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Glucoprivation by insulin leads to trans-synaptic increase in rat adrenal tyrosine hydroxylase mRNA levels

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Abstract

Effects of single or repeated insulin or 2-deoxy-D-glucose administration on adrenal tyrosine hydroxylase mRNA and protein levels were examined in rats. Insulin produced hypoglycemia and an elevation in plasma epinephrine and norepinephrine levels. A significant increase (3–5-fold) in tyrosine hydroxylase mRNA levels was found at 5 h, decreasing to near basal levels at 24 h following the first and also the sixth consecutive injection of insulin or 2-deoxy-D-glucose. Whereas insulin treatment raised tyrosine hydroxylase mRNA levels in intact adrenals, no increase in tyrosine hydroxylase mRNA levels occurred following adrenal denervation by splanchnic nerve transection. Western blot analysis showed that although a single insulin treatment did not affect tyrosine hydroxylase protein levels, a significant increase was observed following the seventh administration. This study shows that insulin-induced hypoglycemia increases tyrosine hydroxylase gene expression and that this process is regulated by a central mechanism via the splanchnic nerve.

Keywords: Insulin; 2-Deoxy-D-glucose; Glucoprivation; Tyrosine hydroxylase gene expression; Tyrosine hydroxylase mRNA; Tyrosine hydroxylase immunoprotein; Stress, acute; Stress, repeated; Adrenal denervation; Plasma catecholamine

1. Introduction

Various psychosocial as well as physical stressors (such as immobilization, exposure to cold, insulin-induced hypoglycemia or 2-deoxy-D-glucose administration) activate the sympathoadrenomedullary system. In addition to increasing catecholamine excretion and secretion (Kvetnansky and Mikulaj, 1970; Silbergeld et al., 1971; Kvetnansky et al., 1978), these stressors also elevate catecholamine synthesis and promote the induction of adrenal medullary catecholamine biosynthetic enzymes (reviewed by Kvetnansky and Sabban, 1993). Acute cellular glucoprivation represents a homeostatic challenge that markedly activates the sympathoadrenomedullary system (Goldstein et al., 1992) and enhances catecholamine release from the adrenals (Scheurink and Ritter, 1993). Although both insulin and 2-deoxy-D-glucose induce glucoprivation and consequently the release of catecholamines, their primary activating stimulus is different. While pharmacological doses of insulin cause glucoprivation via intracellular glucose depletion, 2-deoxy-D-glucose is transported into the cells but not further metabolized. 2-Deoxy-D-glucose elevates blood glucose levels (Breier et al., 1993) and epinephrine is released from the adrenal medulla as a consequence of inhibited glucose utilization. 2-Deoxy-D-glucose is reported to be much more potent than insulin in releasing catecholamines from the adrenal medulla (Silbergeld et al., 1971). Both 2-deoxy-D-glucose and hypoglycemia-inducing doses of insulin increase plasma epinephrine concentrations. Plasma norepinephrine levels rise, most probably secondarily to the rise of epinephrine (Scheurink and Ritter, 1993). Besides releasing catecholamines from the adrenals, insulin and 2-deoxy-D-glucose also have been shown to increase the activity of the adrenal medullary catecholamine biosynthetic enzymes, tyrosine hydroxylase (Silbergeld et al., 1971; Kvetnansky et al., 1971b; Fluharty et al., 1983, 1985a,b), dopamine β-hydroxylase (Viveros et al., 1968) and phenyleth-

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anolamine-*N*-methyltransferase (Silbergeld et al., 1971; Kvetnansky et al., 1971b; Gripois and Valens, 1986).

The major rate-limiting step in catecholamine biosynthesis is the hydroxylation of tyrosine to dihydroxyphenylalanine catalyzed by tyrosine hydroxylase (tyrosine 3-monoxygenase: L-tyrosine, tetrahydropteridine: oxygen oxidoreductase (3-hydroxylating) (Levitt et al., 1965). Tyrosine hydroxylase is regulated primarily by two mechanisms. Short-term exposure to numerous stressors in vivo or to different stimuli in cell culture induces rapid activation by transient covalent modification of the enzyme through its phosphorylation, resulting in an increased affinity of tyrosine hydroxylase for pterin cofactor (Fluharty et al., 1983; Olasmaa et al., 1992; reviewed in Zigmond et al., 1989). Prolonged or repeated stressors (insulin-induced hypoglycemia, immobilization and cold) (Kvetnansky et al., 1970, 1971a,b) gradually elevate the maximal velocity of the tyrosine hydroxylase-catalyzed reaction. Induced formation of additional tyrosine hydroxylase enzyme molecules via elevated tyrosine hydroxylase mRNA levels was shown in several stress models, e.g. cold (Stachowiak et al., 1985; Baruchin et al., 1990; Miner et al., 1992; Kaplan et al., 1987), immobilization (McMahon et al., 1992; Nankova et al., 1994; Kvetnansky et al., 1996) as well as sympathectomy (Stachowiak et al., 1986) and reserpine administration (Tank et al., 1985). Similarly, changes in tyrosine hydroxylase activity and tyrosine hydroxylase protein synthesis were investigated following cold (Kvetnansky et al., 1971a; Fluharty et al., 1985a,b; Miner et al., 1992) and immobilization stress (Kvetnansky et al., 1970; Nankova et al., 1994). However, these two mechanisms of tyrosine hydroxylase activation and increased catecholamine synthesis are not mutually exclusive, nor do they always occur in association with one another (Fluharty et al., 1985a,b).

