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Reduction of blood glucose level by orexins in fasting normal and streptozotocin-diabetic mice

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Abstract

Orexin-A and orexin-B are neuropeptides implicated in the maintenance of energy homeostasis. In the present study, we examined the effects of orexins on blood glucose levels in response to fasting in normal and streptozotocin-induced diabetic mice. After the injection of orexin-A and orexin-B (0.01–1 nmol/kg, i.v.), the blood glucose levels in both normal mice and diabetic mice in the fasting state decreased. In contrast, neither orexin-A nor orexin-B affected the glucose levels in the animals allowed free access to food. Intracerebroventricular administration of orexin-A and orexin-B was associated with glucose-lowering effects in fasting diabetic mice. The serum insulin level did not significantly change following the administration of orexin-A or orexin-B, in either the normal or the diabetic mice in the fasting state. These results demonstrate that orexins lower the blood glucose levels exclusively in the fasting state. The orexins may stimulate some neural and hormonal network and thereby promote blood glucose utilization.
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1. Introduction

Orexins are a pair of neuropeptides (orexin-A and orexin-B) derived from the common precursor prepro-orexin (Sakurai et al., 1998; de Lecea et al., 1998). They exert their biological functions through previously orphaned G-protein-coupled receptors named orexin-1 and orexin-2 receptors. Since the prepro-orexin mRNA and immunoreactive orexin-A are localized in neurons within and around the lateral and posterior hypothalamus in the adult rat brain (Sakurai et al., 1998), most studies of orexin function have been focused on feeding behavior and arousal status (Sakurai et al., 1998; Hagan et al., 1999; Willie et al., 2001). However, recent studies demonstrate a broad extrahypothalamic projection of orexin-immunoreactive neurons and a wide distribution of orexin receptors in the central nervous system (Peyron et al., 1998; Trivedi et al., 1998). It is likely that the orexins contribute to a variety of brain functions, as well as to the control of appetite and the sleep–wake cycle.

A reduction in blood glucose level promotes the urge for food intake (Oomura, 1980). Orexins may mediate this process, because prepro-orexin mRNA levels have been shown to be increased in the rat hypothalamus under both the fasting (Sakurai et al., 1998) and insulin-induced hypoglycemic conditions (Griffond et al., 1999). It has also been demonstrated, using fos-like immunoreactivity as a marker of neuronal activation, that the hypothalamic orexin-containing neurons are activated by insulin-induced hypoglycemia (Moriguchi et al., 1999). By contrast, the hypothalamic prepro-orexin mRNA levels are reduced under hyperglycemic conditions in genetically obese (*ob/ob* and *db/db*) mice (Yamamoto et al., 1999), although they remain unchanged in mice with streptozotocin-induced diabetes (Cai et al., 1999) and Zucker diabetic fatty rats (Cai et al., 2000). It remains to be determined whether the physiological actions of orexins are modified in the diabetic state.

Hara et al. (2001) have recently generated orexin-neuron-ablated mice (orexin/ataxin-3 mice). The mice show late-onset obesity despite the food intake of these mice being

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less than that of their non-transgenic littermates. This may result from lower energy expenditure in the transgenic mice, because the spontaneous motor activity of these mice is decreased. Thus, the lack of orexigenic action may cause the excessive energy store and tip the energy balance toward an abnormal state. To date, there have been no reports indicating whether the orexins affect the regulation of the blood glucose concentrations to maintain energy homeostasis in the fasting state. The present study, therefore, was aimed at examining the effects of orexin-A and orexin-B on the blood glucose levels in mice in the fasting state. The effects of orexins on the blood glucose concentrations under the hyperglycemic condition were also investigated, using mice with streptozotocin-induced diabetes. Furthermore, these orexin effects on the blood glucose concentrations in the fasting state were compared with those on the blood glucose concentrations in the fed state.

