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Effects of Dexamethasone on Pancreatic Growth and Thyroliberin (TRH) Content in Neonatal Rat Pancreas

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Summary

Thyrotropin-releasing hormone (TRH) is also present in pancreatic B-cells and its role and regulation here remain unclear. The rat pancreas displays a peculiar ontogenetic pattern for TRH with a rapid increase after birth up to postnatal day 3 when TRH peak is reached. In the present study, dexamethasone (DXM) treatment (1 μg/100g BW/day) resulted in an increase of pancreatic weight and retardation of the peak of pancreatic TRH concentration by two days. The TRH-degrading system (either in the 10 000 x *g* supernatant or in the pellet of pancreatic homogenate) was not stimulated by *in vivo* DXM treatment. In DXM-treated rats, plasma TSH levels were significantly decreased after postnatal day 1. Plasma glucose concentration was increased on day 1 (i.e. 24 h after the first DXM injection) and decreased to the control level on postnatal day 3. Pancreatic insulin levels were decreased on postnatal day 3 compared to the controls. These results indicate that DXM affects TRH in the neonatal rat pancreas; this effect is probably not mediated through modulation of TRH-degrading activity. The stimulation of pancreatic growth after DXM treatment might be related to the effect on the TRH system.

Key words

Pancreas - Ontogenesis - Thyrotropin-releasing hormone - Dexamethasone

Introduction

Thyrotropin-releasing hormone (TRH), a tripeptide pGlu-His-Pro-NH_2 originally isolated from the hypothalamus (Bøler *et al.* 1969, Burgus *et al.* 1970), has since been identified in other areas of the central nervous system (for review see Jackson 1982) and in the gastrointestinal and reproductive tract of mammals (Morley *et al.* 1977). During the neonatal period extremely high concentrations of TRH have been detected in the pancreas of the rat (Martino *et al.* 1978, Aratan-Spire *et al.* 1984), where is also synthesized (Dutour *et al.* 1987). It is located in the B-cells of the islets of Langerhans probably in the same secretory granules as insulin (Leduque *et al.* 1985). During the development of endocrine pancreas, the TRH mRNA levels and TRH immunoreactivity increase transiently reaching a peak on the day of birth and on postnatal day 3, respectively, and then they decrease rapidly to the low levels observed in adults (Dutour *et al.* 1985, 1987, Martino *et al.* 1980). However, the role and regulation of TRH in this tissue remain poorly understood. Glasbrenner *et al.* (1990) demonstrated that TRH administration in rats induced pancreatic hyperplasia. A similar effect had been reported after treatment with glucocorticoids (Bourry and Sarles 1978). However, no data are available concerning the effects of dexamethasone (DXM) on pancreatic TRH. There have been only sparse reports upon the effects of glucocorticoids on hypothalamic TRH. Mitsuma and Nogimori (1982) and Mitsuma *et al.* (1992) reported a time- and dose-dependent inhibition of TRH release from hypothalamic tissue after DXM treatment. This effect was accompanied by a simultaneous increase in the hypothalamic content of TRH and a decrease of the content of its precursor peptide Lys-Arg-Gln-His- Pro-Gly-Arg-Arg (Mitsuma *et al.* 1992). The present study was designed to evaluate the effects of DXM treatment on TRH in the neonatal rat pancreas in possible relation to its effect on pancreatic growth.

Methods

Experimental animals. Female Wistar rats (VELAZ, Prague, Czech Republic) provided with tap water and commercial pelleted chow and maintained on a 12 h light-dark cycle at 22 °C were bred in our laboratory

and their litters were used in the experiments. The day of birth was designated as postnatal day 0.