Since previous studies did not focus on the transcriptional regulation of the tyrosine hydroxylase gene after insulin or 2-deoxy-D-glucose administration, the primary aim of this study was to investigate changes in adrenal tyrosine hydroxylase mRNA levels after single or repeated insulin or 2-deoxy-D-glucose administration. Our data show that both insulin and 2-deoxy-D-glucose greatly elevated adrenal tyrosine hydroxylase mRNA levels. Unilateral adrenal denervation abolished the insulin-stimulated tyrosine hydroxylase mRNA increase, showing that glucoprivation regulates adrenal tyrosine hydroxylase transcription via the splanchnic nerve.

2. Materials and methods

2.1. Animals

Male, specific pathogen-free, Sprague-Dawley rats (280–320 g) were obtained from Charles River Laboratories (WIGA, Sultzfeld, Germany). The experiments began at least 7 days after arrival of the animals. The animals were housed 3–4 per cage under light-controlled conditions (light from 07:00 to 19:00) with a room temperature of 23 ± 2°C. Food and water were available ad libitum for animals in all experimental groups.

The experimental protocols were approved by the Animal Care and Use Committee of the Slovak Academy of Sciences. Two types of experiments were performed. In one type, blood samples for plasma glucose and catecholamine measurement were collected via an indwelling cannula in the tail artery. Baseline blood samples were obtained 20–24 h after insertion of the cannula and then the animals were administered with insulin, with blood being sampled at the indicated times. In the second type of experiments, animals were killed by decapitation at the indicated intervals after drug administration and the adrenals were removed and assessed for tyrosine hydroxylase mRNA and tyrosine hydroxylase protein levels. Each experiment was repeated at least twice.

2.2. Surgery, cannulation and blood sampling

Blood samples from awake rats were collected into heparinized tubes (Vacutainers) via a chronically indwelling polyethylene catheter that had been inserted into the tail artery 20–24 h before the acute experiment. This allows one to obtain plasma catecholamine levels when the animals are unstressed by manipulations related to blood sampling. Details of the cannulation procedure were described previously (Chiueh and Kopin, 1978; Kvetnansky et al., 1978). In brief, animals were anesthetized with pentobarbital (40 mg/kg i.p.). A catheter (PE 50; 0.75 m long, 0.58 mm in internal diameter) filled with a solution of 0.9% NaCl and containing 300 IU/ml sodium heparin was inserted into the ventral caudal tail artery. The catheter was tunneled under the skin and exited at the nape. A spring wire protected the catheter. After surgery, each rat was housed in an individual plastic cage, with the protected catheter extending out of the cage with a 1-ml syringe at the end. Blood samples (0.5 ml) were collected via the catheter at the indicated times and the same volume of heparinized saline (50 IU/ml) was administered intraarterially after each blood sample was obtained. Repeated blood sampling using this protocol does not affect plasma levels of catecholamines (Dobrakovova et al., 1989; Graessler et al., 1989) or catecholamine metabolites (Kvetnansky et al., 1992). The blood was centrifuged ($3000 \times g$) at 4°C for 20 min (Janetzki Centrifuge model K26) and the plasma was stored at −70°C until assayed.

2.3. Adrenal denervation

In some experiments, the left adrenal glands were denervated by cutting the splanchnic nerve. This operation was performed under pentobarbital anesthesia. A midline incision was made, the adrenal on the left side was ex-

posed and the nerve fibers from the superior mesenteric ganglia were severed about 0.5 cm above the gland. The right adrenal was also exposed; but the nerves were not severed and this adrenal served as control for the denervated left adrenal. The entire operative procedure required only 3–4 min. The procedure was performed 9 days before the beginning of insulin administration.

