Desensitization of the Inhibitory Effect of Norepinephrine on Insulin Secretion From Pancreatic Islets of Exercise-Trained Rats

Yuriko Urano, Tomonobu Sakurai, Hiroshi Ueda, Junetsu Ogasawara, Takuya Sakurai, Megumi Takei, and Tetsuya Izawa

The effect of exercise training (9 weeks of running) on norepinephrine-induced inhibition of insulin secretion was examined in rat islets. Insulin secretions from islets in the presence of glucose (≥5.5 mmol/L) were significantly lower in trained (TR) than in control rats (CR). Norepinephrine inhibited 5.5 mmol/L glucose-stimulated insulin secretions and cyclic adenosine monophosphate (cAMP) contents in a dose-dependent manner in CR. Norepinephrine (10 μmol/L)-induced inhibition of insulin secretion was reversed by the blockade of the α_2-adrenergic receptor in CR, but not in TR. Exercise training substantially shifted the dose-dependent curve for clonidine-induced inhibition of insulin secretions and that of cAMP contents to the right. Exercise training did not alter the density of the α_2-adrenergic receptor either per islet or per protein of islet crude membrane. However, exercise training significantly reduced the protein expression of Gαi-2 without change in Gαi-2 mRNA. In CR but not in TR, norepinephrine significantly inhibited insulin secretions elicited by a combination of high glucose, a protein kinase C activator, and an adenylate cyclase activator under Ca^{2+}-free conditions. Thus, exercise training appears to provoke a decreased expression of Gαi-2 protein. This, at least in part, results in loss of the inhibitory effect of norepinephrine either on cAMP content or on insulin secretion at the post-calcium events in stimulus-secretion coupling, which, in turn, leads to the blunted inhibitory effects of norepinephrine on insulin secretion.

INSULIN PLAYS a crucial role in controlling glucose homeostasis in the body. This secretion is well known to decrease during acute exercise both in humans[1-12] and in experimental animals.[13-18] However, the exact mechanism by which insulin secretion decreases during acute exercise is unknown. Some studies suggest that pancreatic denervation in dogs,[13] adrenomedulation in rodents,[14] and adrenalectomy in humans[1,2] alone do not affect acute exercise-induced changes in insulin secretion. However, the pharmacologic evidence strongly supports the physiologic importance of the adrenergic mediation of insulin secretions during acute exercise. For example, the acute exercise-induced inhibition of insulin secretions can be reversed by the blockade of the α_2-adrenergic receptor in both humans[2-4] and rodents.[15-17] The stimulation of the α_2-adrenergic receptor by either norepinephrine or epinephrine has been well established in vitro to reduce the secretion of insulin.[19-25]

The acute exercise-induced inhibition of insulin secretion has also been reported to occur after chronic exercise training,[5-12] which is a well-known method for reducing the insulin response to arginine or glucose in humans[26] and within islets or β cells in rats.[27,28] However, chronic exercise training results in a blunted response of plasma insulin to acute exercise at the same absolute work rate: a higher plasma insulin level was seen in trained than in untrained humans.[8-11] When trained and untrained human subjects exercised at the same relative intensity, the circulating concentrations of insulin during acute exercise were not different between groups.[5-8] The former findings might result from the lesser plasma level of catecholamines at the same absolute work rate following chronic exercise training. The later findings, however, could not always be explained by the difference of plasma catecholamine levels between groups. Greiwe et al[29] showed that neither plasma epinephrine nor norepinephrine level during acute exercise at the same percentage of $V0_{2max}$ (below 65%) differs between trained and untrained state. This indicates that the inhibitory tone of catecholamines to adrenergic receptors of the pancreatic islet during acute exercise would be identical in trained and untrained subjects. Therefore, if the significantly lower insulin concentration is observed in trained subjects prior to acute exercise, insulin levels should also differ between trained and untrained subjects during acute exercise. In fact, the elegant study by Engdahl et al[5] showed that trained subjects began exercise with lower insulin concentrations, but neither plasma insulin level nor glucose concentration differed between groups during most of a 20-minute exercise period at 60% $V0_{2max}$. Even when plasma epinephrine at identical relative work loads (60%~110% $V0_{2max}$) was higher in trained than in untrained human subjects, no greater decrease in the plasma insulin level was seen in trained subjects.[7]

Considering all of these findings, we hypothesized that a reduction in the inhibitory effects of catecholamines on insulin secretion might occur following chronic exercise training, thereby resulting in the lack of significant differences between trained and untrained subjects in the circulating concentrations of insulin during acute exercise. This adaptation would occur at the level of pancreatic islet. The reduction of in vitro glucose-stimulated insulin secretion at the pancreatic islet or β-cell level has been shown to mimic in vivo responses following chronic exercise training.[28] To our knowledge, however, no biochemical in vitro evidence has so far been obtained. Catecholamines have been suggested to reduce insulin secretions through the inhibition of the following events:[24,25] (1) closure of the adenosine triphosphate (ATP)-sensitive channel and per-

From the Department of Kinesiology, Graduate School of Science, Tokyo Metropolitan University, Tokyo, Japan.

Submitted December 22, 2003; accepted June 17, 2004.

This study was supported in part by a grant-in-aid from the Japanese Ministry of Education, Science, Sports, and Culture.

Address reprint requests to Tetsuya Izawa, PhD, Department of Kinesiology, Graduate School of Science, Tokyo Metropolitan University, Hachioji, Tokyo 192-0397, Japan.

0026-0495/04/5311-0008$30.00/0

doi:10.1016/j.metabol.2004.06.008

haps other K^+ channels, (2) activation of L-type voltage-dependent Ca^{2+} channels, (3) activation of adenylate cyclase, and (4) a distal site in stimulus-secretion coupling. A number of reports in the literature on this issue seem to indicate that the latter 2 effects are the most powerful of the individual inhibitory mechanisms. The present experiments were therefore designed to investigate the effect of chronic exercise training on in vitro norepinephrine-induced inhibition of insulin secretion at the level of adenylate cyclase and at a distal site in stimulus-secretion coupling in the islets isolated from the pancreas of rats. The current study shows that chronic exercise training blunted the inhibitory effect of norepinephrine on insulin secretion at the pancreatic islet level.