2. Materials and methods

2.1. Animals

Male ddY mice (4 weeks old, 25–33 g) were purchased from SLC (Shizuoka, Japan). The animals were housed (3–5 per cage) under a daily cycle of 12 h light and 12 h darkness, with free access to food and water. All procedures were in accordance with the guidelines of the Toyama Medical and Pharmaceutical University Animal Research Committee. Injection of various doses of streptozotocin is able to produce from mild to severe diabetes mellitus in rats (Junod et al., 1969). A group of mice (4 weeks old) was made diabetic by injection of streptozotocin (150 mg/kg, bolus i.v. via the tail vein; Sigma, St. Louis, MO, USA). Four to six weeks after the injection, these mice with streptozotocin-induced diabetes (blood glucose level: 252–466 mg/dl) were alive without injection of insulin, and were used for the experiments as a diabetic model with a relative deficiency in insulin secretion involved in the pathogenesis of Type 2 diabetes (see Ito et al., 1999). Age-matched mice (8–10 weeks old) without any treatment were used as controls.

2.2. Measurement of blood glucose level

Blood samples (20 μl) were collected into capillary glass tubes from the orbital venous plexus of mice anesthetized with ether. The blood glucose levels were measured by the glucose oxidase method using Glucose Analyzer 2 (Beckman Instruments, California, USA). We confirmed that the ether anesthesia did not affect the blood glucose concentrations in the mice (data not shown).

2.3. Reagents

Orexin-A (human, rat, mouse, bovine) and orexin-B (rat, mouse) were purchased from Peptide Institute (Osaka, Japan). Glibenclamide, a sulfonylurea hypoglycemic agent, was purchased from Research Biochemicals (Massachusetts, USA).

2.4. Peripheral administration of drugs

In the experiments conducted under the fasting condition of the mice (Figs. 1, 3 and 4), the mice were deprived of food for 10–14 h, with free access only to water. Then, orexin-A (0.001–1 nmol/kg), orexin-B (0.001–1 nmol/kg), or saline was injected via the tail vein (0.1 ml/10 g body weight). In the experiments conducted under normal feeding condition of the mice (Fig. 2), the mice were maintained with free access to food and water, and then, at the start of the experiment, orexin-A (1 nmol/kg), orexin-B (1 nmol/kg), or saline was administered by bolus i.v. injection. Blood samples (20 μl) were collected four times (before, and 2, 4 and 6 h after the i.v. injection) from each mouse. In all the cases, the animals were deprived of food after the injection of the orexins or saline, so as to prevent confounding of the drug-induced changes in the blood glucose levels by the transient increase in the glucose levels associated with food intake. Also, to minimize the influence of circadian changes in the blood pressure and endocrine factors, the drug administrations were started at 10:00 a.m.

