Role of the Sympathoadrenal System in Exercise-Induced Inhibition of Insulin Secretion

Effects of Islet Transplantation

Harmina Houwing, Kim M.A. Fränkel, Jan H. Strubbe, Paul T.R. van Suylichem, and Anton B. Steffens

The present study was designed to investigate the mechanism leading to inhibition of insulin release during exercise. To investigate the influence of circulating epinephrine and norepinephrine, these catecholamines were infused intravenously in resting islet-transplanted and control rats. The role of neural influences on insulin release was investigated by a swimming exercise study in islet-transplanted and control rats, before and after adrenodemedullation. Streptozotocin-induced diabetic Albino Oxford rats received 5 μl islet tissue into the portal vein, resulting in return of normal basal glucose and insulin levels. Transplanted and control animals were provided with two permanent heart catheters to sample blood and to give infusions. Infusion of epinephrine and norepinephrine did not result in inhibition of plasma insulin levels. Blood glucose levels, as well as nonesterified fatty acids and insulin levels in plasma, were similar in both groups. After the infusion study, the animals were subjected to strenuous swimming. During exercise, plasma insulin levels decreased not only in controls, but also in the islet-transplanted group. Blood glucose and plasma catecholamine responses were identical in both groups. After adrenodemedullation, epinephrine was not detectable and the exercise-induced decrease of insulin was not affected. These results indicate that circulating epinephrine and norepinephrine in physiological concentrations do not cause inhibition of insulin secretion. Since the exercise-induced inhibition of insulin secretion is still present in rats with islet grafts, it seems reasonable to suggest that sympathetic neural influences are responsible for the inhibition of insulin release during exercise and that transplanted islets are sympathetically reinnervated. *Diabetes* 44:565–571, 1995

Exercise involves an increased use of circulating metabolic substrates to provide energy for the active muscles. Since, during exercise, the levels of circulating glucose and nonesterified fatty acids (NEFAs) remain fairly constant, hepatic glucose production and lipolysis must be enhanced. These processes are facilitated by reduced insulin secretion in the early phase of exercise, together with the stimulating effects of the sympathoadrenal system and pancreatic glucagon (1–4). Sympathetic influences play a role in the inhibition of insulin secretion (2,5–10) by contributions from both the neural and adrenomedullary branches of the sympathetic nervous system. Epinephrine, released from the adrenal medulla, and norepinephrine, released from the sympathetic nerve endings, inhibit insulin release via stimulation of α_2-adrenergic receptors on the β-cells of the islets of Langerhans (7,11,12). Intravenous infusion of epinephrine (273 nmol/min) has been shown to decrease plasma insulin levels, whereas intravenous infusion of norepinephrine (300 nmol/min) had no effect on insulin secretion (13). The high norepinephrine concentration in the synaptic clefts near the β-cells suppresses insulin release. A part of the released norepinephrine will leak into the blood, but the concentration is probably not high enough to suppress insulin release. Therefore, only unphysiological high doses of circulating norepinephrine may result in reduced insulin secretion.

Although both epinephrine and norepinephrine can influence insulin release by humoral mechanisms, neural sympathetic influences seem to be the main factor inhibiting insulin release. Electrical stimulation of the sympathetic nerves, innervating the pancreas in dogs, decreases insulin output (14). One method to study the neural influences on insulin secretion is denervation by transplanting islets of Langerhans into diabetic rats. Islets have been transplanted to different sites and with different volumes of endocrine tissue (15–18), resulting in normoglycemia. After islet transplantations, intravenous glucose tolerance tests and/or meal tests were performed (17–21). These studies indicated that glucose tolerance was reduced in rats with islet grafts compared with normal rats. The reduced preabsorptive insulin secretion, possibly due to the absence of cholinergic innervation of the transplanted islets of Langerhans, was the main factor causing this intolerance (17–22).

The main goal of the present study was to investigate the

From the Department of Animal Physiology (H.H., K.M.A.F., J.H.S., A.B.S.), University of Groningen, Haren; and Department of Surgery (P.T.R.V.S.), University Hospital, Groningen, The Netherlands.

Address correspondence and reprint requests to Dr. Harmina Houwing, Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

Received for publication 12 September 1994 and accepted in revised form 19 January 1995.

HPLC, high-pressure liquid chromatography; ID, inside diameter; NEFA, nonesterified fatty acid; NPY, neuropeptide Y; OD, outside diameter; STZ, streptozotocin.

mechanism leading to inhibition of insulin release during exercise. Since exercise leads to increased plasma epinephrine and norepinephrine levels, these levels were mimicked in resting islet-transplanted and control rats by infusion of epinephrine and norepinephrine. In this first part of the study, concentrations of blood glucose, plasma NEFAs, and plasma insulin were measured. In the second part of the study, a swimming exercise study was performed in diabetic rats provided with islets of Langerhans transplanted into the portal vein. Blood glucose concentrations in whole blood and the concentrations of NEFAs, insulin, catecholamines, and corticosterone in plasma were determined. Finally, to investigate the influence of circulating epinephrine during exercise, the islet-transplanted and control rats were subjected to adrenodemedullation and submitted to the swimming exercise experiment again.