MATERIALS AND METHODS

Animal Care and Exercise Training Program

Male Wistar rats (SLC, Shizuoka, Japan) with an initial body weight of 90 to 120 g were housed 2 or 3 to a cage in a temperature-controlled room at 23°C with a 12:12-hour light-dark cycle. Food and water were available ad libitum. The animals were randomly divided into 2 groups: sedentary controls (CR, N = 40) and exercise-trained (TR, N = 40). The rats in the TR group were subjected to exercise on a treadmill set at a 5-degree incline 5 days per week for 9 weeks according to the protocol previously reported.[30] The CR animals were not subjected to running on a treadmill. TR rats were killed at least 36 hours after the last exercise session. The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight; Abbott, North Chicago, IL). All experiments conducted in this study were approved by the Animal Care Committee of the Tokyo Metropolitan University Graduate School of Science.

Isolation of Islets

Islets were isolated by the modified method of Gotoh et al.[31] After a laparotomy to expose the pancreas under anesthesia, the bile duct was cannulated, and a Krebs-Ringer bicarbonate (KRB) buffer (in mmol/L: 11.86 NaCl, 0.5 KCl, 0.12 KH_2PO_4, 25 $NaHCO_3$, 0.025 $CaCl_2$, 0.0012 $MgSO_4$, pH 7.4) containing 2 mg/mL collagenase type IV (Worthington Biochemical Corp, Lakewood, NJ) was injected to distend the pancreas. The pancreas was excised and placed into a conical vial. The digestion was then performed at 37°C in a water-bath shaker for 20 to 25 minutes. After collagenase digestion, the isolated islets were placed onto the dish containing a KRB buffer supplemented with 1% bovine serum albumin. The islets were then checked one by one with the naked eye and collected with tweezers.

Measurements of Insulin Release and Cyclic Adenosine Monophosphate Content

Ten islets were pre-incubated at 37°C for 20 minutes in a KRB buffer supplemented with 1% bovine serum albumin. Thereafter, the islets were incubated for an additional 30 minutes with the various agents indicated at 37°C. To determine the insulin secretions under Ca^{2+}-deprived conditions, the islets were pre-incubated with a Ca^{2+}-free KRB buffer containing 1 mmol/L EGTA and 1% bovine serum albumin for 30 minutes at 37°C. Thereafter, the buffer was removed and replaced with a fresh Ca^{2+}-free KRB/EGTA buffer containing 12.5 mmol/L glucose, 1 μmol/L phorbol 12,13-dibutyrate (PDBu), and 10 μmol/L forskolin. The islets were then incubated with or without 10 μmol/L norepinephrine for 30 minutes at 37°C. In all of the experiments described above, the islet-free incubation medium (100 μL) was assayed for insulin. The insulin concentrations in the medium were measured by using an Insulin EIA kit (Morinaga Biochemical, Yokohama, Japan). For the measurement of cyclic adenosine monophosphate (cAMP) content, the incubation medium was decanted. Thereafter, the lysis buffer included in the cAMP assay kit was added to the tubes containing the islets, and the islets were incubated for 10 minutes at room temperature. An aliquot (100 μL) of islet lysate was then assayed for the total cellular cAMP using a cAMP EIA system kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Measurements of an Adrenergic Receptor

The preparation of islet crude membranes was performed essentially by the method of Duzic et al[32] and Coupry et al.[33] The crude membranes were prepared at 4°C. Isolated islets were washed twice with a washing solution (in mmol/L: 137 NaCl, 2.6 KCl, 10 Na_2HPO_4, 1.8 KH_2PO_4) and centrifuged for 1 minute at 200 × *g*. Thereafter, islets were homogenized in a homogenation buffer (5 mmol/L Tris-HCl, pH 7.5, 5 mmol/L EDTA, 5 mmol/L EGTA, 0.1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL pepstation A, 10 μg/mL aprotinin) and centrifuged for 15 minutes at 17,000 × *g*. The pellet was suspended in a membrane buffer (50 mmol/L Tris-HCl, pH 7.5, 0.6 mmol/L EDTA, 5 mmol/L $MgCl_2$, 0.1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL pepstation A, 10 μg/mL aprotinin) and stored at −80°C until the binding assay and immunobloting were performed. The protein concentration was determined using a Bio-Rad D_C protein Assay (Bio-Rad Laboratories, Hercules, CA). There was no difference in the protein content per islet homogenates in the CR and TR.

The crude membranes were incubated with different concentrations of radioligands in a total volume of 100 μL at 25°C. After 60 minutes, the reaction was terminated by adding ice-cold 10 mmol/L Tris-HCl, pH 7.4, followed by rapid filtration over glass-fiber filters. The filters were washed rapidly twice with ice-cold 10 mmol/L Tris-HCl, pH 7.4, and dried for 30 minutes. They were then placed in 5 mL of scintillation fluid and counted in a liquid scintillation spectrometer. Specific binding was determined by subtracting nonspecific binding from total binding. Nonspecific binding was determined by measuring the radioactivity remaining on a filter when incubated with an α_2-adrenergic antagonist, clonidine (10 μmol/L), or a β-adrenergic agonist, isoproterenol (10 μmol/L). [^{3}H]rauwolsine (Amersham) and [^{3}H]CGP12177 (Amersham) were used to determine the α- and β-adrenergic receptors, respectively.

