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Vasoactive Intestinal Polypeptide in Rat Heart Atria: the Effect of Hyperthyroidism

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Summary

The effects of transient and sustained hyperthyroidism on vasoactive intestinal polypeptide-like immunoreactivity (VIP-LI) levels were studied in the heart atria of developing and adult rats. Newborn rats were divided into 5 groups. Neo-T animals were treated with thyroxine (T4) during postnatal days 1-8 and sacrificed at the age of 60 days. Neo-S rats were treated with T4 during postnatal days 1-60 and sacrificed one day later. Adult-1 and Adult-2 animals received T4 during days 52-60 and were sacrificed 5-6 days and 1 day later, respectively. Control animals were injected with saline. VIP-LI concentrations were determined in extracts from the left and right atria separately. In Neo-S and Adult-2 rats, spontaneous heart rate, the weight of both atria and total T4 serum levels were significantly enhanced, while their body weight was decreased. The ratio atria weight to body weight was significantly increased in all groups except for Adult-1 animals. Hyperthyroidism led to a significant decrease in VIP-LI levels in both atria of Neo-S and Neo-T rats. Hyperthyroidism induced in adult rats also decreased VIP-LI levels in both atria. However, this change was only transient. In conclusion, our data have provided new evidence that hyperthyroidism induced during the early neonatal period interferes with the development of VIP-ergic innervation in rat atria. The period of the first few postnatal days seems to be essential for this effect, since VIP-LI concentrations in 60-day-old animals did not significantly differ between Neo-S and Neo-T atria.

Key words

Vasoactive intestinal polypeptide •Hyperthyroidism • Heart • Rat • Development

Introduction

Hyperthyroidism is usually associated with increased heart rate, enhanced myocardial contractility, reduced vascular resistance and cardiac hypertrophy (Polikar *et al.* 1993). Several studies have shown that the excess of thyroid hormone alters the function of efferent autonomic innervation in the mammalian heart. An increase in β-adrenergic receptor density (Bilezikian and Loeb 1983, Fox *et al.* 1985) and a decrease in α-adrenergic receptor density (Han *et al.* 1995, Limas and Limas 1987) have been reported in the rat heart. Furthermore, impairment of cardiac noradrenaline (NA) turnover was described under conditions of hyperthyroidism in the same species (Gross and Lues 1985, Swann 1988).

Studies concerning the interactions of thyroid hormone excess with parasympathetic innervation in the mammalian heart are less numerous. Several reports have shown diminished vagal activity (Cacciatori *et al.* 1996,

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Maciel *et al.* 1987) and excitability (Kollai and Kollai 1988) in humans. In the rat, the excess of thyroid hormones either decreased the number of muscarinic receptors in the membrane preparations from the whole heart (Sharma and Banerjee 1977) or had no effect (Kastrup and Christensen 1984). In the left ventricle of hyperthyroid rats, the acetylcholine (ACh) content was elevated per chamber due to a decreased clearance of ACh rather than due to a compensatory increase in the density of parasympathetic innervation (Nyquist-Battie *et al.* 1993).

To date, a number of neurochemical substances have been identified in cardiac neurons in addition to classical neurotransmitters NA and ACh. Among them, vasoactive intestinal polypeptide (VIP) has been demonstrated in nerve fibers associated with the atrial myocardium, the conduction system and coronary vessels in various mammalian species. VIP-like immunoreactivity (VIP-LI) has also been found in neuronal cell bodies of intracardiac ganglia (Weihe *et al.* 1984, Slavíková 1997).

VIP is a 28 amino acid peptide that was originally isolated from the porcine intestine (Said and Mutt 1970). It is thought to be co-stored and co-released with ACh from postganglionic parasympathetic neurons in the cardiovascular, digestive, urogenital and respiratory systems (Lundberg 1996). Exogenous VIP exerted positive chronotropic and inotropic effects on the isolated canine and human atrial myocardium (Franco-Cereceda *et al.* 1987, Karasawa *et al.* 1990). VIP was also shown to cause coronary vasodilation in the dog (Anderson *et al.* 1988, Feliciano and Henning 1998) and to protect the myocardium against ischemia and reperfusion injury in the rat (Kalfin *et al.* 1994). Most of the actions of VIP are mediated *via* specific receptors coupled to the membrane-bound adenylate cyclase system. VIP receptors have been demonstrated in various species including man (Christophe *et al.* 1984). Although the role of endogenous VIP is still unclear, the above data suggest that VIP may contribute to the control of cardiac function.