RESEARCH DESIGN AND METHODS

Diabetes was induced in male inbred Albino Oxford (AO/G) rats, body weight 270–380 g, obtained from the Central Animal Laboratory of the University of Groningen, by an intravenous injection of 70 mg/kg streptozotocin (STZ) (Zanozar, Upjohn, Kalamazoo, MI). This resulted in loss of body weight, glucosuria, increased water intake, and blood glucose levels of >19 mmol/l. Then 2–3 weeks after diabetes induction, islet tissue isolated from isogenic islet donors with a body weight of 350–400 g was transplanted into the portal vein.

The animals were housed individually in Perspex cages, and they had free access to water and standard rat food (Hope Farms, Woerden, The Netherlands), except for the 2 h before the start of the experiments. They were maintained on a 12-h/12-h light/dark cycle at a room temperature of ~20°C. Since corticosterone levels show a diurnal variation, experiments were performed between 3 and 6 h after the beginning of the light period, when these levels are low.

Islet isolation and transplantation. The rat islet isolation method used in our laboratory has been described previously (23). Briefly, the pancreas was distended by infusing 10 ml Krebs-Ringer solution, containing 25 mmol/l HEPES and 10% bovine serum albumin, into the pancreatic duct. The pancreas was then excised and cut into small pieces with a pair of scissors. A two-stage collagenase (Sigma type XI, 2,200 U/mg; Sigma, St. Louis, MO) digestion was performed at 37°C at concentrations of 1.2 and 0.7 mg/ml, respectively. Islets were separated from the exocrine tissue using a discontinuous dextran gradient (Sigma industrial grade, molecular weight 70,000). Further purification of the islets was obtained by handpicking to eliminate nonseparated lymph nodes and vascular and ductal tissue from the islet grafts. Islets were identified with the aid of a dissection microscope (Bausch & Lomb 31–28–06) and a fluorescent lamp (Bausch & Lomb 31–33–66). With this illumination, rat islets appear as distinct ocherous bodies, whereas lymph nodes and exocrine tissue are gray. The reliability of this method has been confirmed by histological analysis and by dithizone staining.

The islet volume obtained was determined by measuring the islet diameters, expressed as the mean of two axes, in a 5% aliquot of the islet suspension. Assuming the islets to be perfect spheres, islet volume was calculated. Grafts of 5 μl endocrine tissue were prepared by taking an appropriate portion of the total islet suspension. This volume is ~50% of the content of a normal adult pancreas, as determined by measuring insulin content of a volume of 10 μl (17). Immediately after islet isolation, transplantation into the liver was performed by direct puncture with a 23-gauge butterfly needle in the portal vein. In control animals of similar body weights, a sham operation was performed.

Surgery. The animals were each provided with a silicon heart catheter in a branch of the right jugular vein (0.95 mm outside diameter [OD]; 0.50 mm inside diameter [ID]) to sample blood (24) 3–4 weeks after transplantation or sham operation. A second catheter (0.64 mm OD, 0.28 mm ID) was inserted in a branch of the left jugular vein to give infusions in freely moving animals. After recovery, the animals were submitted to an experiment in which either norepinephrine or epinephrine was infused and to an exercise experiment. Finally, the adrenal medulla was removed by extirpation. After an incision in the adrenal cortex, the adrenal medulla was popped out by slight pressure. None of the adrenodemedullated rats drank the saline solution that was offered in addition to the normal water, indicating that mineralocorticoids were still available. In these animals, the exercise experiment was repeated. All surgery was performed under ether or halothane anesthesia. The animals were allowed to recover for at least 1 week and until they had regained their preoperative body weight.

Experimental design. In nonexercising animals, either epinephrine (109 nmol [20 ng] in 0.1 ml saline/min) or norepinephrine (300 nmol [50 ng] in 0.1 ml saline/min) was infused intravenously during 20 min. Ascorbic acid (0.01% wt/vol) was added to the infusion solution to prevent oxidation of epinephrine and norepinephrine. These doses of epinephrine and norepinephrine were shown to result in similar plasma levels as those found during exercise (25). Blood samples of 0.4 ml were taken before ($t = -11$ and -1 min), during ($t = 2$, 5, 7, 12, and 17 min), and after infusion ($t = 22$, 32, and 42 min) for the determination of blood glucose, plasma insulin, and plasma NEFA levels. After sampling, the withdrawn volume of blood was immediately replaced by an equal volume of citrated donor blood. Citrate was used to prevent the activation of endothelial lipase by heparin.

In a second set of experiments, exercise was performed in a swimming pool (length, 3.0 m; width, 0.4 m; depth, 0.9 m) containing water at 33 ± 2°C. Blood samples of 0.60 ml were taken for determination of blood glucose and plasma NEFAs, insulin, and corticosterone, or samples of 0.75 ml were taken for cases in which determinations of plasma epinephrine and norepinephrine were also made. The withdrawn volume of blood was replaced by citrated donor blood. The samples were taken in the home cage ($t = -23$ and -13 min), at the waiting platform ($t = -11.5$ and -3 min), during lowering of this platform ($t = -1$), during swimming ($t = 1$, 5, 10, and 15 min), and at the resting platform ($t = 19$, 24, 29, and 39 min). The construction of this swimming pool, as well as the experimental procedure, was described previously by Scheurink et al. (8,26).