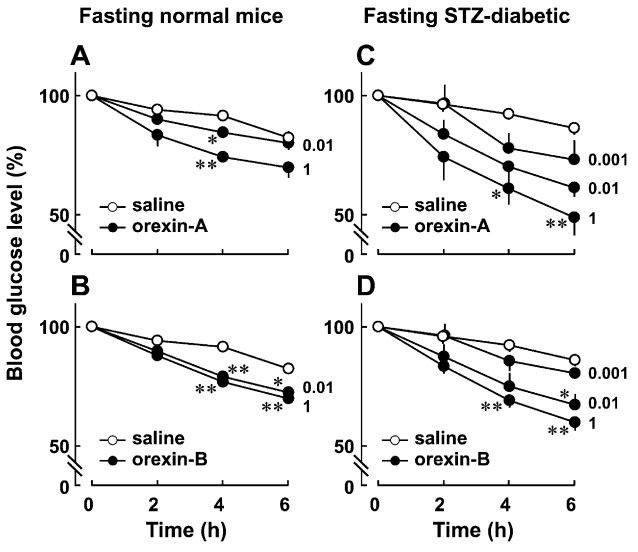

Fig. 1. Blood-glucose-lowering effects of orexin-A and orexin-B in fasting mice. Food deprivation was begun 10–14 h before the administration of orexins at 0 h and continued for 6 h during measurement of the blood glucose levels. Graphs show time-dependent reductions of the blood glucose levels following intravenous injection of orexin-A (A) and orexin-B (B) (0.01 and 1 nmol/kg) in the fasting normal mice, and those following orexin-A (C) and orexin-B (D) (0.001, 0.01 and 1 nmol/kg) injection in the fasting streptozotocin (STZ)-induced diabetic mice. Saline was injected as a vehicle control. The glucose levels are expressed as percentages of the blood glucose concentrations observed before the injection of saline or the orexins (at 0 h) in each mouse, and then averaged. The values represent means ± S.E.M. ($n = 5–6$). $*P < 0.05$, $**P < 0.01$; significantly different from the control response to saline at each time-point, by one-way ANOVA followed by Scheffé multiple-comparison test.

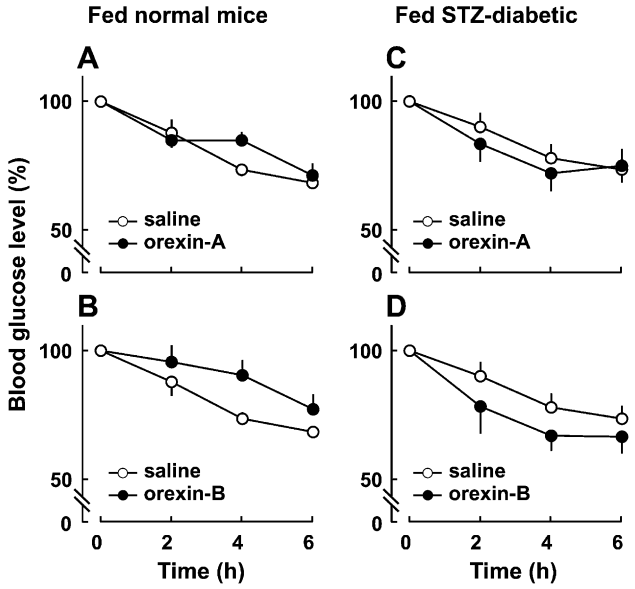

Fig. 2. No significant effects of orexin-A and orexin-B on the blood glucose levels in mice in the fed state. The mice were allowed free access to food before administration of the orexins at 0 h, and then deprived of food during the measurements of the blood glucose levels. Graphs show the time-dependent reductions in the blood glucose levels following intravenous injection of orexin-A (A) and orexin-B (B) (1 nmol/kg, $n=6-8$) in the normal mice in the fed state, and those following injection of orexin-A (C) and orexin-B (D) (1 nmol/kg, $n=4-5$) in the streptozotocin (STZ)-induced diabetic mice in the fed state. Saline was injected as a vehicle control. The glucose levels are expressed as percentages of the blood glucose levels observed before the injection of saline or the orexins (at 0 h) in each mouse, and then averaged. The values represent means ± S.E.M.

In this study, only glibenclamide, an oral anti-diabetic drug, was given perorally to the animals (40 μmol/kg, 0.1 ml/10 g of mouse) with using a mouse feeding needle. The blood glucose-lowering effects were used as positive controls to relatively compare the effects of the orexins, although glibenclamide would react with different mechanisms from those of orexins. The methods of food deprivation and blood-sample collection used were as mentioned above.

2.5. Intracerebroventricular (i.c.v.) administration of orexins

The mice with streptozotocin-induced diabetes were anesthetized with pentobarbital (80 mg/kg, i.p.), positioned in a stereotaxic frame (SR-5, Narishige, Tokyo, Japan), and a guide cannula was implanted into their left lateral ventricles. The coordinates used to achieve the correct positioning of the implants were 1 mm anterior to the lambda, 1 mm lateral to the midline, and 1.5 mm ventral to the skull surface. The mice were then housed for a recovery period of 3 days.

For the measurements of the blood glucose levels, the mice were deprived of food for 10–14 h, and then orexin-A (2 fmol per mouse), orexin-B (200 fmol per mouse), or saline was infused. All of these solutions (2 μl) were delivered into the cerebral ventricle through the cannula guide. Blood samples (20 μl) were collected four times (before, and 2, 4 and 6 h after the i.c.v. administration) from each mouse. The food deprivation was continued throughout the experimental period. At the end of the experimental period, ink was loaded to stain the positions at which the drugs were administered. Then the brains were isolated from the animals anesthetized with ether and cut into slices.