Experimental design. Newborn rats of each litter were divided randomly into experimental and control groups. Using this protocol, the experimental animals were compared with their control siblings from the same litter reared by the same mother. Experimental animals received a daily i.p. injection of dexamethasone (Sigma Chemicals, St Louis, MO) in saline (1 μg DXM/100 g body weight/day, DXM was predissolved in a small quantity of ethyl alcohol) from the day of birth up to decapitation. The control half of each litter received the same volume of saline. The pups were weighed daily and killed by decapitation on postnatal days 1, 3, 5 and 8 (one litter each day). Trunk blood was collected into chilled heparinized tubes, centrifuged and the plasma was stored at −20 °C until TSH and glucose determination. The pancreases were rapidly dissected and weighed. Approximately half of the tissue was extracted for TRH and insulin determination. The other half was homogenized in 50 mM phosphate buffer (PBS, 4 °C, pH 7.5, 1:10 wt:vol) and centrifuged (10 000 x *g* at 4 °C for 20 min). The supernatant was used for the degradation study directly, the 10 000 x *g* pellet after resuspending in 50 mM PBS, pH 7.4 (the resuspending volume was equal to that of the homogenate before centrifugation).

Peptide extraction. Immediately after dissection the pancreas was frozen on dry ice, put into 1 ml cold 1 M acetic acid, homogenized and stored for 24 h at −20 °C. After thawing it was centrifuged (10 000 x *g* at 4 °C for 10 min). The supernatant was saved and the pellet was resuspended in cold 50 % methanol (0.5 ml) and recentrifuged. Both supernatants were pooled and evaporated to dryness using a Speed-Vac (Savant). Dried extracts were reconstituted in 1 ml 10 mM PBS, pH 7.4 before TRH and insulin radioimmunoassay (RIA).

Degradation of TRH. Degradation studies were performed according to Wolf *et al.* (1984) with some modifications. 100 μl of 10 000 x *g* supernatant or resuspended pellet of pancreatic homogenate (100 μl of homogenate representing 10 mg pancreatic wet weight) were incubated with 21.72 ng TRH in 50 mM PBS, pH 7.5, in a final volume of 600 μl at 37 °C. After 0, 10, 20, 30 and 60 min of incubation single 100 μl aliquots were taken and the reaction was stopped with 1.5 ml cold methanol. After centrifugation (900 x *g* for 10 min at 4 °C), the supernatants were evaporated using Speed-Vac (Savant) and the dry extract kept in 1 ml methanol at −20 °C. The methanol solution was then evaporated and the dry extract dissolved in 1 ml 0.05 M PBS, pH 7.5, before RIA. TRH-degrading activity was expressed as the decrease of TRH immunoreactivity in the medium during incubation.

Assay procedures. Pancreatic TRH was measured by RIA. The specific rabbit TRH antibody developed in our laboratory recognizes neither the TRH-degradation products (as TRH free acid and histidyl-proline diketopiperazin or amino acids) nor such putative TRH precursor peptides as TRH-Gly or Gln-His-Pro-Gly-Lys-Arg (cross reactivity less than 0.01 %). Synthetic TRH (a gift from prof. Kasafirek) was labelled with ^{125}I using the chloramine-T method and was purified on Sephadex G-10 (Pharmacia) column (60x1 cm). The sensitivity of the TRH assay was markedly improved by iodinating the standards and unknowns with $Na^{127}I$ as previously described (Grouselle *et al.* 1982, Dutour *et al.* 1985) and was 2 pg per tube. Intra- and interassay variations were 5–8 % and 10–12 %, respectively. Rat TSH RIA was performed using reagents (rat TSH-RP-3, AFP-5512 B and TSH-antibody anti-rat TSH-RIA-6, AFP-329 661 Rb) obtained from NIDDK on the credit of the National Hormone and Pituitary Program. Insulin RIA was performed using the set Ria-ins-kit MI-96 (Radioisotope Production and Distribution Centre Radioimmunology Department, Swierk, Poland). The blood glucose concentration was determined using the set Oxochrom Glucosa Glu 250-E (Lachema, Brno, Czech Republic).

Statistics. The results were expressed as mean ± S.E.M. The data were analyzed by one-way analysis of variance. To determine the levels of significance, the t-values were compared with the values of Dunnett's table for multiple comparison. The number of control animals was 5, 4, 5 and 6 on day 1, 3, 5 and 8, respectively. The number of DXM-treated pups in the same age groups was 6, 5, 6 and 4.

