15 or from day 7 to day 15, the treatment significantly suppressed the DTH response, compared with that in control rats. Indeed, the DTH responses were 0.22 ± 0.02 mm in the saline controls, but 0.14 ± 0.01 (0–15 days, $P < 0.05$) and 0.16 ± 0.01 (7–15 days, $P < 0.05$) in the 2-B4O groups. These data suggest that 2-B4O may suppress EAE by inhibiting the T-cell mediated immune response to MB 68–84.

These observations indicate that 2-B4O has the potential to suppress autoimmune responses, and thus it may have significant potential for the treatment of autoimmune diseases.

Conclusions

Figure 13 outlines our conclusions as to the physiological significance of 2-B4O. During food deprivation, the plasma level of 2,4,5-TP (a hunger substance) peaks at 12 h. Then, 3-DB and 2-B4O (satiety substances) begin to increase at 24 h, and peak at 48 h. The physiological concentration of 2-B4O enters the brain parenchyma, and first reaches the LHA and VMH, where it controls food intake. 2-B4O suppresses the neuronal activity of glucose-sensitive neurons in the LHA (via activation of the Na^+-K^+ pump)

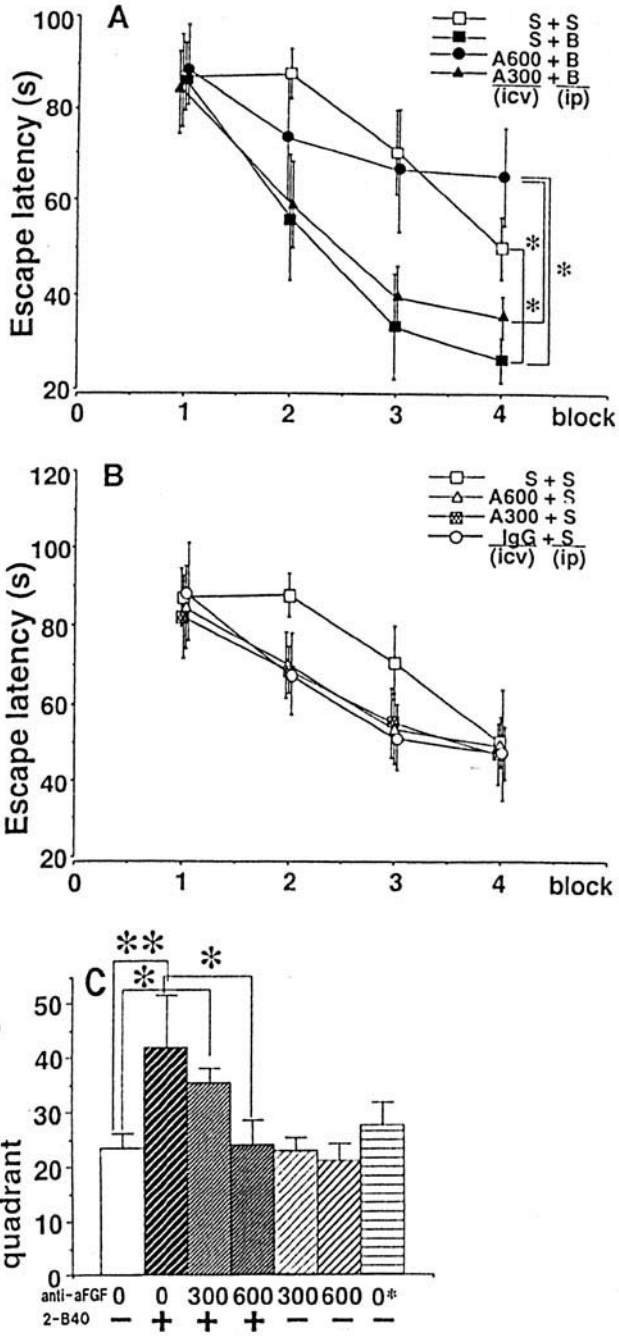

Figure 11. Effect of anti-acidic fibroblast growth factor (aFGF) antibody on spatial-performance facilitated by 2-B4O in (A,B) water-maze task and (C) probe test. 2-B4O or saline was injected 2 h before each block of trials. (A) An icv infusion of anti-aFGF antibody at 300 ng/mouse (A300 + B; A: antibody, B: 2-B4O; n = 8) or 600 ng/mouse (A600+B, n = 5) or saline (S + B, n = 5) was administered 30 min before an ip injection of 2-B4O. The S+S group received saline each time. For each group, n = 10. (B) A300 (A300 + S, n = 8) or A600 (A600 + S, n = 6) or control preimmune IgG (IgG + S, n = 7) or saline (S + S, n = 10) was administered 30 min before an ip injection of saline. Mean ± SEM for escape latency. A600 completely inhibited the memory-facilitating effect of 2-B4O (A). No differences were found among the control group and the groups given