Western Blot Analysis

The islet crude membranes were mixed with Laemmli's sample buffer and then placed in a boiling water bath for 5 minutes at 100°C. To compare TR with CR, identical loading amounts of the pellet fractions were run on the same gel. The samples were cooled and then loaded onto a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel.[34] After electrophoresis, the proteins were then transferred onto a PVDF sequencing membrane (Millipore Corp, CA). The PVDF membrane was first incubated for 1 hour in a washing buffer (80 mmol/L Na_2HPO_4, 20 mmol/L NaH_2PO_4, 100 mmol/L NaCl, 0.1% Tween20) containing 3% bovine serum albumin. After washing, the polyvinylidene difluoride (PVDF) membrane was incubated with specific antisera in the washing buffer for 90 minutes. The following antisera were used at a 1:1,000 dilution: Gαs/olf, Gαi/o/t/z, Gαo, and Gβ (Santa Cruz Biotech Inc, Santa Cruz, CA) and Gαi-1, Gαi-2, and Gαi-3 (Wako Pure Chemical Industries, Osaka, Japan). After washing, the membranes were incubated for 1 hour with goat anti-rabbit immunoglobulin G (1:10,000 dilution)-conjugated horseradish peroxidase or rabbit anti-rabbit immunoglobulin G (1:10,000 dilution)-conjugated horseradish peroxidase antibodies for 90 minutes. The membranes were washed, and the immunoreactive bands were detected by the enhanced chemiluminescence method. The membranes were scanned with a Light-Capture Scanner (ATTO Corp, Tokyo, Japan), and the optical density of each specific band was analyzed with the CS Analyzer (ATTO Corp).

RNA Extraction and Reverse-Transriptase Polymerase Chain Reaction

Gαi-2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNAs were performed using reverse-transcriptase polymerase chain reaction (RT-PCR) analysis. Total RNA was prepared from isolated islets (100 islets) using ISOGEN (NIPPON GENE, Tokyo, Japan). First-strand cDNA was obtained by incubation of 2 μg of total RNA samples with reverse transcriptase (Superscript, GIBCO BRL, Gaithersburg, MD) in 18 μL of a reaction mixture. One microliter of the RT product was subjected to perchloric acid (PCA) using Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ). Thirty-three cycles of amplification were performed for Gαi-2 using the following conditions for each cycle: denaturing at 94°C for 1 minute, annealing at 55°C for 2 minutes, and extension at 72°C for 3 minutes. The PCR products were electrophoresed in 1% agarose gels containing ethidium bromide. The primers used for Gαi-2 were 5′-AATGACCTGGAGCGCATAGC-3′ and 5′-TACTACTGCAGTCACTGCCG-3′.

Expression of Data and Statistical Methods

Values represent the means ± SE. Dose-response curves were evaluated using a 3-way analysis of variance (ANOVA). The differences observed were further assessed by Dancan's multiple comparison. The concentration of clonidine required to inhibit a half-maximal response (IC_{50}) was derived from the mean value of each dose using a 4-parameter logistic equation. The specific maximal binding (Bmax) and K_D for a radiolabeled ligand were determined by a linear regression analysis of the Scatchard plots. To assess the effect of the indicated agents within a group, paired Student's *t* test was applied. The significance of differences between means was assessed by the Scheffé's test after the ANOVA had been performed to establish that there were significant differences between the groups. $P < .05$ was regarded as significant.

RESULTS

Reduced Response to Glucose in Islets From TR

The final mean body weight (g) of the TR rats (321 ± 8) was significantly less than that of the CR animals (365 ± 15). Insulin releases from islets in the presence of various concentrations of glucose were first determined. The basal insulin secretion in the presence of 3 mmol/L glucose was not different between groups: 75.6 ± 22.2 and 70.7 ± 10.1 pg of insulin/islet/30 min in TR and CR, respectively. On the other hand, insulin secretions from islets in the presence of stimulatory concentrations (≥5.5 mmol/L) of glucose were significantly lower in TR than in CR. In the presence of 5.5 mmol/L glucose, the values were 355.6 ± 28.7 and 647.2 ± 34.2 pg of insulin/islet/30 min in TR and CR, respectively (the values, shown as the means ± SE, were calculated from the data obtained in the experiments illustrated in Figs 1 through 3); in the presence of 12.5 mmol/L glucose, the values were 546.1 ± 78.5 and 1337.9 ± 133.9 pg of insulin/islet/30 min in TR and CR, respectively (the values are shown as the means ± SE from 4 independent experiments). The blunted response of islets to glucose is well known. In the present study, the following experiments, in which the effects of indicated agonists on either insulin secretion or cAMP content were examined (Figs 1 to 3), were performed in the presence of 5.5 mmol/L glucose. Such a low concentration was selected because it is able to stimulate insulin secretion, while a higher concentration of glucose would lead to a more significant difference in insulin secretion between groups.

Dose-Response Curves of Insulin Secretions and cAMP Contents to Norepinephrine, Clonidine, and Isoproterenol

Norepinephrine inhibited 5.5 mmol/L glucose-stimulated insulin secretions (Fig 1A and B) and cAMP contents (Fig 1G and H) in a dose-dependent manner in CR. However, no inhibitory effect of norepinephrine on insulin secretions and cAMP contents was found at concentrations from 0.01 to 10 μmol/L norepinephrine in TR. A considerably high concentration of norepinephrine (100 μmol/L) decreased insulin secretions (pg of insulin/islet/30 min) from 385.1 ± 74.5 to 218.3 ± 10.5 (values are shown as the means ± SE from 4 independent experiments) in TR.

Clonidine, an α_2-agonist, inhibited insulin secretions (Fig 1C and D) and cAMP contents (Fig 1I and J) in a dose-dependent manner. However, in TR, the dose-dependent curve for clonidine-induced inhibition of insulin secretion substantially shifted to the right with an increase in the IC_{50} value. The calculated IC_{50} values from the dose-response curves in Fig 1D were 61.5 nmol/L and 1.7 μmol/L in CR and TR, respectively. In addition, the dose-dependent curve for the clonidine-induced inhibition of cAMP content substantially shifted to the right with an increase in the IC_{50} value. The calculated IC_{50} values from the dose-response curves in Fig 1J were 56.6 nmol/L and 7.5 μmol/L in CR and TR, respectively.