Chemical determinations. The blood samples were immediately transferred to chilled centrifuge tubes containing 5 or 10 μl heparin solution (500 IU/ml) as anticoagulant and 0.01% EDTA as antioxidant.

Glucose concentrations were measured in 0.05 ml blood with Hoffman's ferricyanide method in a Technicon AutoAnalyzer TMII. The remaining blood was centrifuged for 10 min at 5,000 rpm at 4°C. For the assay of catecholamines, 100 μl plasma was stored at −80°C. For the assay of NEFAs, 100 μl plasma was immediately extracted according to the method of Antonis (27). The evaporated extracts were stored at −20°C until determination. The remaining plasma was stored at −20°C for the determination of insulin and corticosterone concentrations.

Catecholamine concentrations were analyzed in 90 μl extracted plasma by dihydroxybenzylamine-controlled high-pressure liquid chromatography (HPLC) in combination with electrochemical detection, as previously described (26). The lower detection limit was 54.6 pmol/l for epinephrine and 0.03 nmol/l for norepinephrine.

Insulin concentrations were measured in duplicate in a radioimmunoassay using guinea pig serum as antiserum (M8309, Novo, Copenhagen, Denmark). The bound and free ^{125}I-labeled insulin were separated by means of a polyethylene glycol solution as described by Henquin et al. (28).

Corticosterone was extracted from 75 μl plasma via solid-phase extraction with acetonitrile. The levels of corticosterone were measured by HPLC in combination with ultraviolet detection (29). The lower detection limit was 28.9 nmol/l.

Statistical analysis. Data are expressed as means ± SE and were evaluated using multivariate analysis of variance for repeated measures (MANOVA of SPSS\PC+). The Mann-Whitney U test was used for testing the source of variation between groups. Wilcoxon's matched-pairs signed-rank test was used for testing differences between time points. Differences with a value of $P < 0.05$ were considered significant.

RESULTS

Diabetes induction and transplantation. STZ injection resulted in a body weight loss of 73.9 ± 4.0 g (Table 1). Blood glucose concentrations before transplantation were >19 mmol/l. After transplantation, the animals became normoglycemic (blood glucose <8 mmol/l) within 5 days. The body weight that was lost after STZ injection was gained rapidly after transplantation of islets. After 18 ± 1.6 days, the original body weights were reached again.

Epinephrine and norepinephrine infusions. In Fig. 1, the results of epinephrine infusions on blood glucose, plasma NEFA, and plasma insulin levels in five animals with islet

TABLE 1
Effects of STZ injection on body weight and blood glucose levels, and time period needed to return to the normal state after intraportal islet transplantation

	Before STZ	Before islet transplantation	Time to return to normal state (days)
Body weight (g)	330.0 ± 7.8	256.1 ± 9.5	18.0 ± 1.6
Glucose level (mmol/l)	5.9 ± 0.3	24.1 ± 0.9	4.5 ± 1.1

Data are means ± SE. STZ injection reduces body weight by 73.9 ± 4.0 g ($P < 0.05$) and increases blood glucose levels dramatically ($P < 0.05$). The return to normoglycemia (glucose <8 mmol/l) is much faster than the recovery of normal body weight to the state before diabetes induction.

grafts and six control animals are depicted. Baseline levels of glucose, NEFAs, and insulin were not significantly different in both groups of animals. Intravenous infusion of epinephrine resulted in a similar increase in blood glucose levels ($P < 0.05$) in both groups. Plasma insulin and plasma NEFA levels were not affected by infusion of epinephrine.

Figure 2 shows the influence of norepinephrine infusions on blood glucose, plasma NEFA, and plasma insulin levels. Baseline levels of blood glucose and plasma insulin were similar in the two groups; baseline plasma NEFA levels are not significantly different in the two groups. After the norepinephrine infusion was started, blood glucose levels in the control groups immediately increased ($P < 0.05$). In contrast,

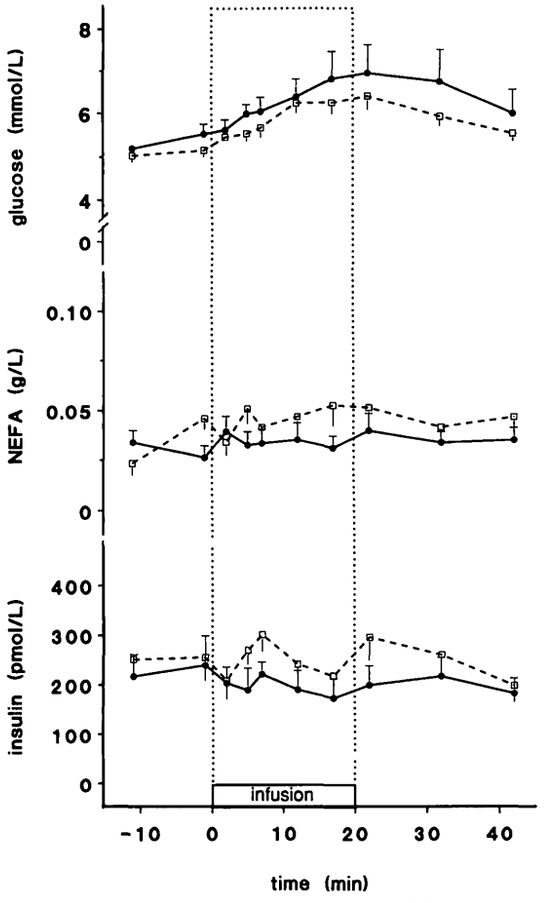


FIG. 1. Effect of intravenous infusion of 109 nmol (20 ng) epinephrine per min on blood glucose, plasma NEFA, and plasma insulin levels in five islet-transplanted (●) and six control (□) rats. Data are means ± SE.