either antibody alone or preimmune IgG (B). $^*P < 0.05$ (C) with regard to the mean time spent in the goal quadrant (shown as a percentage), A600 negated the memory-facilitating effect of 2-B4O. No effect was observed after administration of anti-aFGF alone at either dose or of preimmune IgG (n = 10, 5, 8, 5, 8, 6, 7, respectively, for groups in the order shown in panel C). $^*P < 0.05$; $^{**}P < 0.01$ (Reproduced with permission from Ref. 13).

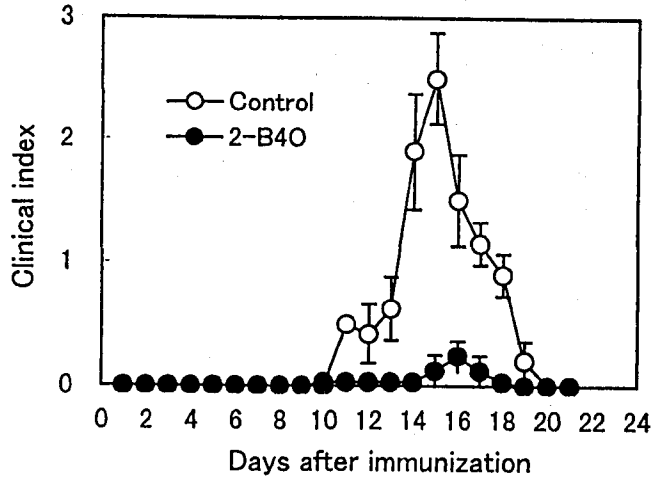

Figure 12. Suppressive effect of 2-B4O on actively induced EAE. Lewis rats were immunized with MB 68–84 in H37Ra on day 0, and then treated with 1.0 mmol/kg 2-B4O (●) or saline (○) daily from day 0 to day 20. Each group consisted of 15 rats. Data are expressed as the mean ± SEM (Reproduced with permission from Ref. 17).

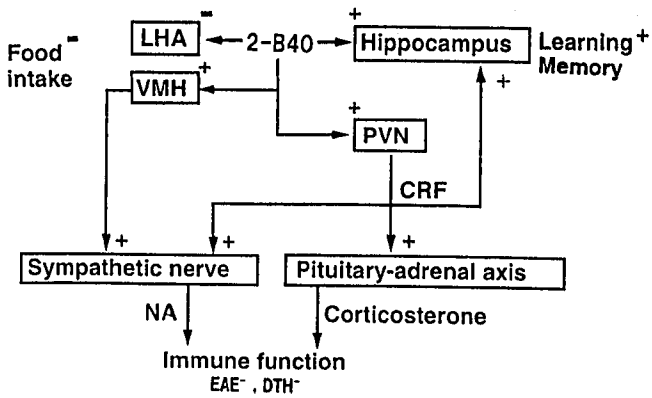

Figure 13. Deduced physiological actions of 2-B4O following its increase in blood during food deprivation. The physiological concentration of 2-B4O enters the hypothalamus. 2-B4O reaches glucose-sensitive neurons in the LHA and glucoreceptor neurons in the VMH, and inhibits or facilitates neuronal activity, respectively. Thus, food intake is suppressed by 2-B4O. 2-B4O reaches the parvocellular neurons in the PVN and activates these neurons, causing CRF to be released. These neurons and the released CRF, as well as the VMH facilitation, activate the efferent sympathetic outflow and the pituitary-adrenal axis. Splenic sympathetic activation and released corticosterone suppress immune function. Actually, 2-B4O suppresses both EAE and DTH reactions. Thus, 2-B4O has the potential to suppress autoimmune responses. 2-B4O facilitates learning and memory performance through actions on the hippocampus. This facilitation can be blocked by pretreatment with anti-aFGF antibody because it is mediated via aFGF released from ependymal cells in the III cerebral ventricle in response to the increase in glucose concentration caused by 2-B4O.