Insulin secretions were not stimulated by isoproterenol at concentrations below 1 μmol/L in either group (Fig 1E and F). Insulin secretions stimulated by isoproterenol (>1 μmol/L) were lower in TR than in CR. However, the stimulatory efficiency of insulin secretions due to isoproterenol, which was determined as a percentage of the value without isoproterenol, was not different between groups. In contrast to the effects of isoproterenol on insulin secretions, isoproterenol stimulated cAMP productions dose-dependently in islets from CR but not from TR (Fig 1K and L). Moreover, exercise training significantly reduced cAMP productions and the stimulatory efficiency of cAMP productions due to isoproterenol.

Effects of Yohimbine or Dibutyryl cAMP on Insulin Secretions

Yohimbine, an α_2-adrenergic blocker, significantly increased the insulin release in the presence of 10 μmol/L norepinephrine in CR but not in TR (Fig 2). The rate of increase in insulin secretions due to yohimbine was considerably lower in TR than in CR: 300% and 20% of the value without yohimbine in CR and TR, respectively. The data indicate that the inhibition of insulin secretion through α_2-adrenergic receptor would be impaired following chronic exercise training.

Dibutyryl (dbt-cAMP) (5 mmol/L) significantly increased insulin secretions in both groups (Fig 3A). However, dbt-cAMP-dependent increases in insulin secretions, which were determined by subtracting insulin secretion with glucose alone, were comparable in CR and TR (Fig 3B).

Exercise training decreases the density of β-adrenergic receptors but not of α-adrenergic receptors. The Bmax of [^{3}H]rauwolsine for either a crude membrane or an islet was not different

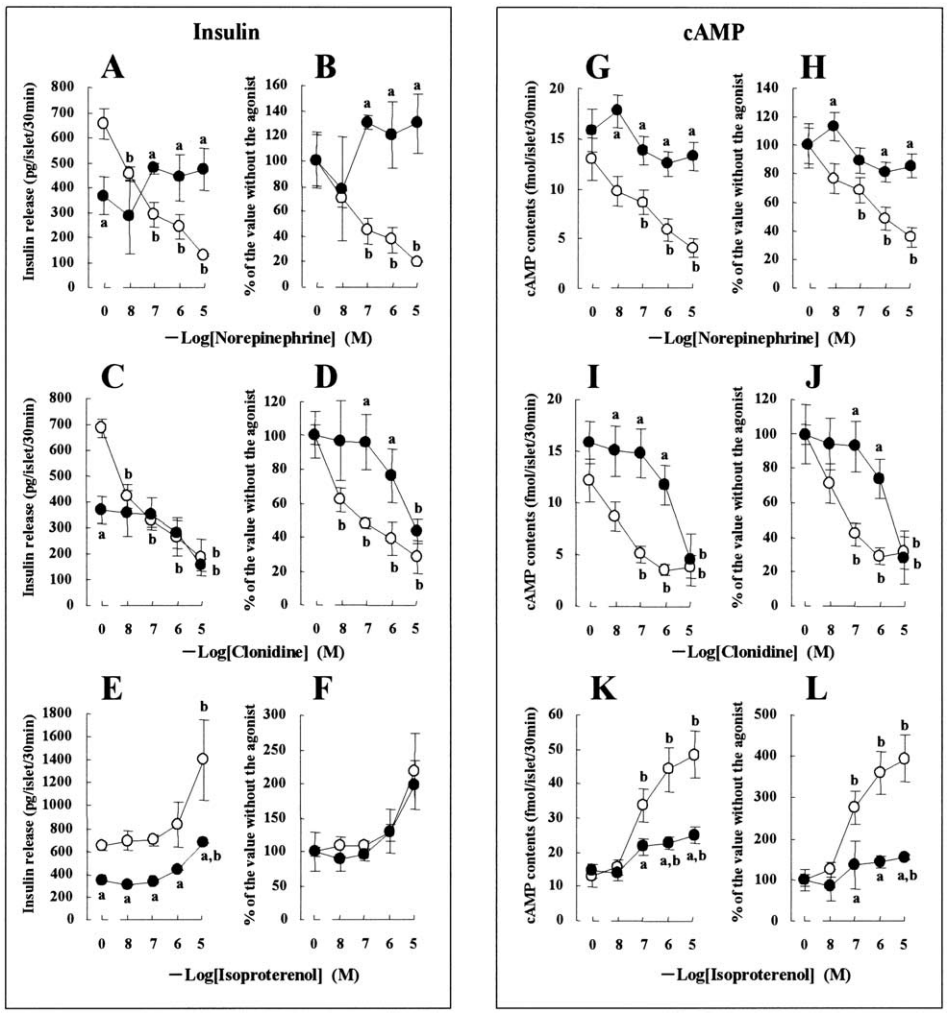

Fig 1. Effects of various agonists on the insulin secretions and the cAMP contents of pancreatic islets from CR (○) and TR (●). Islets were incubated with or without various concentrations of the indicated agonists in the presence of 5.5 mmol/L glucose at 37°C for 30 minutes. (A, C, E) Absolute value of insulin secretions. (B, D, F) Percentages of the values obtained in the absence of any agonist. (G, I, K) Absolute value of cAMP contents. (H, J, L) Percentages of the values obtained in the absence of any agonist. Values represent the mean ± SE of 4 independent experiments. [a]*P* < .05 *v* CR; [b]*P* < .05 *v* without norepinephrine.

between CR and TR (Table 1), indicating that exercise training did not alter the density of the α_2-adrenergic receptor. A reduced K_D value for [^{3}H]rauwolsine was found in TR. The Bmax of [^{3}H]CGP12177 for either a crude membrane or an islet was significantly reduced in TR, indicating that exercise training significantly reduced the density of the β-adrenergic receptor. The K_D value for [^{3}H]CGP12177 was not different between CR and TR.