2.6. Measurement of serum insulin levels

The normal mice and mice with streptozotocin-induced diabetes were deprived of food for 10–14 h, and separated into four groups in order to examine the effects of saline, orexin-A, orexin-B, and glibenclamide. Saline, orexin-A (1 nmol/kg, i.v.) and orexin-B (1 nmol/kg, i.v.) were injected via the tail vein, while glibenclamide was given perorally (40 μmol/kg). The food deprivation was continued even after the administration of these drugs at time zero. At 0.5, 1, 2 and 6 h after the respective drugs were administered, the mice were sacrificed by decapitation under ether anes-

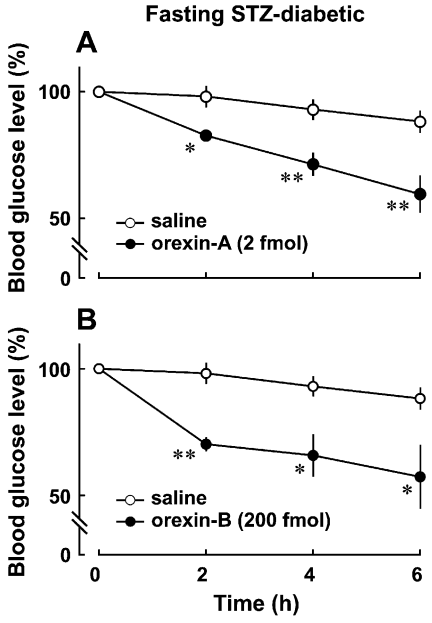

Fig. 3. Blood-glucose-lowering effects of orexins administered by intracerebroventricular injection in fasting streptozotocin-induced diabetic mice. Food deprivation was begun 10–14 h before the administration of orexins at 0 h and continued for 6 h during the measurements of the blood glucose levels. Graphs show the antihyperglycemic effects of orexin-A (A, 2 fmol/mouse) and orexin-B (B, 200 fmol/mouse) delivered to the ventricle in the streptozotocin (STZ)-induced diabetic mice. Saline was administered as a vehicle control. The glucose levels are expressed as percentages of the blood glucose concentrations observed at 0 h in each mouse before the injection of saline (A and B, 272.8 ± 28.0 mg/dl), orexin-A (A, 416.0 ± 28.3 mg/dl) or orexin-B (B, 304.0 ± 68.9 mg/dl), and then averaged. The values represent means ± S.E.M. ($n=4-5$). * $P<0.05$, * * $P<0.01$; significantly different from the vehicle control at each time-point, by one-way ANOVA followed by Scheffé test.