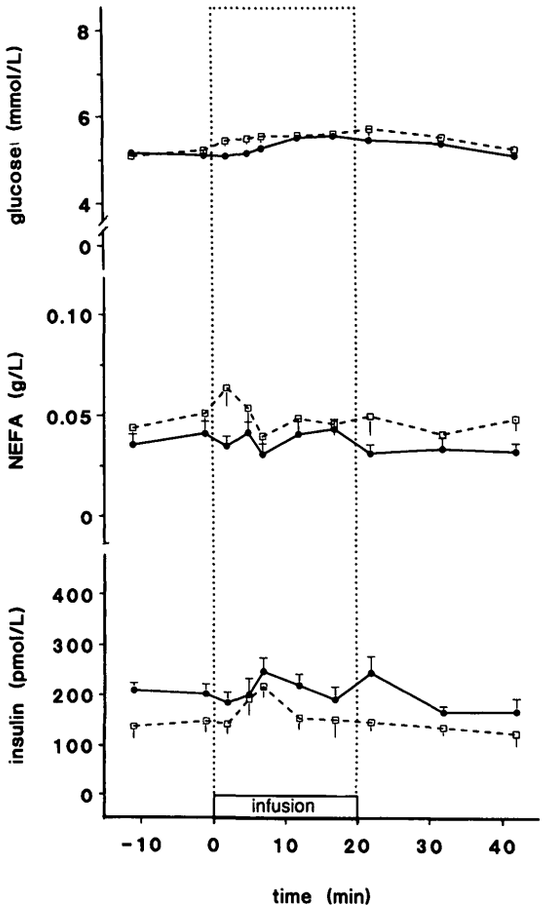
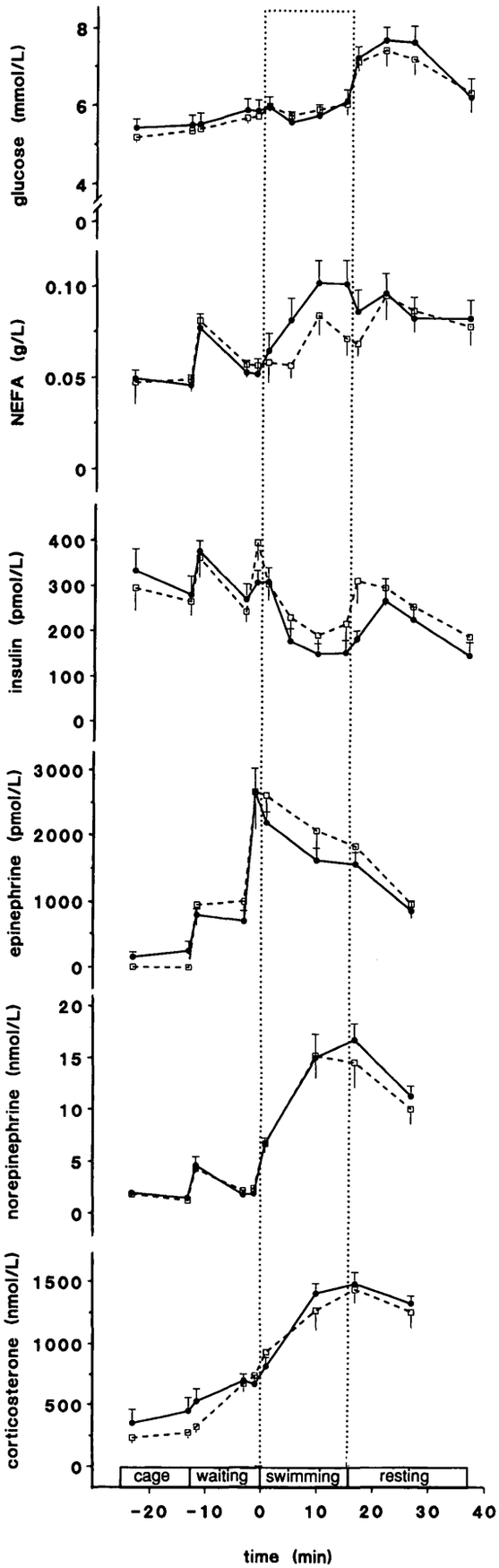


FIG. 2. Effect of intravenous infusion of 300 nmol (50 ng) norepinephrine per min on blood glucose, plasma NEFA, and plasma insulin levels in six islet-transplanted (●) and six control rats (□). Data are means ± SE.

blood glucose levels in the transplanted group started to increase with a delay of 5 min ($P < 0.05$). Plasma NEFA and insulin levels were not affected by infusion of norepinephrine.