Exercise Training Decreases the Protein Expression of Gαi-2

As shown in Fig 4, exercise training significantly reduced the protein expression of Gαi/o/t/z, whereas the protein expressions of Gαs and Gβ were not influenced by exercise training. Exercise training significantly reduced the protein expression of Gαi-2 but not that of other Gαi subunits. On the basis of these results, we performed RT-PCR of Gαi-2 mRNA. However, the expression of Gαi-2 mRNA at least 36 hours after the last bout of exercise in TR was comparable to the expression of Gαi-2 mRNA in CR.

Exercise Training Reduces the Inhibitory Effect of Norepinephrine on Insulin Secretion at a Distal Site

Norepinephrine can also induce the inhibition of exocytosis at a very late stage in stimulus-secretion coupling, where the processes involve translocation, docking, priming, and fusion of the secretory granules through cAMP-independent mechanism(s).[23,35,36] Therefore, for the objective of this study, the effect of exercise training on the inhibitory action of norepinephrine must be examined in islets. Norepinephrine has been shown to inhibit insulin secretion elicited by a combination of high glucose, a protein kinase C (PKC) activator, and an adenylate cyclase activator under Ca^{2+}-free conditions.[23,35] This is an example of norepinephrine-induced inhibition of insulin release at a very late stage in stimulus-secretion coupling.[23,35] In this study, we examined the effect of norepinephrine on insulin secretion elicited by a combination of high glucose (12.5 mmol/L), PDBu, and forskolin under Ca^{2+}-free conditions in 2 types of rats (Fig 5). Under such conditions, the blunted response of islets to glucose was still evident in TR, but the difference in insulin secretion in response to 12.5 mmol/L

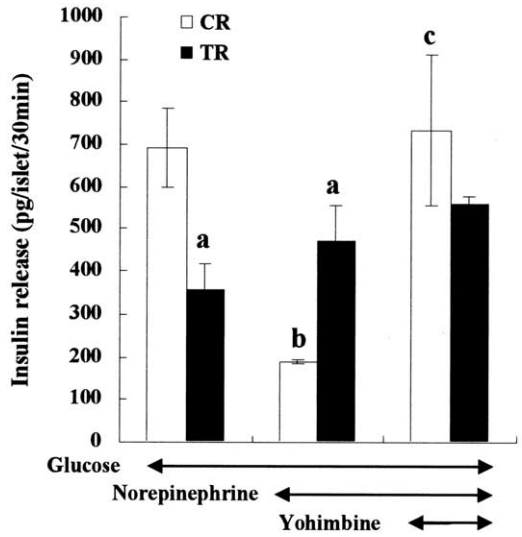

Fig 2. Effects of yohimbine on 10 μmol/L norepinephrine-stimulated insulin secretions of pancreatic islets from CR (□) and TR (■). Islets were pre-incubated with yohimbine at 37°C for 20 minutes and were then incubated with 10 μmol/L norepinephrine in the presence of 5.5 mmol/L glucose at 37°C for 30 minutes. Values represent the mean±SE of 3 independent experiments. [a]*P* < .05 *v* CR; [b]*P* < .05 *v* glucose alone; [c]*P* < .05 *v* without yohimbine.

glucose between CR and TR was similar to that obtained under normal conditions. The insulin secretions of TR were 37% and 44% of those of CR under normal and Ca^{2+}-deprived conditions, respectively. Norepinephrine significantly inhibited insulin secretion under this experimental condition in CR but not in TR.

DISCUSSION

The current study found evidence that exercise training reduces the release of glucose-stimulated insulin from islets. We also discovered that exercise training caused a reduction in the inhibitory effect of norepinephrine on insulin secretion in isolated islets of rats. The mechanism(s) behind this phenomenon involves a decreased expression of Gαi-2 protein, which plays a crucial role in the action of norepinephrine. This, at least in part, results in a reduced sensitivity of the α_2-adrenergic inhibitory effect on the cAMP content and a reduction in the inhibitory effect of norepinephrine on insulin secretion at a very late stage in stimulus secretion coupling mediated through post-calcium events.

We performed the current experiments in islets from TR rats at least 36 hours after their last bout of exercise (from 36 hours to 48 hours). There is little information on whether acute exercise can modulate the effects of cathecholamines on the ability of islets to release insulin. Stich et al[37] showed that during 2 identical successive exercise bouts separated by 1 hour of recovery, the exercise-induced reduction in the plasma insulin level was enhanced, and the increase of plasma epinephrine was dramatically higher during the second exercise bout compared with the first. Thus, the greater decrease in plasma insulin levels during the second bout of exercise was associated with higher plasma epinephrine levels. These results indicate that an initial exposure of isles to high concentrations of catecholamines during the first exercise bout did not lead to desensitization of adrenergic-dependent inhibition of insulin secretion to further catecholamine stimulation during the second bout of exercise. We presume, therefore, that the inhibitory effect of catecholamines on insulin secretion through α_2-adrenergic receptors is intact after a session of acute exercise. The desensitization we observed could not be attributed to the most recent bout of acute exercise performed by a subject. In another study, a single bout of exercise did not affect glucose-stimulated insulin secretion regardless of training status.[38]