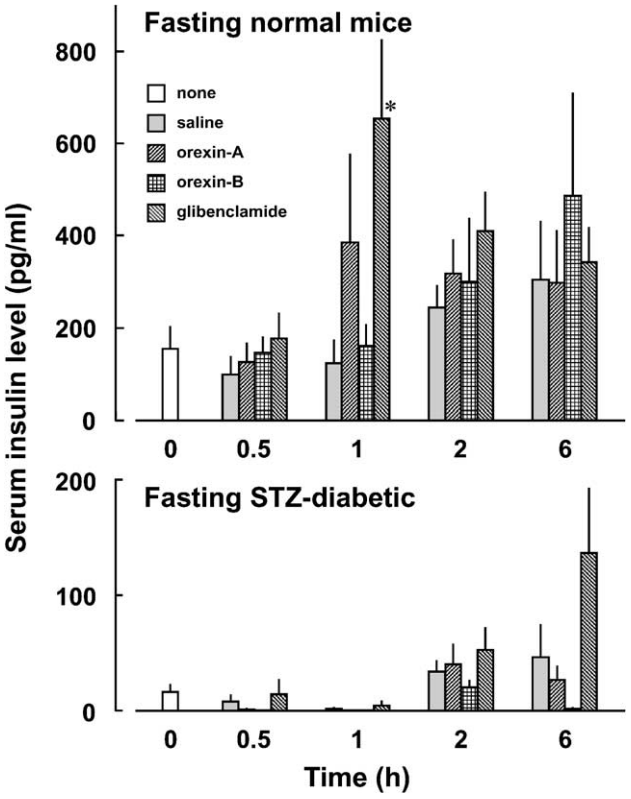

Fig. 4. No involvement of insulin secretion in the orexin-induced reduction of fasting glycemia in mice. Food deprivation was begun 10–14 h before the administration of drugs at 0 h and continued for 6 h during the measurements of the serum insulin levels. Serum insulin levels were measured at 0, 0.5, 1, 2 and 6 h in fasting normal mice (upper) and in fasting streptozotocin (STZ)-induced diabetic mice (lower). No significant increase in the serum insulin levels was observed following injections of orexin-A and orexin-B (1 nmol/kg, i.v.). Saline was injected as a negative control, and glibenclamide (40 μmol/kg) was given orally as a positive control. Data represent means ± S.E.M. ($n=3-5$). * $P<0.05$; significantly different from the control response to saline at each time-point, by the unpaired *t*-test.

thesia, and blood samples (1 ml) were collected and centrifuged at 4 °C. The separated serum specimens were stored at −80 °C until assay. The serum insulin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit for insulin (Morinaga Seikagaku, Tokyo, Japan). In the assay, guinea-pig antiserum against rat insulin was used, because the antiserum cross-react with mouse insulin.

2.7. Statistical analysis

To determine the significance of the effects of orexins on the blood glucose levels 2–4 h after their injection (Figs. 1–3), repeated measures analysis of variance (ANOVA) was performed. When statistical significance was detected by this analysis, further statistical comparison at each measurement time between the groups was conducted by one-way ANOVA, followed by Scheffé test. The data shown in Fig. 4 were analyzed by the unpaired *t*-test, because all blood samples were collected from different mice after 0–6 h of drug administration. $P<0.05$ was considered as denoting statistical significance.

3. Results

3.1. Reduction of blood glucose level by orexin-A and orexin-B in fasting mice

Orexins, or saline as a vehicle control, were injected into the mice after 10–14 h of food deprivation, and the time-course of the changes in the blood glucose levels were recorded in each animal. In the fasting normal mice administered saline, the average glucose levels gradually decreased during the observation period, because of continued food deprivation. The glucose levels were significantly reduced following the injection of orexin-A (1 nmol/kg, i.v.), as compared with the control values (Fig. 1A). Similar reduction of blood glucose levels were also observed following the injection of orexin-B (0.01 and 1 nmol/kg, i.v.) (Fig. 1B). All absolute values of blood glucose levels before and 6 h after administration are presented in Table 1.

In the fasting streptozotocin-diabetic mice, orexin-A and orexin-B (0.001, 0.01 and 1 nmol/kg, i.v.) reduced the blood glucose levels in a dose-dependent manner (Fig. 1C,D;

Table 1
Blood glucose levels before and 6 h after application of orexin-A and orexin-B (1 nmol/kg i.v.), and glibenclamide (40 μmol p.o.) in normal and streptozotocin-induced diabetic mice

	n	0 h	6 h
Normal fasting			
Saline	5	85.2 ± 2.8	70.0 ± 0.6
Orexin-A	6	96.0 ± 3.4	66.7 ± 3.0
Orexin-B	6	81.3 ± 2.6	57.0 ± 2.6
Saline (p.o.)	7	94.3 ± 8.3	85.1 ± 5.2
Glibenclamide (p.o.)	5	82.8 ± 1.9	40.4 ± 4.6
Streptozotocin-diabetic fasting			
Saline	6	315.3 ± 32.0	271.0 ± 26.2
Orexin-A	5	298.8 ± 25.4	142.0 ± 21.4
Orexin-B	6	327.7 ± 39.8	191.3 ± 16.3
Saline (p.o.)	5	294.8 ± 24.7	272.8 ± 21.0
Glibenclamide (p.o.)	5	311.6 ± 38.2	222.8 ± 16.8
Normal fed			
Saline	6	174.7 ± 9.0	119.3 ± 5.7
Orexin-A	7	157.0 ± 4.4	112.3 ± 9.0
Orexin-B	8	167.0 ± 7.8	126.6 ± 9.0
Streptozotocin-diabetic fed			
Saline	4	766.5 ± 33.2	567.3 ± 57.9
Orexin-A	5	765.8 ± 18.3	570.6 ± 37.6
Orexin-B	5	776.4 ± 15.0	517.0 ± 53.8

The values represent means (mg/dl) ± S.E.M.