The aim of this study was to determine whether hyperthyroidism induced in adult albino rats may affect VIP-LI concentrations in the heart atria and whether excess of thyroid hormones during several postnatal days may interfere with the development of VIP-ergic innervation in the heart.

Methods

Animals

Wistar rats of both sexes bred in our laboratory were used. Pregnant rats were housed individually with a free access to food and water. After birth, litters were made up of 8-10 pups each. All experiments were conducted in accordance with the relevant Guidelines of the Czech Ministry of Agriculture for scientific experimentation on animals.

Treatment

After birth, animals were randomly divided into 5 groups. Rats from group 1, subjected to transient neonatal hyperthyroidism (Neo-T), received subcutaneous injections of L-thyroxine (T4) 1 mg/kg b.w./day beginning one day after birth and continuing for 8 consecutive days. They then received injections of saline in the corresponding volume till 60 days of age. Rats from group 2, subjected to sustained neonatal hyperthyroidism (Neo-S), were treated with the same dose of T4 for 60 days and they were sacrificed 24 h after the last injection. Animals from group 3 (Adult-1) were treated with T4 from day 52 for 8 days and were sacrificed 5-6 days after the last injection. Age-matched animals from group 4 (Adult-2) were injected daily with saline till the age of 52 days and for further 8 days with the same dose of T4. They were sacrificed 24 h after the last injection. Animals from group 5 were treated with saline from the first postnatal day till the age of 60-65 days and served as controls.

Spontaneous heart rate

One day prior to dissection, rats were placed in a small chamber and their heart rates were recorded using electrodes located in the floor of the chamber. Repeated measurements of the heart rate were made until stable values were attained. The values mentioned in the results represent the average of these measurements.

Preparation

At the age of 60-65 days, the rats were decapitated and their hearts rapidly excised. Tissues were rinsed with ice-cold 155 mmol/l NaCl, placed in an ice-cold SET buffer containing 0.1 mmol/l EDTA, 5.0 mmol/l Tris-HCl, pH 7.4 and 0.25 mol/l sucrose. The hearts were freed of connective tissue and fat, and separated into left atria with the interatrial septum, right

atria, and free walls of both ventricles. Immediately after dissection, the tissues were frozen on dry ice and weighed. The samples were then placed in 0.1 mol/l HCl containing 100 μmol/l EDTA and 0.01 % $Na_2S_2O_5$ 1:10 (w/v) and briefly pulverized. Test tubes with the tissues were heated in a water bath at 95 °C for 15 min and then cooled on ice. Content of the tubes was homogenized for 30 s using an Ultra-Turrax homogenizer. The homogenate was centrifuged at 10 000 x *g*, 4 °C, 20 min. The supernatant was neutralized with 1 mol/l Tris-base and centrifuged again at 5 000 x *g*, 4 °C, 15 min. The clear supernatant was aspirated, lyophilized, and stored at –70 °C until radioimmunoassay.

Biochemical assays

Protein concentrations were determined according to Lowry *et al.* (1951) using bovine serum albumin as a standard.

The Chiron Diagnostics ACS:180® Automated Chemiluminiscent System (USA) was employed for assessing total thyroxine levels in the serum.

VIP-LI was assayed in tissue extracts by radioimmunoassay using the commercial kit (Phoenix Pharmaceuticals, USA). Lyophilized tissue extracts were dissolved in 250 μl of assay buffer. Assay tubes were set up in duplicate, each containing 100 μl of an unknown sample or the standard and 100 μl of rabbit anti-peptide serum. After incubation (20 h) at 4 °C, 100 μl of the tracer solution was added to each tube and the tubes were incubated for further 20 h. Bound radioactivity was separated by adding goat anti-rabbit IgG serum and centrifugation.

Recovery was assessed in another set of measurements (n=7) by addition of exogenous VIP at the time of heating in HCl. About 65 % of the added exogenous VIP could be detected in the final extract. The results were not corrected for recovery. Intra-assay variations did not exceed 10 %.