Results

Fig. 1. shows the effect of DXM treatment on pancreatic growth. On postnatal day 1, the pancreatic weight in the experimental and control groups was comparable. At later intervals, the absolute and relative pancreatic weight of DXM-treated animals was significantly ($P<0.05$) higher, while the body weight was slightly lower on days 3 and 5 (data not shown). Analysis of variance revealed that DXM affected the overall postnatal development of pancreatic weight ($P<0.01$).

Changes in pancreatic TRH immunoreactivity after DXM treatment are illustrated in Fig. 2. In the control animals, TRH immunoreactivity increased rapidly from postnatal day 1 up to postnatal day 3 when its peak was reached. It then decreased sharply to the initial level on days 5 and 8. In DXM-treated animals the TRH concentration curve exhibited a similar shape but with a delay of two days. The differences between

the control and DXM-treated groups measured on days 3 and 5 were statistically significant ($P<0.05$).

Incubation of 10 000 x *g* supernatant or pellets of pancreatic homogenates with exogenous TRH failed to reveal any significant TRH degradation in both the control and DXM-treated groups (data not shown).

As is shown in Fig. 3, plasma TSH concentrations in DXM-treated animals decreased from postnatal day 1 to postnatal day 3 and was lower till the end of the experiment, while it remained stable in the controls. The overall difference between the control and experimental groups was statistically significant ($P<0.01$). The individual differences at specific age (3, 5 and 8 days) were significant at the level $P<0.05$. In control rats, plasma glucose levels remained stable during the whole experiment (Fig. 4). In DXM-treated animals, the plasma glucose level was significantly increased only on postnatal day 1. Pancreatic insulin concentration in control rats increased from the day 1, reaching its peak on day 3 and subsequently returned to the initial levels (Fig. 5). Insulin concentration development in DXM-treated animals was similar (no difference between the treated groups). However, the peak on day 3 was significantly lower in comparison to the controls ($P<0.05$).