Table 1). These results demonstrated that the blood-glucose-lowering effects of orexin-A and orexin-B were more evident in the diabetic mice than in normal mice.

We used glibenclamide to establish positive controls to study the effects of the orexins in animals in the fasting state. In the normal mice, the blood glucose level 6 h after glibenclamide administration was significantly lower than that obtained in normal mice in response to saline administration (Table 1). In the mice with streptozotocin-induced diabetes also, the blood glucose level at 6 h in response to the oral administration of glibenclamide was significantly lower than the control level (Table 1).

3.2. No effect of orexin-A and orexin-B on blood glucose levels in mice with free access to food

We investigated whether the orexins also affect the blood glucose levels in mice with free access to food. Food was provided to the animals as usual, and then removed from measurement-time zero when saline or orexins were injected (Fig. 2). The food deprivation was continued for the following 6 h during measurement of the blood glucose levels. In the normal mice, even a high concentration of orexin-A or orexin-B (1 nmol/kg, i.v.) did not induce any lowering of the blood glucose level during the observation for period of 6 h (Fig. 2A,B; Table 1). Similar results were obtained in the mice with streptozotocin-induced diabetes (Fig. 2C,D; Table 1). None of these time-dependent changes in the blood glucose levels in the mice administered orexin-A (Fig. 2C) or orexin-B (Fig. 2D), as reflected by the curves, were significantly different from those in the control mice administered saline. These results indicate that the orexins do not reduce the blood glucose levels in mice in the fed state.

3.3. Antihyperglycemic effects of intracerebroventricularly administered orexin-A and orexin-B in fasting mice with streptozotocin-induced diabetes

Central administration of orexins induces hyperphagia via the hypothalamic neural pathway (Flier and Maratos-Flier, 1998). To investigate the central orexin effect on blood glucose levels, we used the mice with streptozotocin-induced diabetes, because the orexin effects after i.v. injection in fasting diabetic mice were more evident rather than those in fasting normal mice (see above). The diabetic mice were deprived of food for 10–14 h, and then administered orexin-A or orexin-B by i.c.v. injection. The food deprivation was continued for the 6 h during measurement of the blood glucose levels. The blood glucose levels were reduced 2–6 h after the i.c.v. administration of orexin-A (2 fmol/mouse), while i.c.v. saline administration did not affect the blood glucose levels (Fig. 3A). Orexin-B (200 fmol/mouse, i.c.v.) also induced lowering of the blood glucose level (Fig. 3B), although at a lower dose (2 fmol/mouse), it had no effect (data not shown). Therefore, orexin-A seems to have a stronger antihyperglycemic action than orexin-B.

3.4. No effect of orexin-A and orexin-B on the serum insulin level

To investigate how orexin induced the reduction in blood glucose levels, serum insulin levels were measured in vivo in both normal mice and mice with streptozotocin-induced diabetes. The animals were deprived of food for 10–14 h before, and for 6 h after the administration of drugs. The serum insulin level was increasing over time in these fasting mice even after the saline injection (Fig. 4). In the normal mice, the serum insulin levels remained unaffected from 0.5 to 6 h after the intravenous injection of orexin-A or orexin-B (1 nmol/kg), as compared with the control response to saline (Fig. 4, upper); the insulin concentrations measured at 1 and 6 h tended to be increased following administration of orexin-A and orexin-B, respectively, but the increases did not attain statistical significance. A significant increase in the serum insulin levels was observed 1 h after the peroral administration of glibenclamide (40 μmol/kg) as the positive controls.