2.4. Drug treatments

Insulin-Actrapid (Novo Nordisk, Denmark) diluted in saline (0.1 ml per 100 g body weight) or saline alone was administered i.p. at a dose of 5 IU per kg of body weight. 2-Deoxy-D-glucose (Sigma, St Louis, MO) dissolved in saline was administered i.p. at a dose 500 mg per kg of body weight. The concentrations of insulin and 2-deoxy-D-glucose were based on previous studies showing their maximal effects on catecholamine release and activation of catecholamine biosynthetic enzymes (Brown et al., 1986; Scheurink and Ritter, 1993; Silbergeld et al., 1971). Animals were injected 1, 6 or 7 times and killed 2.5, 5 or 24 h after the drug administration. In cannulated animals, blood was collected before insulin injection and then 30, 60, 120, 180 and 300 min after its administration. In the present experiments, only one dose of drug was used.

2.5. Analytical methods

2.5.1. Determination of plasma glucose

Plasma glucose levels were measured by the enzymatic colorimetric test (GOD-PAP) (Trinder, 1969), using the automatic analyzer BM/Hitachi 911 and the kit SYS 1 (Boehringer Mannheim, Germany).

2.5.2. Determination of plasma epinephrine and norepinephrine

Catecholamines were measured in 50 μl aliquots of plasma by a modification of the radioenzymatic assay described previously (Peuler and Johnson, 1977). Catecholamines present in plasma aliquots were converted into their labeled *O*-methylated derivatives by *S*-[^{3}H]adenosyl methionine (Amersham, UK) and lyophilized catechol-*O*-methyltransferase isolated from rat liver. The *O*-methylated derivatives of the amines were then extracted along with unlabeled carrier compounds, separated by thin-layer chromatography, eluted and reacted with periodate. The detection limit was 5 pg norepinephrine and epinephrine per tube.

2.5.3. Isolation of RNA and Northern analysis

Isolation of mRNA and Northern blot analysis were as previously described (McMahon et al., 1992; Nankova et al., 1994). Briefly, after decapitation of the rats, the adrenals were removed, cleaned of fat tissue, transferred into sterile Eppendorf tubes and immediately frozen in liquid nitrogen. Total RNA was isolated by the procedure of Chomczynski and Sacchi (1987) using RNAzol (Tel-Test) and analyzed by Northern blots. Aliquots of total RNA samples (one-fourth) were fractionated on 1.3% agarose gels containing 2.2 M formaldehyde, 1 × MOPS buffer (20 mM 3-[*N*-morpholino]propanesulfonic acid (MOPS), pH 7.0, 5 mM sodium acetate, 1 mM ethylenedinitrilo-tetraacetic acid (EDTA)) as described previously (Kilbourne and Sabban, 1990), transferred to nitrocellulose and baked for 2 h at 80°C in a vacuum oven. Northern blot filters were prehybridized in 0.08 ml/cm^2 of 50% formamide, 5 × Denhardt's, 0.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA (1 × SSPE), 0.4% sodium dodecyl sulfate (SDS) and 100 μg/ml salmon sperm DNA at 42°C for 4 h. Hybridizations were performed using the following probes: 5′ 1.1-kb *Eco*RI fragment from rat tyrosine hydroxylase cDNA (Leonard et al., 1987) and cyclophilin cDNA (Danielson et al., 1988). The autoradiograms were scanned with a LKB Pharmacia Uppsala scanning densitometer, using exposures that were within the linear range.

2.5.4. Measurement of immunoreactive tyrosine hydroxylase protein levels

Adrenals were homogenized in 0.05 M potassium phosphate, pH 6.65/0.2% Triton X-100 and centrifuged at 10 000 × *g* for 20 min at 4°C. Protein concentrations were measured (Lowry et al., 1951) and equal amounts of supernatant protein were fractionated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred to nitrocellulose membranes. The levels of immunoreactive protein were measured by using a monoclonal antibody to rat tyrosine hydroxylase (Boehringer Mannheim), visualized by using the Western light chemiluminiscent detection system (Amersham, UK) and analyzed by densitometry. The amounts of immunoreactive tyrosine hydroxylase protein used were within the linear range of the dose-response curve produced by the chemiluminiscent signal.

2.6. Statistical analysis

The significance of differences in the values from blood samples collected from the same animal at different intervals was calculated by one-factor, repeated-measures analysis of variance (ANOVA), followed by Fisher's post-hoc test. Statistical differences between experimental groups and controls killed by decapitation were determined by one-way ANOVA followed by Fisher's post-hoc test. Statistical significance was considered at $P < 0.05$.

3. Results

3.1. Changes in plasma glucose and catecholamine levels in response to insulin administration

A significant up to 50% decrease in blood glucose levels was found 30–120 min following insulin injection with a return to basal levels by 3 h after injection (Fig. 1).