The inhibitory mechanisms of catecholamines on insulin secretion are involved in the interaction of the α_2-adrenergic receptor and pertussis toxin–sensitive G proteins (Gαi-1, Gαi-2, Gαi-3, Gαo-1, and Gαo-2). Of pertussis toxin–sensitive G proteins (Gαi-1, Gαi-2, Gαi-3, Gαo-1, and Gαo-2), Gαi-2 is implicated in adenylate cyclase activation and is associated with α_2-adrenergic receptors.[24] The present study showed that exercise training significantly reduced the protein expression of Gαi-2 but not that of other G proteins examined. On the other hand, the density of α_2-adrenergic receptors, which was determined using [^{3}H]rauwolsine, for either a crude membrane or an islet, was not different between CR and TR. A reduced K_D value for [^{3}H]rauwolsine was also found in TR. However, this value represents the affinity of receptors for rauwolsine, an antagonist, but not for an agonist. The fact that the dose-dependent curve for clonidine-inhibited cAMP contents substantially shifted to the right without a change in the α_2-adrenergic receptor density in TR, therefore, must be due to the reduced affinity of the α_2-adrenergic receptor with the decreased expression of Gαi-2 protein. The blunted effect of norepinephrine on cAMP contents in TR would be mediated through the same mechanism. A comparison of the inhibitory effect of clonidine versus that of norepinephrine on cAMP contents showed that norepinephrine had a less inhibitory effect (see Fig 1G, H, I, and J). This was due to the differences in the affinity of norepinephrine for the receptor and that of clonidine

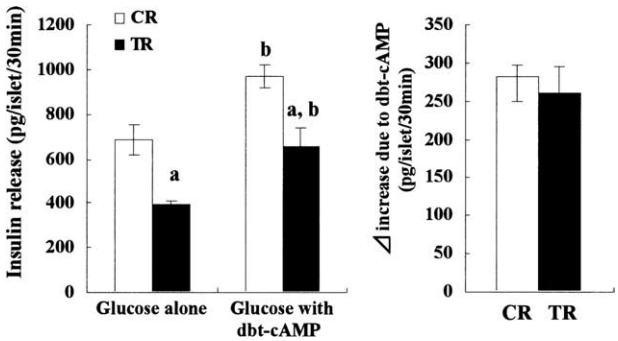

Fig 3. Effects of dbt-cAMP on the insulin secretions of pancreatic islets from CR (□) and TR (■). The left panel shows the absolute value of insulin secretions due to dbt-cAMP, and the right panel, the increases in insulin secretions due to dbt-cAMP. Islets were incubated with 5 mmol/L dbt-cAMP in the presence of 5.5 mmol/L glucose at 37°C for 30 minutes. Reactions were terminated, and insulin release was determined as described in the Methods. Values represent the mean ± SE of 3 independent experiments. [a]*P* < .05 *v* CR; [b]*P* < .05 *v* glucose alone.

Table 1. Binding Characteristics of Either [^{3}H]Rauwolsine or [^{3}H]CGP12177 on Crude Membranes of Islets of Each Conditioned Rat

	CR	TR
[^{3}H]rauwolsine		
Bmax (fmol/mg protein)	975.32 ± 92.64	945.09 ± 58.62
Bmax (fmol/islet)	3.97 ± 0.49	4.25 ± 0.37
K_D (nmol/L)	2.08 ± 0.31	0.93 ± 0.15*
[^{3}H]CGP1217		
Bmax (fmol/mg protein)	837.76 ± 61.48	570.72 ± 74.95*
Bmax (fmol/islet)	2.28 ± 0.19	1.38 ± 0.21*
K_D (nmol/L)	3.97 ± 0.49	4.25 ± 0.37

Note. Bmax represents the specific maximal binding of each radiolabeled ligand to crude membranes. Bmax and K_D were determined by linear regression analysis of the Scatchard plots. Other experimental conditions were described in the Methods. Each value represents the mean ± SE of 3 independent experiments.

Abbreviations: CR, control rats; TR, exercise-trained rats.

*P<.05 *v* CR.

for the receptor. According to the reported apparent dissociation constants of the agonist-receptor complex, as estimated on the basis of the inhibition of cAMP accumulation, clonidine exhibited 33 times stronger affinity for the receptor than norepinephrine.[22]

cAMP is well known to potentiate glucose-stimulated insulin secretion. The exact mechanism behind the augmentation of insulin secretion by cAMP remains uncertain. Some studies suggest that cAMP opens Na^+ channels and Ca^{2+} channels via the phosphorylation of protein kinase A (PKA),[36,39] which immediately enhances glucose-induced insulin secretion. PKA has also been shown to play a positive role in the control of the insulin granule movements in the pancreatic β cell.[40-42] In addition to the PKA-dependent pathway, cAMP may potentiate glucose-induced insulin secretion through a PKA-independent mechanism.[40,43-45] This PKA-independent pathway may di-