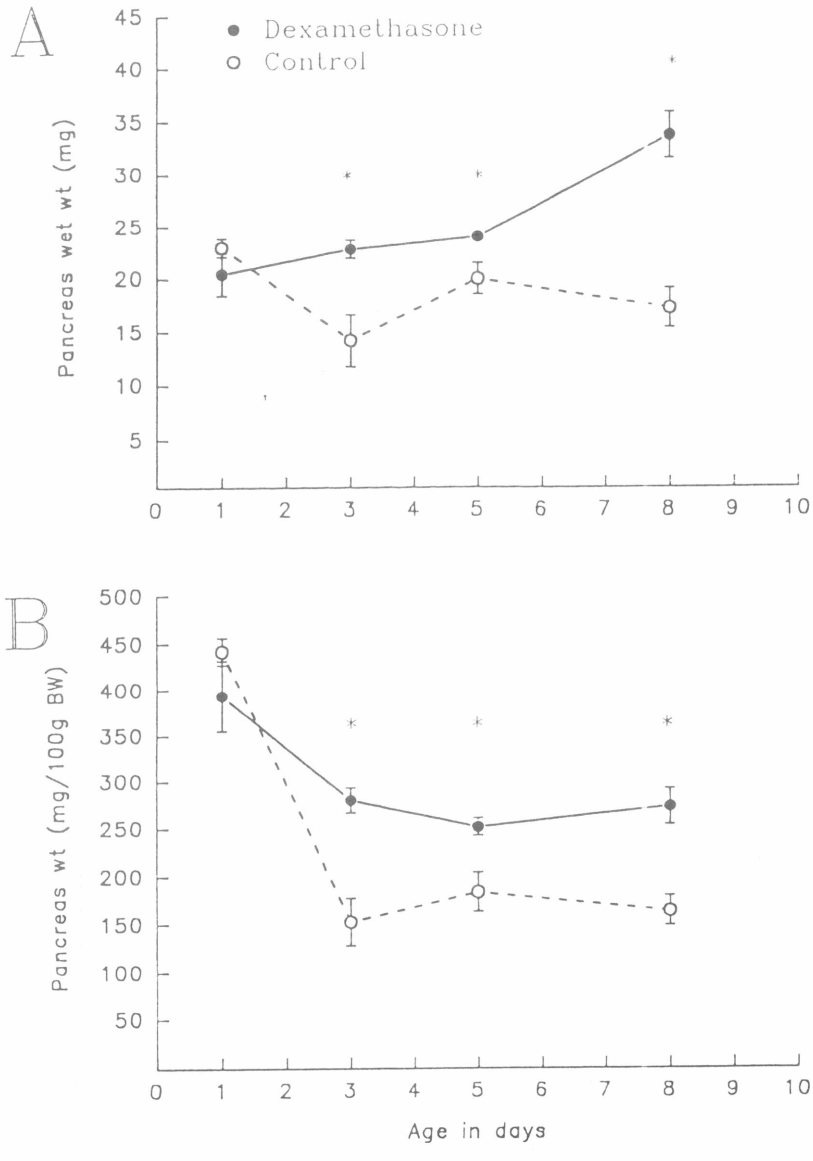

Fig. 1
Effect of DXM treatment (1 μg DXM i.p./100 g BW/day from the day of birth) on the growth of neonatal pancreas. Controls obtained the same volume of saline i.p.. **A** - shows the development of absolute pancreatic wet weight during ontogenesis. Results are expressed in mg (mean ± S.E.M.). **B** - shows the development of relative pancreatic wet weight. Results are expressed in mg/100 g (mean ± S.E.M.). * $P<0.05$ for individual differences between age-matched DXM and control groups.

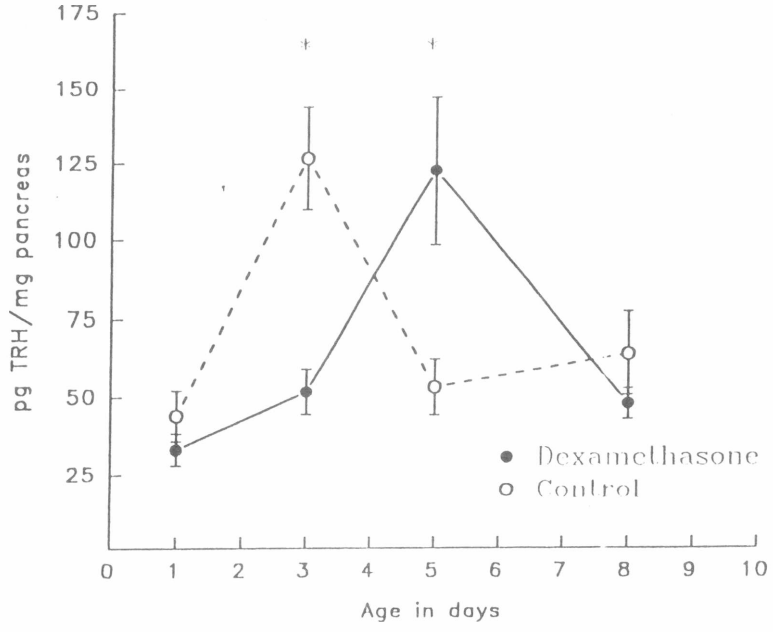

Fig. 2
Effect of DXM treatment on the development of pancreatic immunoreactive TRH concentration during ontogenesis. TRH immunoreactivity was measured by RIA after acidic extraction of the pancreatic homogenate. Results are expressed in pg of TRH per mg of pancreatic wet weight (mean ± S.E.M.). * $P<0.05$ for individual differences between age-matched DXM and control groups.