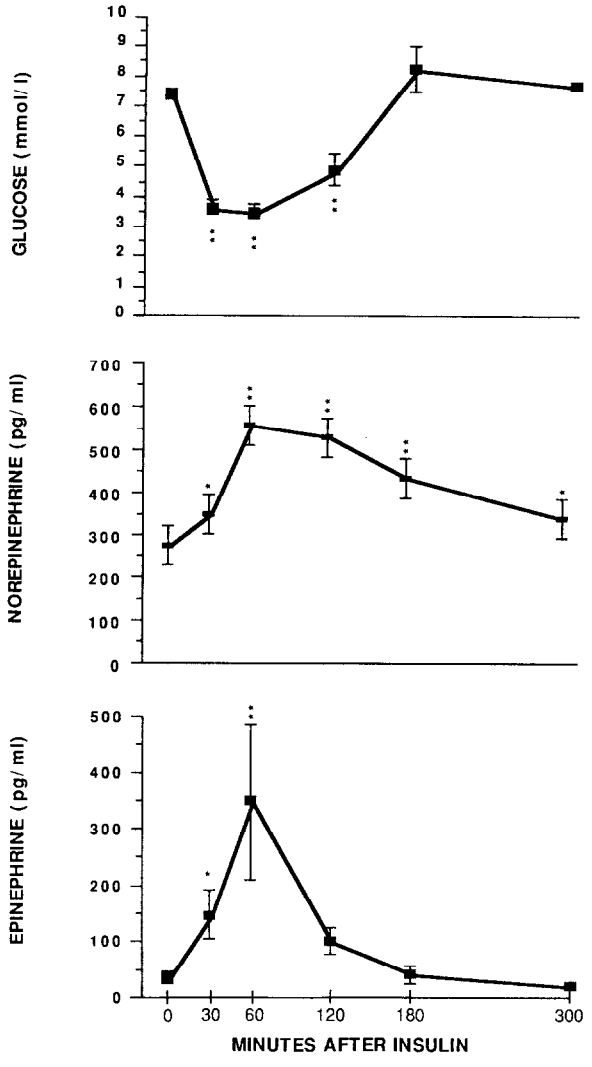

Fig. 1. Effect of a single insulin administration on plasma catecholamines and glucose levels. The tail artery of rats was cannulated and the next day the rats were intraperitonealy injected with a single dose of insulin (5 IU/kg). Blood (0.5 ml) was collected before the insulin injection and at indicated times afterwards. Plasma catecholamine and glucose concentrations were determined as described in Section 2. Values are expressed as means ± S.E.M. ($n = 11$). Statistical significance was calculated with repeated-measures ANOVA: * $P < 0.05$; ** $P < 0.01$ vs. untreated control values.

In contrast, insulin elicited a marked increase in both epinephrine and norepinephrine levels, with the largest effects being observed 60 min after insulin injection. Plasma epinephrine displayed a substantial but transient rise. Epinephrine levels were significantly elevated 30 and 60 min after insulin injection, returning to the basal level by 3 h. The rise in plasma norepinephrine levels was about 2-fold; the levels remained elevated for at least 3 h and did not return to baseline values even 5 h after insulin administration.

3.2. Effect of a single insulin treatment on adrenal tyrosine hydroxylase mRNA levels

Although plasma glucose levels were reduced significantly already at 30 min and maximally at 1 h following the insulin injection, there was no significant difference in adrenal tyrosine hydroxylase mRNA levels even 2.5 h after the injection (Fig. 2). However, tyrosine hydroxylase mRNA levels were elevated over 4-fold 5 h after a single insulin injection. Ten hours after the single insulin injection adrenal tyrosine hydroxylase mRNA levels started to decline and, by 24 h, they were not significantly different from the basal level.

3.3. Effect of repeated insulin administration on adrenal tyrosine hydroxylase mRNA levels

The effect of seven consecutive daily insulin injections was examined. At 5 h following the first or seventh daily insulin injection, there was a large increase in adrenal tyrosine hydroxylase mRNA levels compared to control levels (Fig. 3). When tyrosine hydroxylase mRNA levels were measured 24 h following the sixth insulin injection ('adapted controls') and statistically compared by ANOVA test to absolute control values no significant difference was found. However, when relative values of adrenal tyrosine hydroxylase mRNA levels in these two groups were compared by Student's t-test the difference was statistically significant ($P < 0.01$). The decrease in blood glucose levels in rats given repeated injections of insulin was identical to that after a single insulin injection (data not shown), indicating that the rats did not develop resistance to insulin. Seven daily injections of insulin elevated tyrosine hydroxylase mRNA in a pattern very similar to that observed after a single insulin injection. There was no significant increase after 2.5 h (data not shown). By 5 h after a