Exercise. In Fig. 3, the results of the exercise experiment in eight rats with portal islet grafts and in eight control rats are presented. Baseline concentrations of glucose in whole blood and baseline concentrations of NEFAs, insulin, epinephrine, norepinephrine, and corticosterone in plasma were similar in both groups of animals.

Blood glucose levels remained at similar levels during exercise but increased after swimming (resting platform) to >130 mg/dl in both groups ($P < 0.05$). During swimming, plasma NEFA concentrations increased more in the rats with islet grafts than in the control rats ($P < 0.05$); however, these levels were similar again in both groups after swimming. In the control animals, the plasma insulin levels peaked ($P < 0.05$) when the waiting platform was lowered. During the swimming period, concentrations of plasma insulin decreased in both groups. In the control animals, the rebound of plasma insulin levels after swimming (at $t = 17$ min) was faster than in the animals with transplanted islets ($P < 0.05$). As observed previously (26), lowering the waiting platform into the water resulted only in an epinephrine response ($P < 0.05$), whereas no increases in plasma norepinephrine concentrations were observed. Physical exercise resulted in a continuous increase of plasma norepinephrine concentra-



tions ($P < 0.05$), whereas plasma epinephrine dropped gradually. The concentrations of these catecholamines were similar in both groups. During exercise, concentrations of plasma corticosterone increased ($P < 0.05$) similarly in both groups.

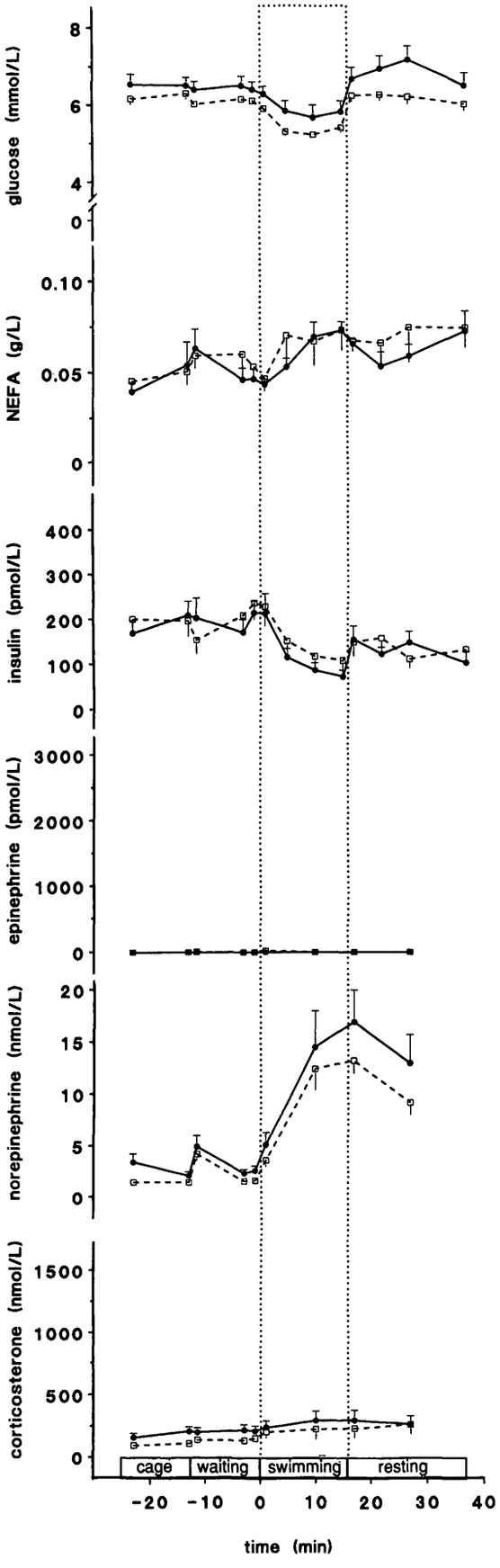
Exercise in adrenodemedullated rats. After adrenodemedullation, seven animals with portal islet grafts and eight control animals were submitted to a second swimming experiment. The results are shown in Fig. 4. As in the previous experiments, there were no differences in the baseline concentrations of blood glucose, plasma NEFAs, plasma insulin, plasma norepinephrine, and plasma corticosterone between control animals and animals with islet grafts. However, basal plasma insulin and corticosterone concentrations were lower in the adrenodemedullated than in the nonadrenodemedullated rats ($P < 0.05$), and plasma epinephrine levels were undetectable during the whole experiment.

During exercise, the levels of blood glucose decreased ($P < 0.05$), and they increased again immediately after exercise ($P < 0.05$). The concentrations of plasma NEFAs did not change throughout the experiment and were similar in both groups. Plasma insulin levels decreased during exercise ($P < 0.05$) and followed the same pattern in both the controls and the animals with islet grafts. The increase in plasma norepinephrine was not significantly different between the groups. Adrenodemedullation resulted in a reduced baseline plasma corticosterone concentration. The response to swimming, as depicted by an only minor increase in plasma corticosterone levels, was attenuated.