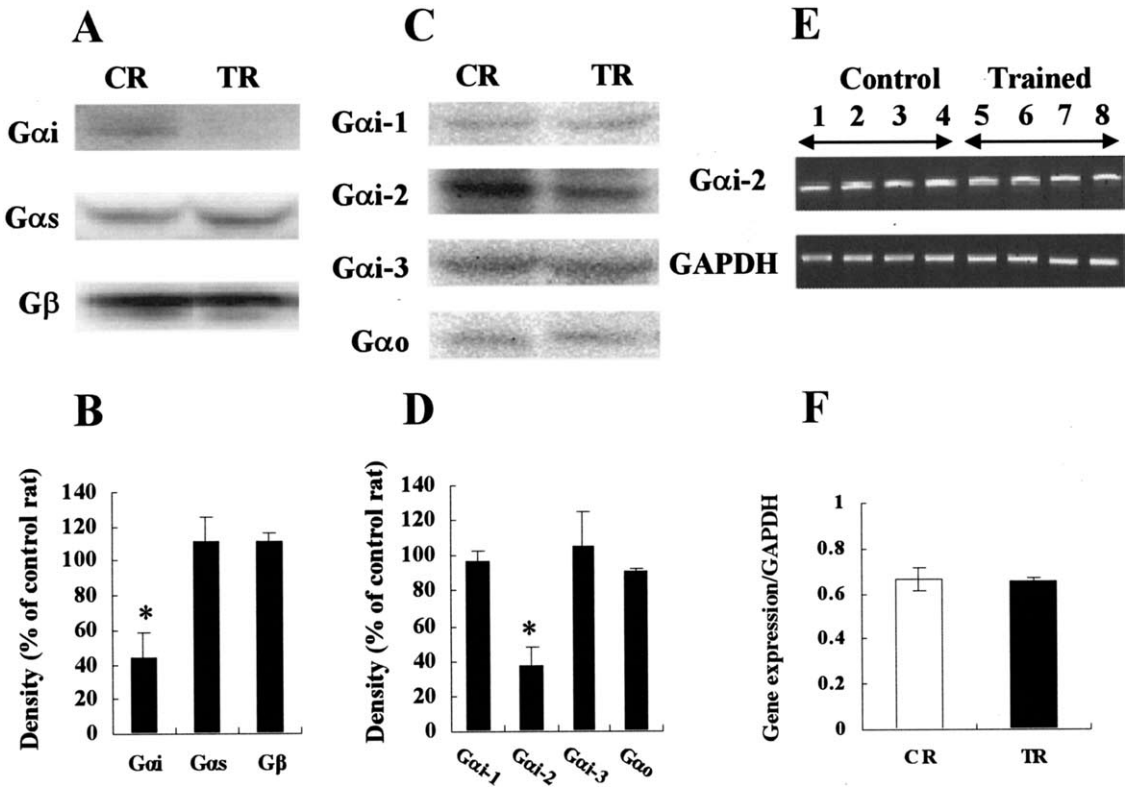

Fig 4. Expression of some subunits of G proteins in the pellet fractions of islet homogenates and expression of Gαi-2 mRNA in cellular extract islets from CR and TR. (A) Representative immunoblot data of the Gαi, Gαs, and Gβ subunits. (B) Relative density of each subunit. (C) Representative immunoblot data of the Gαi-1, Gαi-2, Gαi-3, and Gαo subunits. (D) Relative density of each subunit. The representative immunoblot data are from pooled samples of 2 individual rats in each group. Values are the means ± SE. *P < .05 *v* CR. (E, F) Representative data of Gαi-2 mRNA and gene expression/GAPDH, respectively, in CR (□) and TR (■). The data for RT-PCR correspond to 4 individual rats from each group. Values are means ± SE (n = 4 for each group).

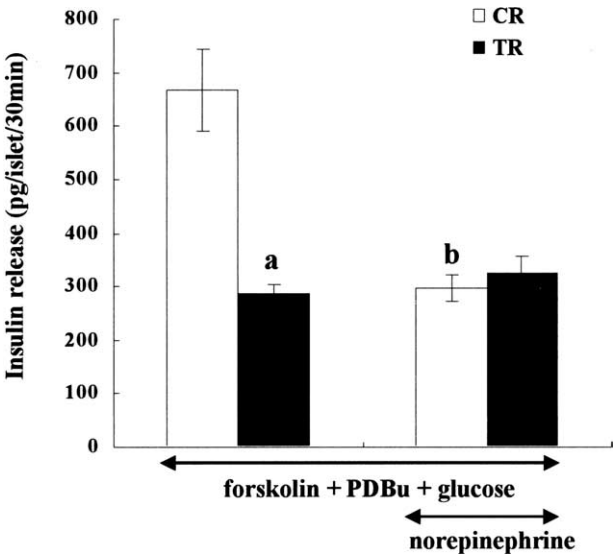

Fig 5. Inhibitory effect of norepinephrine on insulin secretion elicited by a combination of high glucose, a protein kinase C activator, and an adenylate cyclase activator under Ca^{2+}-free conditions in CR (□) and TR (■). Islets were pre-incubated with the indicated agents without norepinephrine at 37°C for 10 minutes and then incubated with 10 μmol/L norepinephrine in the presence of 12.5 mmol/L glucose at 37°C for 30 minutes. Values represent the mean ± SE of 4 independent experiments. [a]*P* < .05 *v* CR; [b]*P* < .05 *v* without norepinephrine. PDBu, phorbol 12,13-dibutyrate.

rectly activate exocytosis.[45] Thus, cAMP may potentiate glucose-stimulated insulin secretion through both PKA-dependent and PKA-independent pathways. A loss in the reducing effect of α_2-agonists on cAMP content, therefore, leads to a reduced inhibitory action of these agonists on insulin secretion. Indeed, the alteration in the dose-dependent curve for clonidine-inhibited cAMP contents was well in accord with the alteration in the dose-dependent curve for clonidine-inhibited insulin secretions with a salient increase in the IC_{50} value. The blunted effect of norepinephrine on insulin secretion was also accompanied by a loss in the reducing effect of norepinephrine on cAMP content. Thus, the present data clearly show that the blunted inhibitory effects of α_2-agonists on insulin secretion in TR were accompanied by a loss in the reducing effect of α_2-agonists on cAMP content through the decreased expression of Gαi-2 protein without a change in the α_2-adrenergic receptor density.

Norepinephrine is also able to inhibit exocytosis at a very late stage in stimulus secretion coupling through cAMP-independent mechanism(s).[23,35,36] This inhibitory effect of norepinephrine is also mediated through some G proteins, Gi-1, Gi2, Gi-3, and Go-2.[24] Therefore, we tested the effect of norepinephrine on the PKA/PKC-induced Ca^{2+}-independent glucose-stimulated insulin secretion in both groups. Under this condition, norepinephrine has been shown to inhibit Ca^{2+}-independent glucose-stimulated insulin secretion at a very late stage in stimulus secretion coupling through pertussis toxin–sensitive G proteins.[23,35,36,46] As would be expected, owing to the reduction of Gαi-2 protein expression, no inhibitory effect of norepinephrine was found in TR.