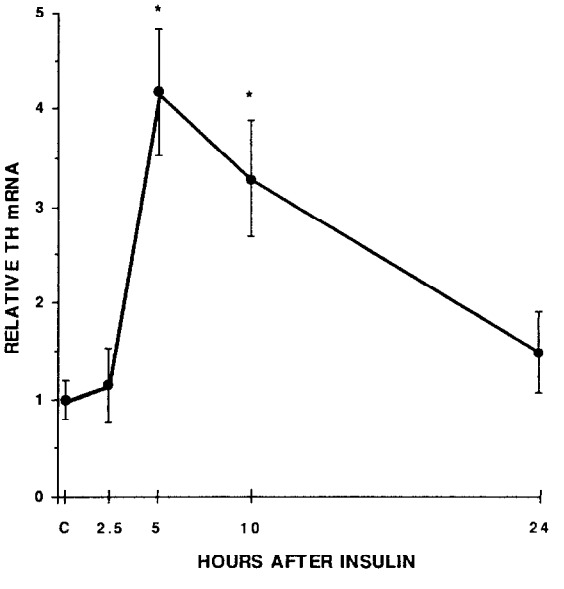

Fig. 2. Effect of a single insulin administration on adrenal tyrosine hydroxylase mRNA levels. Rats were injected once with insulin (5 IU/kg) and euthanized at the indicated times. The first point of the line refers to a control group which was injected with saline and decapitated 5 h later. The tyrosine hydroxylase mRNA levels are expressed in arbitrary densitometric units relative to those of the nonstressed control group of animals. Values are expressed as means ± S.E.M. ($n = 5$–7). Statistical significance was calculated with one-factor ANOVA: * $P < 0.01$ vs. control group.

seventh insulin injection, tyrosine hydroxylase mRNA levels were similar to those after a single insulin injection (Fig. 3) and, by 24 h, the levels had declined toward the basal level (data not shown).

3.4. Effects of a single or repeated 2-deoxy-D-glucose administration on adrenal tyrosine hydroxylase mRNA levels

Since 2-deoxy-D-glucose has similar effects as insulin on catecholamine excretion and on the increase of tyrosine hydroxylase activity in the adrenal medulla (Fluharty et al., 1983, 1985b; Scheurink and Ritter, 1993), we analyzed the effects of single or repeated 2-deoxy-D-glucose treatment on adrenal tyrosine hydroxylase mRNA levels. At 5 h following a single injection of 2-deoxy-D-glucose, tyrosine hydroxylase mRNA levels were about 4–5-fold increased compared to the control levels (Fig. 4). This rise in tyrosine hydroxylase mRNA levels produced by 2-deoxy-D-glucose is similar to that seen after insulin injection. At 24 h after the sixth treatment with 2-deoxy-D-glucose, tyrosine hydroxylase mRNA levels were somewhat, although not significantly, elevated compared to the values in the control group which received a single saline injection. In response to the seventh 2-deoxy-D-glucose administration, tyrosine hydroxylase mRNA levels were increased markedly (3-fold increase, $P < 0.01$; Fig. 4) when compared to those of the group of adapted control animals (rats treated with 2-deoxy-D-glucose six times and left untreated for an additional 24 h).

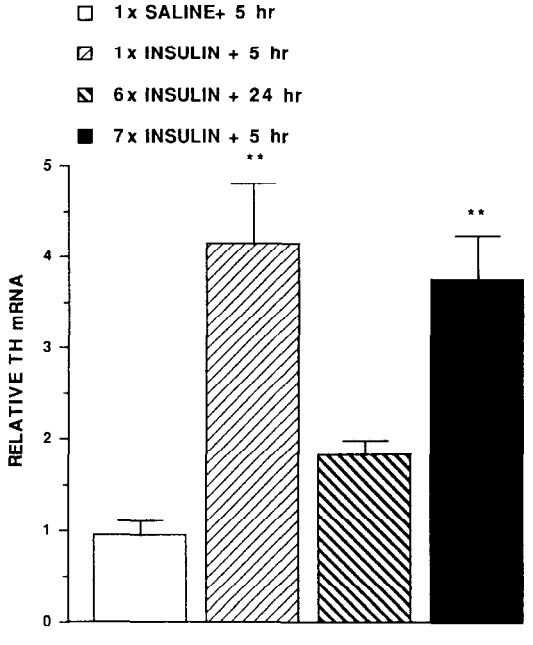

Fig. 3. Effect of repeated insulin administration on adrenal tyrosine hydroxylase mRNA levels. Rats were injected with saline (1×SALINE+5 h; $n = 4$) or insulin once (5 IU/kg) and killed 5 h following the injection (1×INSULIN+5 h; $n = 7$). 'Adapted Control' animals were injected 6 times with insulin and killed 24 h following the last injection (6×INSULIN+24 h; $n = 7$). Seven times insulin-injected animals were killed 5 h following the last insulin injection (7×INSULIN+5 h; $n = 7$). Statistical significance was calculated with one-factor ANOVA: $^{**}P < 0.01$ vs. control groups. Each experiment was repeated at least twice. For the purpose of comparison of effects of the repeated vs. a single treatment with insulin, we have used the same data for 1×INSULIN+5 h in both Figs. 2 and 3. These results are from one representative experiment.