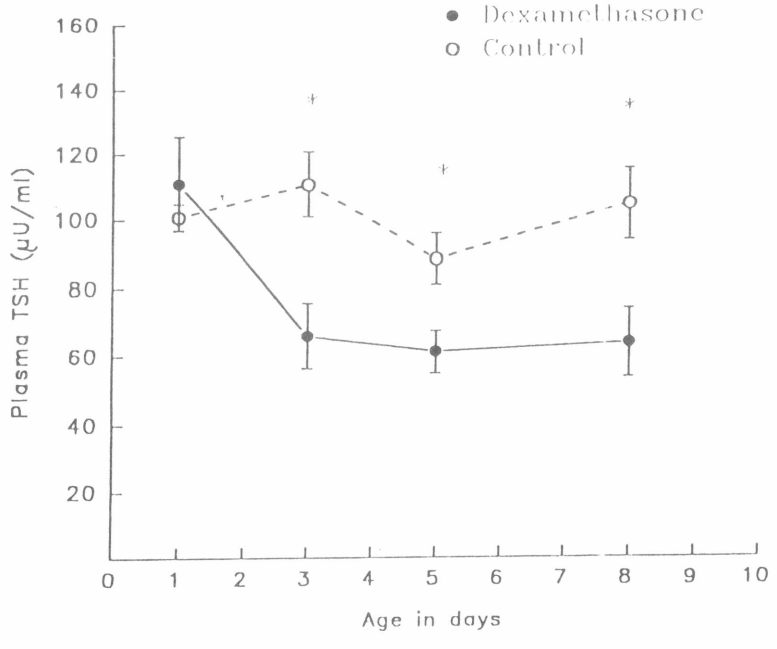

Fig. 3
Effect of DXM treatment on the development of plasma TSH concentration. After decapitation the trunk blood was collected and plasma TSH was measured by RIA. Results are expressed in μU TSH per ml of plasma (mean ± S.E.M.). * $P<0.05$ for individual differences between age-matched DXM and control groups.

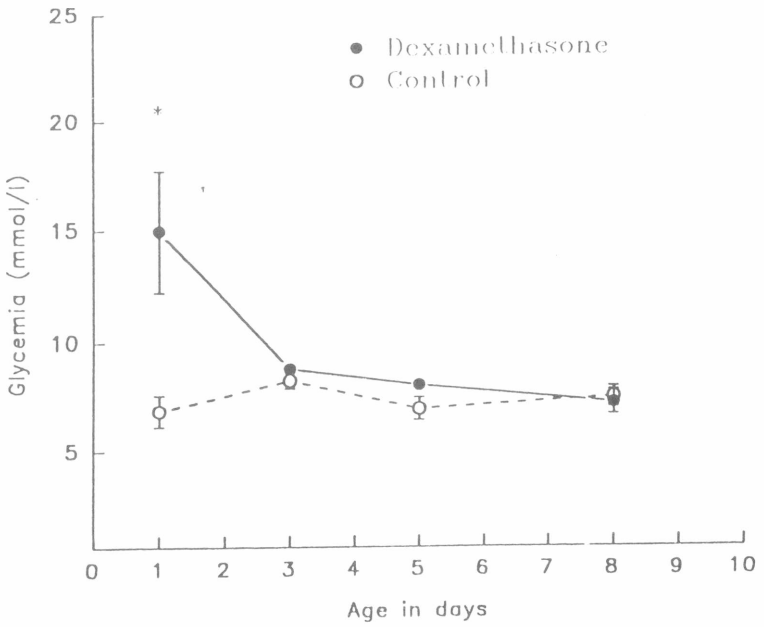

Fig. 4
Effect of DXM treatment on plasma glucose concentration. After decapitation the trunk blood was collected and plasma glucose was measured. The results are expressed in mmol/l (mean ± S.E.M.). * $P<0.05$ for individual differences between age-matched DXM and control groups.