Data analysis

Tissue content of VIP was expressed in pg/mg protein, total serum thyroxine level in nmol/l. Results are reported as means ± S.E.M. with levels of significance calculated by Student's t test (two-tailed, unpaired). $P \leq 0.05$ values were considered to be significant.

Table 1. Body weight, weight of both atria, serum total thyroxine concentration, spontaneous heart rate and the ratio of atria weight to body weight in adult female rats.

	Body weight (g)	**Weight of atria (mg)**	**Serum T4 (nmol/l)**	**Heart rate (beats/min)**	**Atria weight/ body weight (%)**	**n**
Controls	223±4	67±2	64.3±2.2	345±7	0.0309	18
Neo-T	190±6*	68±4	61.2±2.0	365±6	0.035*	11
Neo-S	198±4*	77±4*	194±14**	469±7*	0.039*	8
Adult-1	221±4	68±3	60±3	349±5	0.0305	7
Adult-2	196±4*	73±3*	64.3±2.2	447±5*	0.037*	18

*Controls, rats treated with T4 for 8 postnatal days (Neo-T), rats treated with T4 for 60 postnatal days (Neo-S), rats treated with T4 during postnatal days 52-60 and killed 5 days later (Adult-1), and rats treated with T4 during postnatal days 52-60 and killed 1 day after the last injection (Adult-2). Significant differences vs. controls: *p<0.01, **p<0.001.*

Results

Basal parameters

Several parameters were established to evaluate thyroid status resulting from transient and sustained neonatal T4 treatment, administration of T4 to both groups of adult animals and the controls. Values obtained in female rats are shown in Table 1. The body weight of T4-treated rats was significantly reduced and their heart rate was increased (compared to the controls) in groups of

animals either treated for 60 postnatal days (Neo-S) or on days 52-60 and sacrificed one day after the last injection (Adult-2) ($P<0.01$). The weight of atria was significantly increased in the Neo-S and Adult-2 groups ($P<0.01$). Total T4 serum levels were significantly higher in the Neo-S and Adult-2 animals compared to the controls ($P<0.001$). Neonatal transient hyperthyroidism did not lead to changes in total T4 serum levels and the heart rate but resulted in a significant increase in the atria weight/body weight ratio in 60-day-old animals.

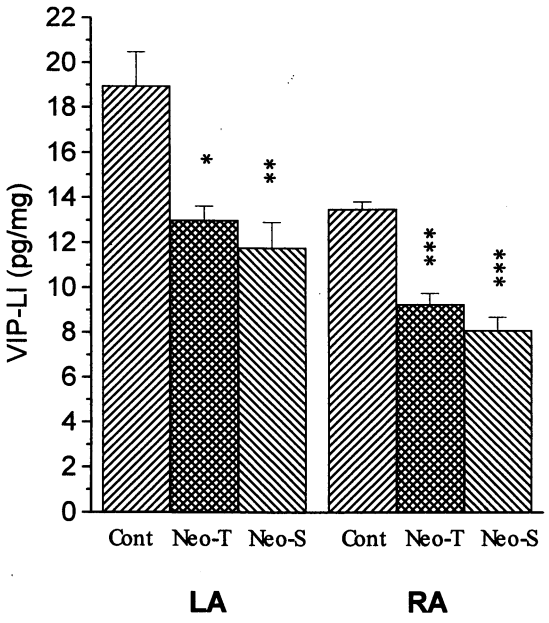

Fig. 1. *Effects of thyroxine (T4) administration on VIP-LI concentrations in the rat right (RA) and left atria (LA) in controls (Cont), rats treated with T4 either during days 1-9 and sacrificed at the age of 60 days (Neo-T) or during days 1-60 and sacrificed 1 day later (Neo-S). Data are means ± S.E.M., *p< 0.05, **p< 0.01, ***p< 0.001 vs controls.*

Effects of different protocols of T4 treatment on tissue VIP-LI concentrations

In the control rats, the VIP-LI levels expressed in pg/mg protein were 18.9±1.5 (n=18) in the left atria (LA) and 13.5±0.3 (n=13) in the right atria (RA). Values of VIP-LI concentrations in both atria did not differ between males and females. Tissue concentrations of VIP-LI were significantly higher in the LA than in the RA ($p<0.01$).