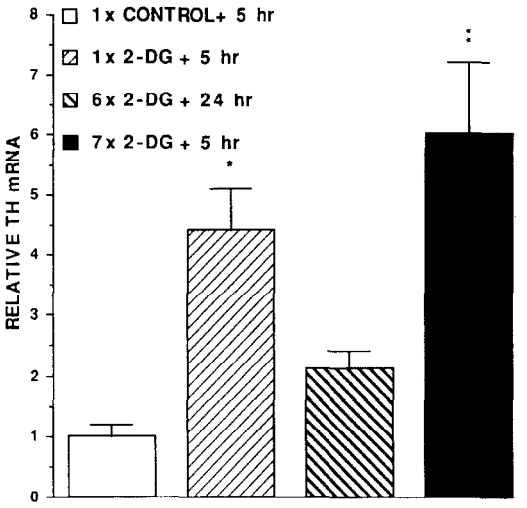

Fig. 4. Effects of single or repeated 2-deoxy-D-glucose administration on adrenal tyrosine hydroxylase mRNA levels. Rats were injected either with saline (1×SALINE+5 h; $n = 6$) or with 2-deoxy-D-glucose once (500 mg/kg) and killed 5 h following the injection (1×2-deoxy-D-glucose+5 h; $n = 6$) $^{*}P < 0.05$ vs. 1×SALINE+5 h. 'Adapted Control' animals were treated 6 times with 2-deoxy-D-glucose and killed 24 h following the sixth injection (6×2-deoxy-D-glucose+24 h; $n = 6$). Seven times 2-deoxy-D-glucose-injected animals were killed 5 h following the last 2-deoxy-D-glucose injection (7×2-deoxy-D-glucose+5 h; $n = 6$). Statistical significance calculated by one-factor ANOVA: $^{*}P < 0.05$; $^{**}P < 0.01$ vs control groups.

3.5. Effect of adrenal denervation on insulin-induced increase in tyrosine hydroxylase mRNA levels

To determine whether the observed effects on tyrosine hydroxylase gene expression are neuronally mediated by the splanchnic nerve, all animals had their left adrenal surgically denervated by transection of the splanchnic nerve. The right adrenals were left intact and served as internal controls. There was no effect of adrenal denervation on tyrosine hydroxylase mRNA levels in the group of animals injected with saline only. However, the effect of a single insulin injection on tyrosine hydroxylase mRNA elevation was completely blocked by the adrenal denervation (Fig. 5).

3.6. Effect of a single and repeated insulin treatment on adrenal tyrosine hydroxylase protein levels

Adrenal tyrosine hydroxylase protein concentration was examined by immunoassay in controls and in animals exposed to a single or repeated (seven times) insulin injections. There was no statistically significant increase in

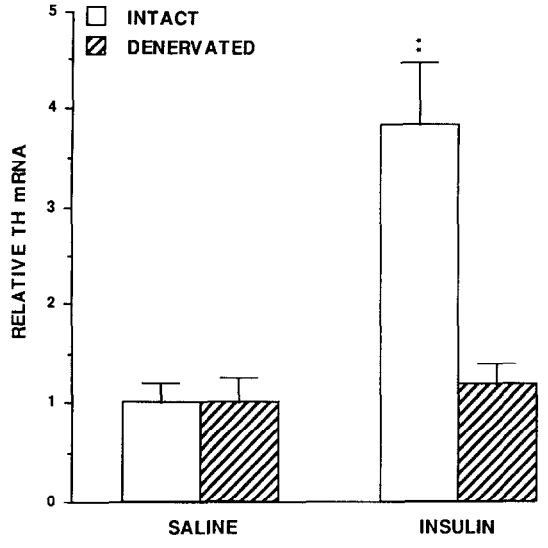

Fig. 5. Effect of adrenal denervation on insulin-induced elevation of tyrosine hydroxylase mRNA levels. All rats underwent splanchnectomy and a recovery period as described in Section 2; the intact right adrenal served as the sham-operated control. Insulin-treated animals received a single injection of insulin (5 IU/kg), and all animals were euthanized 5 h following the injection (Control = INTACT SALINE; $n = 9$; denervated control = DENERVATED SALINE; INTACT INSULIN; $n = 11$; DENERVATED INSULIN); ** $P < 0.01$. Values in all groups are related to values in intact adrenals of the saline-treated animals.

adrenal tyrosine hydroxylase protein levels compared to controls following a single administration of insulin. However, both 24 h after the sixth daily consecutive injection and 5 h following the seventh insulin administration, there were statistically significant ($P < 0.01$) increases in tyrosine hydroxylase protein levels (Fig. 6). Thus, the rise in tyrosine hydroxylase mRNA is reflected by a significant (about 2-fold) elevation in tyrosine hydroxylase protein.