In the mice with streptozotocin-induced diabetes, the basal insulin levels were lower than those in normal mice, and the levels were not elevated following the injection of orexin-A or orexin-B (1 nmol/kg) (Fig. 4, lower). The maximal insulin level was obtained 6 h after the administration of glibenclamide (40 μmol/kg, p.o.), which tended to be increased as compared with the control response to saline. The effect was weaker in streptozotocin-treated mice than in normal mice.

The insulin-induced glucose uptake was further measured in murine 3T3-L1 adipocytes in vitro, as described previously (Wada et al., 2001). Insulin stimulated the uptake of tritiated 2-deoxyglucose into the adipocytes in a concentration-dependent manner (1–17 nM). However, this response to insulin was changed by treatment neither with orexin-A (30 nM) nor with orexin-B (30 nM) (data not shown).

4. Discussion

Accumulating evidence indicates that orexin-A and orexin-B play a role as mediators in the central mechanisms that regulate feeding behavior and sleep control (Sakurai et al., 1998; Chemelli et al., 1999). In the present study, we explored the role of the orexins in the maintenance of energy homeostasis, and demonstrated that both orexin-A and orexin-B induce a reduction of blood glucose levels in mice under the fasting condition. Since the antihyperglycemic effects were also observed following i.c.v. administration of the orexins in fasting diabetic mice, the orexin effects appear to be related to some central regulatory pathway.

Centrally administered orexins are known to stimulate food intake (Sakurai et al., 1998). However, the effects of orexins on the blood glucose and insulin levels had not been studied under the fasting condition. Haynes et al. (1999) reported no change in blood glucose levels following i.c.v. injection of orexin-A into normally fed rats for 8 days, and Nowak et al. (2000) showed that a subcutaneous injection of orexin-A increased the glycemia associated with food intake in rats. It should be noted here that some of the blood-glucose-lowering effect would be hidden by postprandial hyperglycemia. Yoshimichi et al. (2001) used incompletely fasting rats that were deprived of food only for a daylight period, and observed that i.c.v. injection of orexin-A increased the blood glucose concentrations in these rats. This administration paradigm is close to our feeding experiments illustrated in Fig. 2. Indeed, we observed that the blood glucose levels exhibited a tendency to increase following orexin-A and orexin-B administration only in the fed normal mice (Fig. 2A,B), although the differences did not attain statistical significance.

The mechanism underlying the blood-glucose-lowering actions of the orexins remains unclear. But at least stimulation of insulin secretion can be excluded, because the present results demonstrate that both orexin-A and orexin-B reduced the blood glucose levels without affecting the serum insulin levels in fasting normal mice, whereas glibenclamide, a stimulant of insulin release from the islets of Langerhans in the pancreas, reduced the glucose levels with a parallel significant increase in the serum insulin levels. A similar tendency was observed in fasting diabetic mice. The serum insulin level was increasing over time both in fasting normal mice and fasting diabetic mice even after the saline injection. This is probably due to sympathetic activation in the period after injection leading to suppression of insulin release, since this effect is strongest at time-points immediately after injection.

Nowak et al. (2000) have reported that orexin-A and orexin-B stimulate insulin secretion from in vivo and in vitro experiments on rats, which is inconsistent with our observation. We consider that orexins exhibit different properties depending on the concentrations used. In the aforementioned study, 10–1000 times higher concentrations (1–2 nmol per rat weighing ca. 150 g) of orexins than those used in our study were used. Additionally, since fed rats were used in their experiments (Nowak et al., 2000), it is possible that orexins promote feeding behavior and induce an increase in blood glucose levels, causing secondary insulin secretion.