Since the VIP-LI levels in the ventricles were just at the detection boundary of the method, the effects of different T4 treatments on VIP-LI tissue levels were assessed in the atria only.

Tissue concentrations of VIP-LI in the Neo-T hearts were 13.00±0.62 pg/mg protein in the LA and 9.26±0.49 pg/mg in the RA. Compared to the controls, neonatal transient T4 treatment resulted in a significant decrease in tissue VIP-LI levels (LA/RA: $p<0.05$/$p<0.001$). The percentage reduction in the content of VIP-LI was comparable in both atria (LA/RA: 31.37 %/31.44 %). Sustained T4 treatment from birth till adulthood led to a slightly more pronounced decrease in VIP-LI in both atria (LA/RA: 37.81 %/40.00 %). Values obtained for the Neo-S group were 11.78±1.13 and 8.1±0.58 pg/mg protein for LA and RA, respectively. The levels of tissue VIP-LI did not significantly differ between the groups subjected to transient neonatal and sustained neonatal treatment protocols (Fig. 1).

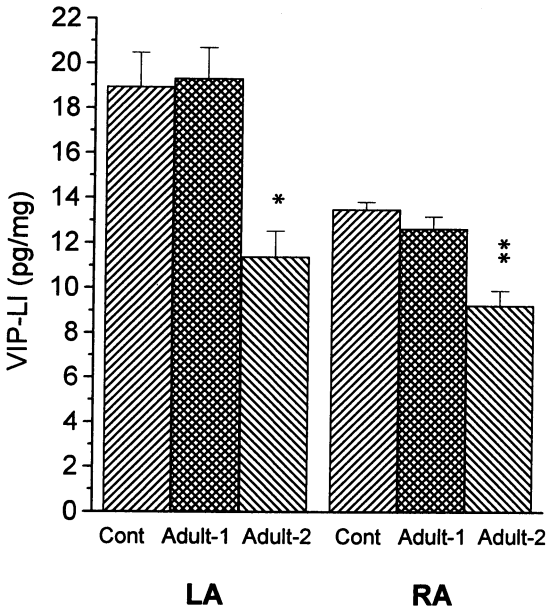

Fig. 2. *Effects of T4 administration on VIP-Li concentrations (in pg per 1 mg of protein) in the rat right (RA) and the left (LA) heart atria in controls (Cont), adult rats treated with T4 either during days 52-60 and sacrificed 5 days later (Adult-1) or from day 52 to 60 and sacrificed 24 hours later (Adult-2). Data are means ± S.E.M., *p< 0.05, **p< 0.01 vs controls.*

Exogenous hyperthyroidism induced in adult animals sacrificed 24 h after the last T4 administration (Adult-2) resulted in a significant decrease in VIP-LI in both atria compared to values obtained from control rats

($p<0.01/p<0.0001$; LA/RA). In contrast, no differences in VIP-LI were observed in adult animals subjected to T4 treatment for 8 days and sacrificed 5 to 6 days after the cessation of treatment (Adult-1) when compared to the controls. Values of VIP-LI obtained from the Adult-2 group were 9.25±0.64 and 11.39±1.14 pg/mg protein for the RA and LA, respectively. These concentrations represented 68.5 % and 60.17 % of the control values in the respective atria (Fig. 2).

Discussion

All the measured basal parameters of the thyroid status were significantly changed in rats treated with T4 either for 60 postnatal days (Neo-S) or during days 52-60 and killed one day after the last injection (Adult-2). These findings suggest that these protocols of T4 administration were sufficient to cause hyperthyroidism associated with its typical symptoms. In the rats given T4 only transiently after birth (Neo-T), the atria weight/body weight ratio remained significantly increased even when the treatment had been discontinued. This is in accordance with the observation that hyperthyroidism induced in neonatal rats evoked cardiac hypertrophy that was accompanied by a marked increase in cell number and a slight increase in cell size, suggesting cardiac hyperplasia (Slotkin *et al.* 1992). This thyroid hormone-induced increase in cardiac growth has been shown to be more pronounced in the atria than in the ventricles (Canavan *et al.* 1994).