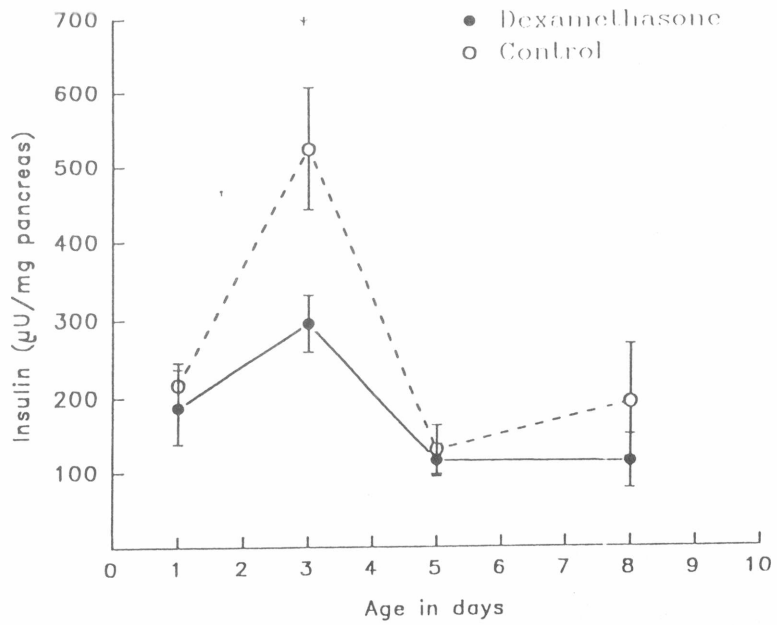

Fig. 5
Effect of DXM treatment on the development of pancreatic insulin concentration. Insulin was measured by RIA after acidic extraction of pancreatic homogenate. Results are expressed in μU insulin per mg of pancreatic wet weight (mean ± S.E.M.). * $P<0.05$ for individual differences between age-matched DXM and control groups.

Discussion

The rat pancreas displays a peculiar ontogenetic pattern for TRH with a rapid increase after birth up to postnatal day 3–4 when the peak is reached (for review see Oliver *et al.* 1986). In our study, postnatal DXM treatment retarded the typical neonatal TRH changes by two days, i.e. the peak of TRH immunoreactivity was achieved on postnatal day 5 instead of 3, yet both peaks were comparable. This effect is underlined by the fact that both control and DXM-treated animals of each age group came from the same litter and were reared by the same mother in the same cage. Therefore, the effects of genetic background or environmental conditions could be excluded. The rapid changes in pancreatic TRH levels appear to be regulated (at least in part) by biosynthetic steps. Indeed, the ontogenetic evolution of TRH mRNA and peptidyl-glycin-amidating monooxygenase (PAM), an enzyme involved in the last step of the posttranslational processing of TRH precursor, parallels that of TRH (Dutour *et al.* 1987, Ouafik *et al.* 1987). However, hormones can also be degraded in their cells of origin before secretion (Bienkowski 1983). Thus, the variations in the activity of TRH-degrading enzymes may be involved in the control of intra- and extracellular levels of TRH in the pancreatic tissue. However, we observed no TRH degradation in any of the age groups examined. This observation is in good agreement with that published previously by Aratan-Spire *et al.* (1984) and Fuse *et al.* (1990) who failed to detect any appreciable TRH-degrading activity in the rat postnatal pancreas. On the other hand, Salers et al. (1992), were able to detect high specific activity of pancreatic soluble unspecific pyroglutamyl peptidase I and prolyl endopeptidase in postnatal rats. These authors, however, suppose that these enzymes are not involved in the control of TRH levels in the neonatal rat pancreas and that the pancreatic TRH content appears to be primarily regulated by biosynthetic steps. Our data show that glucocorticoids are probably not involved in the regulation of pancreatic TRH-degrading activity at this age and that their effect is more likely due to changes in the regulation of TRH release and/or biosynthesis. Mitsuma *et al.* (1992) reported inhibition of TRH release from the hypothalamus accompanied by a parallel increase in the hypothalamic TRH content and a decrease in the hypothalamic content of the TRH precursor peptide Lys-Arg-Gln-His-Pro-Gly-Arg-Arg (p-8) after DXM treatment. From these data the authors concluded that DXM administration may induce a reduction of pro-TRH biosynthesis in association with enhanced maturation of p-8 into TRH. On the other hand, DXM treatment increased both TRH mRNA and cellular and secreted TRH in CA77 neoplastic thyroidal C cell line producing TRH (Tavianini *et al.* 1989) and elevated the levels of TRH mRNA in cultured anterior pituitary cells (Bruhn *et al.* 1992). The possibility that DXM affects TRH biosynthesis is supported by the fact that the proposed consensus sequence for glucocorticoid receptor binding is present near the promoter of the rat TRH gene (Lee *et al.* 1989). However, the effects of glucocorticoids on TRH biosynthesis appear to be controlled in a site-specific manner and further investigation should be done to elucidate its authentic role in the regulation of pancreatic TRH. On the other hand, the possibility that TRH secretion is changed after DXM treatment should also be considered. Whatever was the mechanism of DXM effect in our experiments, it was only transient despite continuous treatment with the drug. This seems to be confined only to the neonatal pancreas, since the effect on pituitary TSH secretion lasted till the end of the experiment. It would be of interest to obtain insight into the mechanism of this escape from the DXM effect in the pancreas.