It is of interest to point out that for a similar effect on blood glucose levels, 100-times higher concentration of orexin-B was required after i.c.v. administration than that of orexin-A, whereas after i.v. injection nearly the same concentrations of orexin-A and orexin-B were needed. The expressed mRNA of orexin-1 receptor was more abundant than that of orexin-2 receptor in several brain areas, containing ventromedial hypothalamic nucleus and amygdalo-hippocampal area (Trivedi et al., 1998). Moreover, after 20 h of fasting, the levels of orexin-1 receptor mRNA are increased in these areas (Lu et al., 2000). Because the affinity of orexin-A for orexin-1 receptor is 100 times higher than that of orexin-B (Sakurai et al., 1998), more potent effects by i.c.v. injection of orexin-A than orexin-B may be explicable by the abundance of orexin-1 receptor, although the reason of similar effects of peripheral orexins is yet uncertain. Orexin-A rapidly crosses the blood–brain barrier and reaches brain tissue by the process of simple diffusion because of its high lipophilicity (Kastin and Akerstrom, 1999). Orexin-A may affect mainly centrally, but orexin B does peripherally the fasting blood glucose levels.

Although orexin-A has been reported to increase food intake during the first 4 h after i.c.v. administration in rats, the food intake decreased over the next 20 h, suggesting that the importance of orexins as a central stimulant of food intake is questionable (Taheri et al., 2000). Orexins appear to be involved to a greater extent in the control of energy metabolism than in that of food intake (Lubkin and Stricker-Krongrad, 1998). It has been reported that orexin-neuron-deficient mice exhibit decreased spontaneous locomotor activity (Hara et al., 2001). In addition, central administration of orexins promotes several behavioral activities, including face washing, grooming and searching behaviors (Ida et al., 1999), increases the arterial blood pressure and heart rate (Samson et al., 1999; Shirasaka et al., 1999; Chen et al., 2000), and also the body temperature (Yoshimichi et al., 2001) in rats. These results suggest that orexins increase the arousal status and promote energy consumption. Thus, the most plausible mechanism underlying the blood-glucose-lowering action of the orexins is the possible enhancement of basal energy metabolism by these peptides which considerably increase the glucose consumption.

The observed orexin actions occurring only in the fasting state may lead to the proposed mechanism, as follows. Since the administration of orexins has been reported to increase the plasma epinephrine concentrations in rats (Shirasaka et al., 1999), orexins may also stimulate the epinephrine-induced glycogenesis in the liver in order to maintain normal levels of blood glucose in the face of greatly increased glucose consumption by the orexin actions. It is well known that after 12–18 h of fasting, the liver becomes almost totally depleted of glycogen in humans (Mayes, 1985). It seems, therefore, that in the fasting state, the glycogenesis promoted by orexins cannot maintain blood glucose homeostasis, resulting in anti-hyperglycemic phenomena.

Recently, the existence of a peripheral orexin system has been revealed, based on the findings that orexin receptor mRNAs are expressed in several peripheral tissues of the rat (Jöhren et al., 2001). This is supported by the detection of orexin-A in the rat plasma (Jöhren et al., 2001). The orexin-1 and orexin-2 receptors are expressed in the adrenal glands, in addition to hypothalamus and pituitary (Jöhren et al., 2001). Russell et al. (2001) reported that i.c.v. injection of

orexin-A in rats affects the hypothalamic–pituitary–adrenal function, and increases the plasma corticosterone level, followed by an increase in the plasma level of adrenocorticotropic hormone. It is likely that orexin can promote the corticosterone-induced glycogenesis in the liver of fed mice. We also suggest that the hypothalamic–pituitary–adrenal axis in fasting mice is stimulated by the prolonged food-deprivation stress. Under the condition, the administered orexins may readily enhance negative-feedback modulation on the hypothalamic–pituitary–adrenal axis, and reduce the plasma corticosterone and glucose levels.

The nuclear receptor known as peroxisome proliferator-activated receptor α (PPARα) is reported to play a vital role in the hepatic fatty acid oxidation after prolonged deprivation of food. The oxidation of fatty acids is tightly coupled to glucose synthesis, and fasted PPARα-null mice display severe hypoglycemia (Kerster et al., 1999). If the fatty acid oxidation is inhibited by orexin actions, the blood glucose level could be reduced only in the fasting state, as observed in our study. Thus, it will be of great interest to study further the effect of orexin on lipid metabolism for the energy store during fasting.

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