and facilitates that of glucoreceptor neurons in the VMH (by closing K_{ATP} channels). Thus, 2-B4O acts on these neurons in the same way as glucose does, and 2-B4O is used a brain fuel during hypoglycemia. Secondly, 2-B4O reaches the parvocellular neurons in the PVN, facilitates CRF release, and activates not only the hypothalamo-pituitary-adrenal axis, but also the efferent hepatic, pancreatic, splenic, and adrenal sympathetic outflows. These effects produce hyper-

glycemia, together with increases in the plasma epinephrine, norepinephrine, and corticosterone levels. Thirdly, 2-B4O facilitates emotional and spatial learning and memory performance in the hippocampus through the aFGF release that occurs in response to the hyperglycemia induced by 2-B4O. In addition, 2-B4O acts as an immunomodulator, suppressing the activity of the immune system. This is achieved by corticosterone attenuating the immune functions involving antigen-antibody reactions, and by activation of the splenic sympathetic outflow suppressing splenic natural-killer cytotoxicity. By these immune-system suppressions, autoimmune reactions may be reduced. 2-B4O potentially suppresses the clinical severity of EAE, as well as the DTH reaction, which is an indicator of the T-cell immune response. Thus, 2-B4O has the potential to suppress autoimmune responses.

Figure 14 outlines our conclusions as to the physiological significance of the aFGF release from the ependymal cells in the III cerebroventricle that occurs in response to the increased glucose concentration in the CSF during food intake (14). The released aFGF diffuses into the brain parenchyma, reaches the LHA, and suppresses the activity of glucose-sensitive neurons. Thus, aFGF acts as one of the endogenous satiety substances. aFGF then reaches parvocellular neurons in the PVN, and also the hippocampus, and acts in the same way as 2-B4O to activate hypothalamo-pituitary-adrenal axis (18) and the efferent sympathetic outflows (19), and to facilitate learning and memory performance (15, 16). The immune system is also modulated by aFGF via corticosterone release and facilitation of the splenic sympathetic outflow. This modulation involves, as in the case of 2-B4O, suppression of autoimmune responses

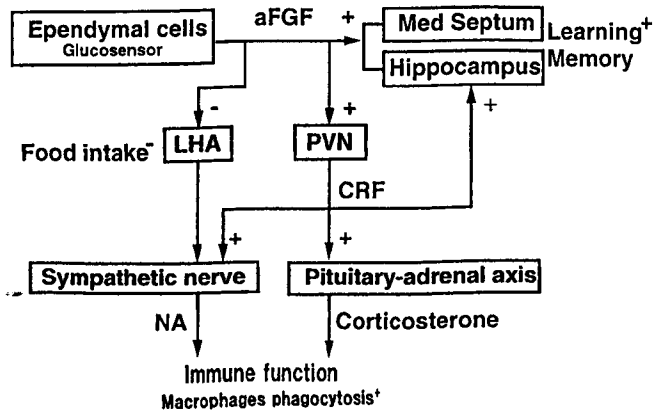

Figure 14. Deduced physiological actions of aFGF following its release during food intake. Ependymal cells located in the III cerebral ventricle release aFGF in response to an increase in the glucose in the CSF during food intake. The aFGF reaches the glucose-sensitive neurons in the LHA and inhibits their neuronal activity. Thus, food intake is suppressed. In addition, aFGF reaches the hippocampus, and thereby facilitates learning and memory performance. aFGF also reaches the parvocellular neurons in the PVN and activates these neurons, causing CRF to be released. These neurons and the released CRF activate the sympathetic outflow and the pituitary-adrenal axis. Splenic sympathetic activation and released corticosterone suppress immune function, as does 2-B4O (See Fig. 13). However, aFGF also stimulates the phagocytosis of macrophages.

(20). Unlike 2-B4O, aFGF has no effect on VMH neurons (because they lack FGF receptor-1) and facilitates the phagocytosis of macrophages (i.e., activation of a biodefense mechanism). Therefore, aFGF maintains not only the body's energy homeostasis through food-intake regulation, but also primes the brain for important functions. Finally, we can say that 2-B4O and aFGF play quite similar physiological roles, albeit in the absence (food deprivation) or presence (following food intake) of alimentation, respectively.