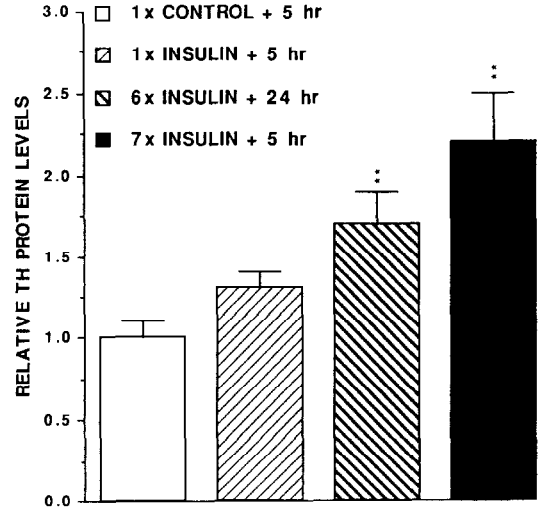

Fig. 6. Effects of single or repeated insulin treatment on immunoreactive tyrosine hydroxylase protein levels. The rats were treated as described in Fig. 3. One adrenal of each rat was taken to determine tyrosine hydroxylase mRNA levels (Fig. 3) and the second to determine immunoreactive tyrosine hydroxylase protein as described in Section 2 (1×SALINE+5 h; $n = 4$; 1×INSULIN+5 h; $n = 7$; 6×INSULIN+24 h; $n = 7$; 7×INSULIN+5 h; $n = 5$); ** $P < 0.01$. Values in all groups are compared to values in intact adrenals of saline-treated animals. Statistical significance calculated by one-factor ANOVA: ** $P < 0.01$ vs. control group.

4. Discussion

Single or repeated glucoprivation produces a significant activation of the adrenal medulla. This is manifested by an elevation of plasma epinephrine levels (Goldstein et al., 1992; Scheurink and Ritter, 1993) and an increase in the activity of catecholamine-synthesizing enzymes in the adrenal medulla (Kvetnansky et al., 1971a,b; Silbergeld et al., 1971; Fluharty et al., 1983, 1985b). Soluble proteins of the chromaffin granule are also elevated by hypoglycemia (Laslop et al., 1989; Sietzen et al., 1987).

In this study, we investigated the effects of glucoprivation stress on regulation of the expression of the catecholamine biosynthetic enzyme tyrosine hydroxylase. Concentrations of insulin and 2-deoxy-D-glucose used in our current experiments were based on previously studies works showing that these concentrations stimulated the sympathoadrenal system and also increased the activity of catecholamine synthesizing enzymes in the adrenals (Brown et al., 1986; Scheurink and Ritter, 1993; Silbergeld et al., 1971).

Our data reveal a marked increase in tyrosine hydroxylase mRNA levels induced by insulin- or 2-deoxy-D-glucose-caused glucoprivation. A single dose of insulin was sufficient to trigger a large rise in tyrosine hydroxylase mRNA levels, evident after 5 h. A common feature of hypoglycemia and immobilization was that, if applied only once, they had a transient effect that decreased toward pre-stress levels within 24 h (McMahon and Sabban, 1992; Nankova et al., 1994; Kvetnansky et al., 1996). The different kinetics of changes in adrenal tyrosine hydroxylase mRNA levels in the glucoprivation stress model, compared to other stress stimuli, may be due to a different intensity and/or complexity of the particular stress stimulus.

Repeated insulin injection did not diminish the response of the sympathoadrenomedullary system with respect to the induction of tyrosine hydroxylase mRNA levels. The seventh daily administration was still able to cause a rapid and substantial increase in tyrosine hydroxylase mRNA levels. The magnitude of the rise of tyrosine hydroxylase mRNA levels following the seventh insulin administration (compared to the group of adapted controls) was similar to that after a single insulin treatment. These results indicate that with respect to tyrosine hydroxylase gene expression the adrenals of the animals did not adapt and still responded to repeated hypoglycemia.