Several authors (Brown and Hedge 1974, Mitsuma and Nogimori 1982, Brabant *et al.* 1989, Mitsuma *et al.* 1992) reported an inhibitory effect of glucocorticoids on pituitary TSH secretion, resulting in lowered levels of TSH in the blood. This effect might be partially due to an inhibition of hypothalamic TRH release (Mitsuma and Nogimori 1982, Mitsuma *et al.* 1992, Brabant *et al.* 1987). Our observation of the significant decrease in plasma TSH levels after DXM treatment is in agreement with this finding. Moreover, the low dose of DXM used in our study (1 μg/100 g BW in contrast to 25–500 μg/100 g in previous studies) proved to be sufficient to affect TSH secretion.

TRH and insulin coexist in the B-cells of islets of Langerhans, probably in the same secretory granules (Leduque *et al.* 1985, 1987). Insulin and TRH were reported to be presumably cosecreted during the early neonatal period (Ebiou *et al.* 1992). While in DXM-treated animals the peak of TRH concentration was retarded, no retardation was observed for insulin. The pancreatic insulin level in DXM-treated rats reached its peak on the same day as in the controls, although it was significantly lower. It is known that chronic DXM treatment causes insulin resistance, in which hyperglycaemia coexists with hyperinsulinaemia and B-cell hyperplasia. However, in our experiment, the increased glucose levels were observed only 24 h after the first DXM injection, returning afterwards to control levels. Thus, it is likely that the decrease in pancreatic insulin content observed in DXM-treated rats might be a consequence of its increased release.

DXM profoundly affects pancreatic growth. The considerable increase in pancreatic weight could hardly be explained by hyperplasia of pancreatic B-cells, since the islets of Langerhans represent less than two per cent of the total pancreatic cell mass. Glucocorticoids are known to induce hyperplasia and

hypertrophy of the acinar and ductal cells (Bourry and Sarles 1978, Morisset and Jolocoeur 1980). However, endocrine cells located in the islets of Langerhans contain a substantially larger number of glucocorticoid receptors (Matthes *et al.* 1994) and are more sensitive to glucocorticoids as compared to exocrine and acinar cells. Therefore, the effects of glucocorticoids on exocrine cells could be mediated by an indirect pathway modulating the synthesis and paracrine secretion of endocrine pancreatic hormones. The fact that chronic TRH treatment induced pancreatic hyperplasia (Glasbrenner *et al.* 1990) supports this hypothesis. It remains to be established how the changes of pancreatic TRH after DXM are related to changes in pancreatic growth.

In conclusion, postnatal DXM treatment retarded the typical neonatal ontogenetic changes in the pancreatic TRH content. This effect was not mediated through stimulation of the TRH-degrading activity and might result from changed biosynthesis and/or release of TRH. The significant stimulation of the pancreatic growth observed in DXM-treated rats might be related to the concomitant TRH changes.

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