1. Shimizu N, Oomura T, Sakata T. Modulation of feeding by endogenous sugar acids acting as hunger or satiety factors. Am J Physiol **246:**R532–R550, 1984.
2. Plata-Salaman CR, Oomura T, Simizu N. Endogenous sugar acid derivative acting as a feeding suppressant. Physiol Behav **38:**359–373, 1986.
3. Oomura Y. Regulation of feeding by neural responses to endogenous factors. News Physiol Sci **2:**197–203, 1987.
4. Sakata T, Arase K, Fujimoto K, Kurata K, Fukagawa K, Fukushima M, Steffans AB, Oomura Y. Structural characteristics of endogenous sugar acids and relations to feeding modulation. Brain Res **473:**43–50, 1988.
5. Arai I, Ueki I, Muramatsu M, Otomo S, Shiraishi T, Oomura Y. Effects of an endogenous satiety substance, 2-buten-4-olide, on gastric acid secretion and experimental ulceration in rats. Physiol Behav **47:**197–202, 1990.
6. Puthuraya KP, Oomura Y, Shimizu N. Effects of endogenous sugar acids on the ventromedial hypothalamic nucleus of the rat. Brain Res **332:**165–168, 1985.
7. Fukuda A, Oomura Y, Minami T, Ito C. A novel endogenous sugar acid depolarizes ventromedial hypothalamic neurons *in vitro*. Am J Physiol **255:**R134–R140, 1988.
8. Minami T, Oomura Y, Nabekura J, Fukuda A. Direct effects of 3,4-dihydroxybutanoic acid γ-lactone 2,4,5-trihydroxypentanoic acid γ-lactone on lateral and ventromedial hypothalamic neurons. Brain Res **462:**258–264, 1988.
9. Matsumoto I, Oomura Y, Nishino H, Nemoto S, Aou S, Aikawa T. Effects of 2-buten-4-olide, an endogenous satiety substance on plasma glucose, corticosterone and catecholamines. Am J Physiol **266:**R413–R418, 1994.
10. Ono N, Samson WK, McDonald JK, Lumpkin MD, Bedran de Castro JC, McCann SM. Effects of intravenous and intraventricular injection of antisera directed against corticotropin-releasing factor on the secretion of anterior pituitary hormones. Proc Natl Acad Sci USA **82:** 7787–7790, 1985.
11. Wiener R, Hirsch HJ, Spitzer JJ. Cerebral extraction of ketones and their penetration into CSF in the dog. Am J Physiol **220:**1542–1546, 1971.
12. Brown MR, Fisher LA, Spiess J, Rivier C, Rivier J, Vale W. Corticotropin-releasing factor; actions on the sympathetic nervous system and metabolism. Endocrinology **111:**928–981, 1982.
13. Li X-L, Aou S, Li A-J, Hori T, Tooyama I, Oomura Y. 2-Buten-4-olide, an endogenous feeding suppressant, improves spatial performance through brain acidic fibroblast growth factor in mice. Brain Res Bull **56:**531–536, 2001.
14. Hanai K, Oomura Y, Kai Y, Nishikawa K, Morita H, Plata-Salaman CR. Central action of acidic fibroblast growth factor in feeding regulation. Am J Physiol **256:**R217–R223, 1989.
15. Li A-J, Oomura Y, Sasaki K, Suzuki K, Tooyama I, Hanai K, Kimura H, Hori T. A single pre-training glucose injection induces memory facilitation in rodents performing various tasks: contribution of acidic fibroblast growth factor. Neuroscience **85:**785–794, 1998.
16. Sasaki K, Oomura Y, Figrov A, Yagi H. Acidic fibroblast growth

factor facilitates generation of long-term potentiation in rat hippocampal slices. Brain Res Bull **33:**505–511, 1994.
17. Naiki M, Takeoka Y, Yago H, Kurimoto Y, Gershwin ME. Suehiro S. 2-Buten-4-olide (2-B4O) inhibits experimental allergic encephalomyelitis (EAE) in Lewis rats. J Autoimmun **8:**727–739, 1995.
18. Matsumoto I, Oomura Y, Niijima A, Sasaki K, Aikawa T. Acidic fibroblast growth factor activates hypothalamo-pituitary-adrenocortical axis in rats. Am J Physiol **274:**R503–R509, 1998.
19. Matsumoto I, Niijima A, Oomura Y, Sasaki K, Tsuchiya K, Aikawa T. Acidic fibroblast growth factor activates adrenomedullary secretion and sympathetic outflow in rats. Am J Physiol **275:**R1003–R1012, 1998.
20. Ichinose M, Sawada M, Sasaki K, Oomura Y. Enhancement of phagocytosis in mouse peritoneal macrophages by fragments of acidic fibroblast growth factor (aFGF). Int J Immunopharmacol **20:**193–204, 1998.