2-Deoxy-D-glucose was used in this study to elicit glucoprivation by a different mechanism. This glucose analogue is transported into cells where it is phosphorylated to 2-deoxy-D-glucose-6-phosphate by hexokinase, but

is not able to be further metabolized by the glycolytic pathway. With pharmacological doses of 2-deoxy-D-glucose, 2-deoxy-D-glucose-6-phosphate accumulates to levels sufficient to inhibit glucose-6-phosphate isomerase, an enzyme that converts glucose-6-phosphate to fructose-6-phosphate. Subsequently glycolysis and the oxidative metabolism of glucose are inhibited. Blood glucose levels are elevated, presumably secondary to glycogenolysis, the reduced body metabolism of glucose and gluconeogenesis. Thus, 2-deoxy-D-glucose directly inhibits the glycolytic flux, which has effects similar to hypoglycemia despite elevated blood glucose levels (Breier et al., 1993).

It has been previously reported that adrenal tyrosine hydroxylase activity increases more after repeated 2-deoxy-D-glucose than after insulin administration (Silbergeld et al., 1971). In the present study, we found a large rise in adrenal tyrosine hydroxylase mRNA levels following single or repeated 2-deoxy-D-glucose injections. The stimulatory effect of 2-deoxy-D-glucose on adrenal tyrosine hydroxylase mRNA levels was very similar to that of insulin. The rise in tyrosine hydroxylase mRNA levels following a single 2-deoxy-D-glucose treatment was transient. Even 24 h following the sixth consecutive daily administration of 2-deoxy-D-glucose, tyrosine hydroxylase mRNA levels still decreased to near basal values. Similarly, as in the case of hypoglycemia induced by repeated insulin treatment, repeated (seven times) 2-deoxy-D-glucose injection caused an increase in tyrosine hydroxylase mRNA levels. Repeated saline injection did not affect tyrosine hydroxylase mRNA levels, indicating that neither repeated injection of the vehicle nor daily handling of animals affected tyrosine hydroxylase mRNA levels.

It was known from earlier studies that stress-induced catecholamine release can activate tyrosine hydroxylase activity via at least two different mechanisms. Acute stress results in rapid activation of tyrosine hydroxylase activity (Fluharty et al., 1983) and chronic stress stimulates formation of additional tyrosine hydroxylase molecules (Fluharty et al., 1985b). Western blot data presented in this paper provide direct evidence that repeated administration of insulin elicits a considerable increase in tyrosine hydroxylase protein levels. In contrast, a single injection of insulin does not significantly alter tyrosine hydroxylase protein concentrations. An increase in tyrosine hydroxylase protein has been observed also with other models of chronic or repeated stress such as cold (Baruchin et al., 1990), immobilization (Nankova et al., 1994) or heat (Garcia et al., 1994).

Earlier studies suggested that trans-synaptic pathways mediate the effect of different stressors on increased tyrosine hydroxylase activity (reviewed by Axelrod and Reisine, 1984; Kvetnansky and Sabban, 1993). Long-term effects of cold stress (Chuang and Costa, 1975), insulin-induced hypoglycemia (Fluharty et al., 1985b), 2-deoxy-D-glucose administration (Silbergeld et al., 1971) or immobilization (Kvetnansky et al., 1970) on adrenal tyrosine hydroxylase activity are abolished by adrenal denervation by section of the splanchnic nerve.

To determine if neuronal input is an important, probably major component for regulation not only of tyrosine hydroxylase activity, but also of gene expression, we examined changes in insulin-stimulated tyrosine hydroxylase mRNA levels following adrenal denervation. Splanchnectomy completely blocked insulin-induced gene expression. The increase of tyrosine hydroxylase gene expression in the intact adrenal of splanchnectomized animals was not higher than in control animals. Our results indicate the essential role of neuronal input in the large rise in tyrosine hydroxylase mRNA levels after insulin administration. As shown earlier, the cold-induced increase in tyrosine hydroxylase mRNA levels is also abolished by adrenal denervation (Baruchin et al., 1993). However, it has been recently demonstrated that the increase in adrenal tyrosine hydroxylase gene expression induced by a single immobilization is not blocked by denervation (Kvetnansky et al., 1996).

The present paper provides new data on tyrosine hydroxylase regulation in glucoprivation stress at the level of gene expression. It shows that single and repeated insulin or 2-deoxy-D-glucose administration induces tyrosine hydroxylase gene expression and that repeated glucoprivation stimulates tyrosine hydroxylase protein synthesis. It is likely that the large rise in steady-state tyrosine hydroxylase mRNA observed within hours reflects increased transcription. It still remains to be determined which transcription factors are involved in the induction of tyrosine hydroxylase gene expression following the glucoprivation stress. In this study, we also showed that adrenal denervation abolishes insulin-induced tyrosine hydroxylase gene expression.

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