It is well known that the appropriate level of the thyroid hormones is essential for the normal development and function of most tissues. Many endocrine and neural alterations induced by neonatal hyperthyroidism have been reported in adult rats, including impaired adrenomedullary function (Lau *et al.* 1988), hyperresponsiveness of the sympathoadrenal medullary system to acute stress (McCarty *et al.* 1983) and a decrease in the brain weight (Pascual-Leone *et al.* 1985). Diminished thyroid function has been reported in rats receiving thyroid hormones 12 days after birth which persisted till the age of 120 days (Walker and Courtin 1985). In our experiments, total T4 levels were normal in adult Neo-T rats, and the weight of their atria did not differ significantly from the controls. Thus, the period of thyroid hormone administration for 9 postnatal days does not seem to be sufficient for evoking hypothyroidism in adult animals.

In the rat, neonatal transient hyperthyroidism was shown to accelerate the maturation of sympathetic neurotransmission which is followed by long-term functional impairment of the function of sympathetic innervation of the heart (Lau and Slotkin 1980). The noradrenaline content in the whole rat heart was also found to be significantly reduced even 50 days after neonatal T4 treatment had been discontinued (Volín *et al.* 1982). However, at the postsynaptic level, hyperthyroidism during first 5 postnatal days elicited an initial increase in β-adrenergic receptor density with subsequent deficits and an eventual return to normal values in early adulthood (Pracyk and Slotkin 1991).

Little is known about the development of VIP-ergic innervation in the mammalian heart. In the human fetal heart at gestation age of 10-12 weeks, nerve fibers with VIP-LI, but not neuronal cell bodies, were associated with the atrial myocardium (Gordon *et al.* 1993). In contrast, Chow *et al.* (1993) reported that the conducting system of the human heart is not innervated by VIP-ergic fibers at birth. In the rat right atrium, VIP-LI was first detected in both nerve fibers and nerve cell bodies of 10-day-old animals by means of immunohistochemistry (Slavíková 1997). It has recently been shown that the atrial VIP-LI concentrations did not differ in one-week-old puppies and adult dogs (Kralios *et al.* 1999).

Our study shows that the excess of thyroid hormones in the early neonatal period results in a long-term decrease of VIP-LI concentrations in both atria. It provides the first evidence that excess of thyroid hormones during several postnatal days may interfere with the development of VIP-ergic innervation in the rat atria. Tissue VIP-LI levels in our experiments did not change substantially even 6 weeks after T4 administration had been discontinued and they did not differ significantly from values found in the atria of animals given T4 continuously. Thus, the first postnatal week seems to be crucial for the effect of hyperthyroidism on the atrial VIP-LI content present in adult animals.

Only a few studies have dealt with the effects of abnormal thyroid status on tissue VIP-LI levels in adult animals. The excess of thyroid hormones was shown to cause VIP-LI depletion in cultured cerebral cortical cells from fetal rats (Lorenzo *et al.* 1992) and in the anterior pituitary of the same species (Buhl *et al.* 1995). In contrast, no changes in VIP-LI concentrations were observed in the hypothalamus, anterior pituitary (Jones *et al.* 1989) and adrenal medulla (Tsuchiya *et al.* 1990) of hyperthyroid rats.

In the present study, hyperthyroidism in adult rats reduced VIP-LI levels in both atria. This effect only seems to be transient, since no differences in atrial VIP-LI concentrations were found between animals killed 5-6 days after cessation of T4 administration and the controls. The heart rate, total serum T4 levels, the body weight and weights of the atria did not differ from control values. This is in accordance with the observation that many symptoms associated with exogenous hyperthyroidism in adults, including cardiac hypertrophy and increased β-adrenergic receptor density regress within 3 days after cessation of T4 administration (Atkins *et al.* 1983).

In conclusion, this paper is the first report demonstrating the effects of excess thyroid hormones on VIP-LI concentrations in newborn and adult rat heart atria. The mechanism underlying the thyroid hormone effect on VIP-ergic innervation (altered gene expression, release and/or degradation of the peptide) remains to be elucidated.

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