DISCUSSION

The results of the experiments in which epinephrine and norepinephrine were infused intravenously in nonexercising rats revealed that plasma insulin concentrations did not decrease. It is proposed that, in the rat, epinephrine is released by the adrenal medulla and that all norepinephrine in blood originates from the peripheral nerve endings from the sympathetic nervous system (26). After sympathetic nerve stimulation (e.g., during exercise), only a small part leaks into the blood. Scheurink et al. (25) showed previously that intravenous infusions of 109 nmol/min epinephrine and 300 nmol/min norepinephrine resulted in increments of catecholamines that were similar to the increases seen during swimming. These infusions resulted in maximal plasma levels of 2,840 pmol/l epinephrine and 10.2 nmol/l norepinephrine, respectively. Only the infusion of epinephrine resulted in a slight reduction in plasma insulin levels (25). These circulating epinephrine and norepinephrine levels were not adequate to reduce insulin secretion in the present study. One might suggest that since insulin levels did not follow the rising glucose levels after epinephrine infusion, the possibility of a direct α_2-mediated effect of circulating epinephrine levels still exists. However, the exercise experiments in adrenodemedullated rats showed that, although epinephrine was absent, insulin levels still decreased during exercise. For non-islet-transplanted rats, this was also shown

FIG. 3. Blood glucose, plasma NEFA, plasma insulin, plasma epinephrine, plasma norepinephrine, and plasma corticosterone concentrations before, during, and after exercise in eight islet-transplanted (●) and eight control rats (□) before adrenodemedullation. At $t = -0.5$, just before the onset of swimming, the waiting platform was lowered into the water. Data are means ± SE.



in previous studies (8–10). Taken together, these studies indicate that the reduction of insulin levels during exercise is not the result of α_2-adrenergic inhibition by humoral influences via circulating catecholamines, but that other mechanisms must be involved.

Islet grafts in the liver become revascularized from the hepatic arterial system (30). Therefore, it is assumed that the infused catecholamines reach both the liver and the pancreas in the same concentrations. Pipeleers et al. (31) suggested that the β-cells in transplanted islets become more sensitive to stimulation of adrenergic receptors. The results of the infusion experiments revealed that the sensitivity of the postsynaptic adrenoceptors was similar in both groups. Thus, the inhibition of insulin release in the exercise experiments cannot be due to enhanced sensitivity of adrenoceptors in transplanted islets.

Inhibition of insulin release during exercise is also caused by stimulation of the neural branch of the sympathetic nervous system, leading to norepinephrine outflow. Norepinephrine, via α_2-adrenergic receptors, is not solely responsible for the inhibition of insulin secretion (6,32,33); galanin (34,35), which is co-localized (36) and co-released (37) with norepinephrine in dogs, is also responsible. In mice, galanin contributes to the impairment of insulin secretion during swimming (38), and in rats, galanin inhibits basal and stimulated insulin release (39). Neuropeptide Y (NPY) is also co-localized with norepinephrine and appeared to be a slight inhibitor of insulin secretion in rats as well (40). We expect that the neural input is eliminated by the procedure of transplanting pancreatic islets into diabetic rats. Because insulin release is inhibited during exercise in both control animals and animals with islet grafts and because circulating epinephrine and norepinephrine levels cannot be responsible for the inhibition, reinnervation of the transplanted islets is probably the only remaining possibility to explain the observed results. There is already some evidence for noradrenergic reinnervation of islet grafts in the liver after 10 days in isogenic rats (41) or after 300 days in allogenic rats (42). Ingrowth of nerve fibers was also found in transplanted pancreatic tissue in the spleen in dogs after 4 weeks (43) and in transplanted islets in the spleen, in the liver, or under the kidney capsule in mice after >8 weeks (44,45). A low density of cholinergic nerve fibers was observed in the liver (45), but the physiological function remains unclear (17,18). However, noradrenergic nerve fibers, as observed in islets in the pancreas, have not been found in direct contact with the transplanted islet cells in the liver but rather with the capillaries within the islets (41). Moreover, it is not clear whether the ectopic site and innervation of the islet grafts result in the same functional effects as in control animals. In the present study, the exercise experiments were performed after a time period of at least 8 weeks. Since after this period the possibility of reinnervation can not be excluded and the decrease in insulin levels in islet-transplanted animals is similar to that in control animals, it seems reasonable to suggest that the nerve fibers in transplanted islets are functioning normally. Thus, not only noradrenergic innerva-

FIG. 4. Blood glucose, plasma NEFA, plasma insulin, plasma epinephrine, plasma norepinephrine, and plasma corticosterone concentrations before, during, and after exercise in seven islet-transplanted (●) and eight control rats (□) after adrenodemedullation. At $t = -0.5$, just before the onset of swimming, the waiting platform was lowered into the water. Data are means ± SE.

tion of islets in the pancreas, but also noradrenergic reinnervation of the islet grafts, can be responsible for the inhibition of insulin secretion during exercise.

Adrenodemedullation resulted not only in the absence of epinephrine but also in low plasma corticosterone concentrations. These attenuated responses might be due to damage of the adrenal cortex. However, the animals still showed a small response to exercise and did not drink from the presented 1% saline solution, indicating that enough mineralocorticoids were available. Corticosterone has only long-term effects on glucose levels, while epinephrine has more short-term increasing effects on glucose levels (1). Since blood glucose levels decrease during exercise in the absence of epinephrine, it can not be excluded that the reduction in glucose levels is at least partly responsible for inhibition of insulin secretion during and also immediately after exercise. The effects of circulating epinephrine may be potentiated by the rise in corticosterone, but if that is the case, there must be redundancy in the control of insulin secretion, because the adrenodemedullated rats still showed a reduction in insulin during exercise.