In the present study, we did not try to determine whether exercise training would alter insulin secretory responses at a site distal to cAMP production or exocytosis at a very late stage in stimulus secretion coupling, where the processes involve translocation, docking, priming, and fusion of the secretory granules. In the current study, dbt-cAMP–dependent increases in insulin secretions were comparable in the CR and TR. This could indicate that exercise training does not alter a response at the sites distal to cAMP production. However, a lack of any other data for the effect of exercise training on several pathways distal to either cAMP or exocytosis at a very late stage does not permit us to elicit such a conclusion. Further studies of this nature may shed light on the mechanisms behind the adaptation of insulin secretory cells to exercise training.

The mechanism(s) by which exercise training reduced the protein expression of Gαi-2 remains unclear at present. When exercise training alters G-protein expression, 2 broad mechanisms should be involved: an alteration in the rate of synthesis and/or an increase in the rate of degradation of G proteins. The lack of alteration of Gαi-2 mRNA expression may indicate that the reduced protein expression of Gαi-2 is due to a change at the post-transcriptional levels. Some recent in vitro studies indicate that the downregulation of Gi2α protein is not always accompanied by a decrease in Gi2-α mRNA.[47,48] Botion et al[47] showed that in rat adipocytes, either tumor necrosis factor-α– or adenosine-induced down-regulation of Gi was due to an increase in the rate of proteolysis mediated by the proteasome pathway, rather than decreased transcription or translation. Specific degradation of Gα-subunit protein by ubiquitin/proteasome-dependent pathway has also been documented in the yeast G protein Gpa1[49,50] and in Go.[51] Interestingly, exercise training tends to enhance the activity of proteasome complex in skeletal muscles.[52] Moreover, Jewell-Motz et al[48] showed that the physical interaction between the receptor and Gi reduces the down-regulation of Gi protein without alteration of Giα-2 mRNA in transfected Chinese hamster ovary cells expressing the human α_{2A}-adrenergic receptor. They suggest that repetitive receptor Gi high-affinity binding events that occur with long-term agonist exposure target Gαi to a degradation pathway. The agonist-induced dissociation of α-subunit of G protein from $\beta\gamma$ could make the α-subunit a better substrate for ubiquitination. In this respect, exercise training can also induce repetitive exposure of α_2-adrenergic receptors in islets to the relatively high concentrations of catecholamines that occur during repeated exercise. These events could yield the increase in the amount of agonist-induced dissociated α-subunit of G protein from $\beta\gamma$. Taken together, it is tempting to speculate that both the repetitive receptor Gi high-affinity binding events and the ubiquitin/proteasome complex, at least in part, could act as the modulators that cause the adaptive response of Gαi-2 to chronic exercise.

Exercise training did not alter the protein expression of Gsα coupled with the β-adrenergic receptor. The reduction of isoproterenol-induced cAMP production appears to be due to the decrease in the density of the β-adrenergic receptor. However, cAMP production in response to isoproterenol increased in a dose-dependent manner, but insulin secretion did not. Thus, there was no obvious correlation between the β-receptor–stimulated increases in cAMP production and those in insulin

secretion. The exact reason for this phenomenon is unknown at present. However, it is questionable whether β-adrenergic receptors play a physiologic role in insulin secretion. The action of norepinephrine, a physiologic agonist, on α_2-adrenergic receptors has been shown to predominate,[19] while insulin secretion is normally reduced during exercise.[1-18]

The main goal of this study was to assess the inhibitory effect of norepinephrine on insulin secretion in vitro in isolated islets from exercise-trained rats. In this initial study, we did not test whether the observed in vitro–blunted response of islets to norepinephrine is involved in the acute exercise-induced changes in in vivo insulin secretion in exercise-trained rats. However, blockade of the α_2-adrenergic receptors has been shown to prevent an acute exercise-induced decrease in blood insulin concentrations in rats.[15-17] We conducted a preliminary experiments using fasted rats ($n = 5$ for each group) with a training protocol identical to that used in the present study. In that study, we found that the peripheral insulin concentration (ng/mL) from a retro-orbital puncture was less in the trained (0.55 ± 0.03) than in the untrained rats (0.79 ± 0.04); however, the insulin concentrations immediately after treadmill running (17 to 20 m/min, for 60 minutes) were higher in the trained (0.34 ± 0.03) than in the untrained rats (0.25 ± 0.04) (unpublished results). Thus, the obtained in vitro data could be reflected in the insulin secretory responses observed in vivo. Given that the observed findings are also evident in portal vein plasma, we assume that the higher insulin concentration could result in lesser activation of hepatic glycogenolysis and glyconeogenesis during acute exercise after chronic exercise training.[53] Depletion of hepatic glycogen stores during exercise has been shown to occur more slowly in trained than in untrained rats.[54] However, a large number of important regulatory factors of in vivo insulin secretion exist, and the effects of insulin on metabolism interact with some other hormonal factors and metabolites. In order to relate in vitro observations with in vivo tests of insulin secretion and to address the exact physiologic significance of higher insulin levels during submaximal acute exercise in trained individuals, further studies will be required in our animal model or in human subjects.

In conclusion, the inhibition of norepinephrine-induced insulin secretion was not observed in TR. This event was associated with the reduced sensitivity of the α_2-adrenergic inhibitory effect on the cAMP content in islets of TR. The desensitization of the norepinephrine effect may be due to the decreased expression of Gαi-2 protein but not to the down-regulation of receptors. Moreover, exercise training reduced the inhibitory effect of norepinephrine on insulin secretion at a very late stage in stimulus secretion coupling mediated through post-calcium events. This would also be attributable to the decreased expression of Gαi-2 protein. Thus, one mechanism behind the observed desensitization of the inhibitory effect of norepinephrine on insulin secretion in islets of TR rats may be the reduced expression of the Gαi-2 protein, thereby resulting in losses in the inhibitory effects of norepinephrine on either cAMP or Ca^{2+}-independent glucose-stimulated insulin secretion.

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