Another effect of adrenodemedullation was the absence of increases in NEFA levels during exercise, which indicates a permissive action of epinephrine for physiological NEFA responses or a dependence on increasing corticosterone levels for normal NEFA elevations (1).

In summary, this study provides evidence that sympathetic inhibition of insulin secretion, as observed during exercise, is not mediated by circulating epinephrine and norepinephrine in blood. Transplantation of islets of Langerhans into diabetic rats failed to prevent the exercise-induced inhibition of insulin secretion, even after adrenodemedullation. The most probable explanation is that, as a result of reinnervation of the transplanted islets, the inhibition of insulin levels during exercise was similar in both groups. Hence, it is suggested that increased sympathetic activity, releasing norepinephrine, galanin, and NPY at the sympathetic nerve endings in the islets of Langerhans, is the main cause of diminishing insulin release during swimming exercise.

ACKNOWLEDGMENTS

This research was supported by the Diabetes Foundation of the Netherlands.

We thank J.E. Bruggink for excellent technical assistance and Dr. G.J. Taborsky for critically reading the manuscript.

REFERENCES

1. Galbo H: *Hormonal and Metabolic Adaptation to Exercise.* Stuttgart, Germany, Thieme, 1983
2. Vranic M, Wasserman D, Bukowiecki L: Metabolic implications of exercise and physical fitness in physiology and diabetes. In *Diabetes Mellitus, Theory and Practice.* Rifkin H, Porte D, Eds. New York, Elsevier Science, 1990, p. 198–219
3. Wasserman DH, Cherrington AD: Hepatic fuel metabolism during muscular work: role and regulation. *Am J Physiol* 260:E811–E824, 1991
4. Wasserman DH, Williams PE, Lacy DB, Goldstein RE, Cherrington AD: Exercise-induced fall in insulin and hepatic carbohydrate metabolism during muscular work. *Am J Physiol* 256:E500–E509, 1989
5. Ahrén B, Ericson LE, Lundquist I, Lorén I, Sundler F: Adrenergic innervation of pancreatic islets and modulation of insulin secretion by the sympatho-adrenal system. *Cell Tissue Res* 216:15–30, 1981
6. Ahrén B, Taborsky GJ Jr, Porte D Jr: Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* 29:827–836, 1986
7. Scheurink AJW, Steffens AB, Benthem B: Central and peripheral adrenoceptors affect glucose, free fatty acids, and insulin in exercising rats. *Am J Physiol* 255:R547–R556, 1988
8. Scheurink AJW, Steffens AB, Bouritius H, Dreteler G, Bruntink R, Remie R, Zaagsma J: Sympathoadrenal influence on glucose, FFA, and insulin levels in exercising rats. *Am J Physiol* 256:R161–R168, 1989
9. Hoelzer DR, Dalsky GP, Schwartz NS, Clutter WE, Shah SD, Holloszy JO, Cryer PE: Epinephrine is not critical to prevention of hypoglycemia during exercise in humans. *Am J Physiol* 251:E104–E110, 1986
10. Järhult J, Holst J: The role of adrenergic innervation to the pancreatic islets in the control of insulin release during exercise in man. *Pflüg Arch Eur J Physiol* 383:41–45, 1979
11. Havel PJ, Taborsky GJ Jr: The contribution of the autonomic nervous system to changes of glucagon and insulin secretion during hypoglycemic stress. *Endocr Rev* 10:332–350, 1989
12. Miller RE: Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocr Rev* 2:471–493, 1981
13. Steffens AB, Damsma G, Van der Gugten J, Luiten PGM: Circulating free fatty acids, insulin and glucose during chemical stimulation of hypothalamus in rats. *Am J Physiol* 247:E765–E771, 1984
14. Ahrén B, Veith RC, Taborsky GJ Jr: Sympathetic nerve stimulation versus pancreatic norepinephrine infusion in the dog. I. Effects of basal release of insulin and glucagon. *Endocrinology* 121:323–331, 1987
15. Mellgren A, Schnell Landström AH, Petersson B, Andersson A: The renal subcapsular site offers better growth conditions for transplanted mouse pancreatic islet cells than the liver or spleen. *Diabetologia* 29:670–672, 1986
16. Federlin KF: Islet transplantation: the connection of experiment and clinic exemplified by the transplantation of islets of Langerhans. *Exp Clin Endocrinol* 101:334–345, 1993
17. Van Suylichem PTR, Strubbe JH, Houwing H, Wolters GHJ, Van Schilfgaarde R: Insulin secretion by rat islet isografts of a defined endocrine volume after transplantation to three different sites. *Diabetologia* 35:917–923, 1992
18. Van Suylichem PTR, Strubbe JH, Houwing H, Wolters GHJ, Van Schilfgaarde R: Rat islet isograft function: effect of graft volume and transplantation site. *Transplantation* 57:1010–1017, 1994
19. Strubbe JH, Van Wachem P: Insulin secretion by the transplanted neonatal pancreas during food intake in fasted and fed rats. *Diabetologia* 20:228–236, 1981
20. Trimble ER, Siegel EG, Berthoud HR, Renold AE: Intraportal islet transplantation: functional assessment in conscious unrestrained rats. *Endocrinology* 106:791–797, 1980
21. Trimble ER, Berthoud HR, Siegel EG, Jeanrenaud B, Renold AE: Importance of cholinergic innervation of the pancreas for glucose tolerance in the rat. *Am J Physiol* 241:E337–E341, 1981
22. Strubbe JH: Neural control of insulin secretion. *Horm Metab Res* 25:507–512, 1993
23. Van Suylichem PTR, Wolters GHJ, Van Schilfgaarde R: The efficacy of density gradients for islet purification: a comparison of seven density gradients. *Transpl Int* 3:156–161, 1990
24. Steffens AB: A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 4:833–836, 1969
25. Scheurink AJW, Steffens AB, Roossien B, Balkan B: Sympathoadrenal function in genetically obese Zucker rats. *Physiol Behav* 52:679–685, 1992
26. Scheurink AJW, Steffens AB, Bouritius H, Dreteler G, Bruntink R, Remie R, Zaagsma J: Adrenal and sympathetic catecholamines in exercising rats. *Am J Physiol* 256:R155–R160, 1989
27. Antonis A: Semiautomated method for the colorimetric determination of plasma free fatty acids. *J Lipid Res* 6:307–312, 1965
28. Henquin JC, Malvaux P, Lambert AE: Glucagon immunoassay using polyethylene glycol to precipitate antibody-bound hormone. *Diabetologia* 10:61–68, 1974
29. Dawson R, Kuntur P, Monjan A: High-performance liquid chromatographic (HPLC) separation and quantitation of endogenous glucocorticoids after solid-phase extraction from plasma. *Horm Res* 20:89–94, 1984
30. Andersson A, Korsgren O, Jansson L: Intraportally transplanted pancreatic islets revascularized from hepatic arterial system. *Diabetes* 38 (Suppl. 1):192–195, 1989
31. Pipeleers DG, Pipeleers-Marichal MA, Karl IE, Kipnis DM: Secretory capability of islets transplanted intraportally in the diabetic rat. *Diabetes* 27:817–824, 1978
32. Lorrain J, Angel I, Duval N, Eon MT, Oblin A, Langer SZ: Adrenergic and nonadrenergic cotransmitters inhibit insulin secretion during sympathetic stimulation in dogs. *Am J Physiol* 263:E72–E78, 1992
33. Tate CA, Scherer NM, Stewart G: Adrenergic influence on hormonal and hepatic metabolic response to exercise in rats. *Am J Physiol* 250:R1060–R1064, 1986
34. Dunning BE, Ahren B, Veith RC, Taborsky GJ Jr: Non-adrenergic sympathetic neural influences on basal pancreatic hormone secretion. *Am J Physiol* 255:E785–E792, 1988
35. Dunning BE, Taborsky GJ Jr: Galanin release during pancreatic nerve stimulation is sufficient to influence islet function. *Am J Physiol* 256: E191–E198, 1989
36. Ahrén B, Bötcher G, Kowalyk S, Dunning BE, Sundler F, Taborsky GJ Jr: Galanin is co-localized with noradrenaline and neuropeptide Y in dog

pancreas and celiac ganglion. *Cell Tissue Res* 261:49–58, 1990
37. Scheurink AJW, Mundinger TO, Dunning BE, Veith RC, Taborsky GJ Jr: α_2-Adrenergic regulation of galanin and norepinephrine release from canine pancreas. *Am J Physiol* 262:R819–R825, 1992
38. Dunning BE, Karlsson S, Ahrén B: Contribution of galanin to stress-induced impairment of insulin secretion in swimming mice. *Acta Physiol Scand* 143:145–152, 1991
39. Lindskog S, Ahrén B: Effects of galanin on insulin and glucagon secretion in the rat. *Int J Pancreatol* 4:335–344, 1989
40. Skoglund G, Gross RA, Bertrand GR, Ahrén B, Loubatières-Mariani M-M: Comparison of effects of neuropeptide Y and norepinephrine on insulin secretion and vascular resistance in perfused rat pancreas. *Diabetes* 40:660–665, 1991
41. Griffith RC, Scharp DW, Hartman BK, Ballinger WF, Lacy PE: A morphological study of intrahepatic portal-vein islet isografts. *Diabetes* 26:201–214, 1977
42. Noda N, Ina K, Yasunami Y, Ryu S, Ono J, Takaki R: Reinnervation of intraportal islet allografts in rats. *Transplantation* 55:664–665, 1993
43. Madureira MLC, Adolfo A, Dias J, Sebe M, Carvalhais HA, Von Hafe P: Reinnervation of the endocrine pancreas after autotransplantation of pancreatic fragments in the spleen of the dog: a morphofunctional study. *World J Surg* 9:335–347, 1985
44. Korsgren O, Andersson A, Jansson L, Sundler F: Reinnervation of syngeneic mouse pancreatic islets transplanted into renal subcapsular space. *Diabetes* 41:130–135, 1992
45. Korsgren O, Jansson L, Andersson A, Sundler F: Reinnervation of transplanted pancreatic islets: a comparison among islets transplanted into the kidney, spleen or liver. *Transplantation* 56:138–143, 1993