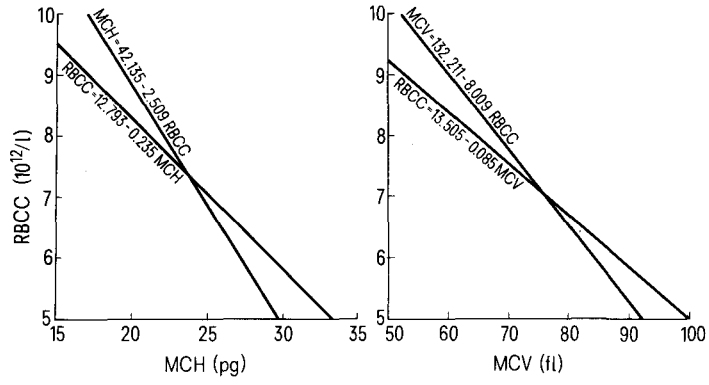

Graph of the regression lines for laboratory dogs showing the linear relation of mean cell haemoglobin (MCH) and red blood cell counts (RBCC) or the linear relation of mean corpuscular volume (MCV) and RBCC.

haemoglobin concentration ($V_M = 10.7$, $V_F = 8.8\%$) and haematocrit ($V_M = V_F = 7.7\%$). These data suggest that correlations among MCV, RBCC and MCV, which are computed above, might be decisive in the controlling mechanism in the red blood picture of normal adult dogs.

Discussion. We have found no report on a negative coefficient of correlation between MCH and RBCC in any animal species or in human subjects. There is one report of such a relationship between human MCV and RBCC[3] but no report for other species has been found. The MCH values depend upon the haemoglobin synthesis and the cell volume (MCH = MCHC × MCV). As the MCHC is relatively constant (cf. variation coefficients), the basic relationship is between MCH and MCV. Thus, the erythrocyte volume might be important in the controlling mechanism. Nevertheless, the MCV depends upon haematocrit and RBCC (MCV = haematocrit/RBCC). As both haematocrit and haemoglobin levels are relatively constant (cf. variation coefficients), both MCV and MCH seem to be decisive in controlling the mechanisms of RBCC, which we confirmed by the higher statistical significance of correlation between RBCC and MCV or MCH as well. Our findings partially explain clinical experience that RBCC are usually a less responsive determination than other characteristics made in the examination of the red blood picture, particularly haematocrit[4] and MCHC[5].

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Hypothalamus-adenohypophysis-thyroid axis in spontaneously hypertensive rats (SHR)

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Summary. Increased basal TSH levels and an enhanced response to cold-exposure were apparent in the SHR accustomed to +30 °C, whereas goitres and an increased amount of the anterior pituitary TSH were measured in SHR kept at +20 °C.

Spontaneously hypertensive rats (SHR) which were originally isolated by Okamoto and Aoki[2], have been widely used as an animal model for essential hypertension. From the very beginning small but definitive abnormalities were observed in the endocrine function of SHR[3], e.g. basal TSH levels were somewhat higher in SHR than in the various control rats[4-7]. In 1 study[6], the TSH-response to exogenous TRH was enhanced in SHR. Furthermore, SHR have had small goitres[2,4,5] or an increased uptake of radioiodine[2,4]. Although there has not been very marked hypertrophy of the anterior pituitaries in SHR[2,3], basophilic[3] or more specifically thyrotrophic cells[8] have been relatively increased. The levels of serum thyroid hormones have sometimes been low[4,5,9]. These data from several sources have not been easy to interpret, and definitive conclusions about the level of the disturbance have not been drawn.

We have now compared the dynamics of the hypothalamus-anterior pituitary-thyroid gland axis in the SHR and the Wistar-Kyoto (WKY) control rats, trying to localize the possible error of this axis. We have measured hypothalamic TRH levels, anterior pituitary TSH concentrations and serum TSH and thyroid hormone levels both under basal conditions and during cold- and TRH-stimulation.

Materials and methods. SHR and WKY control rats were originally obtained from NIH and then inbred in our department. The rats were used at the age of about 8 months. They were fed with common laboratory pellets (iodine content 0.5-1 mg/kg) and tap water ad libitum. Blood pressure of SHR was between 150 and 210 mmHg and that of WKY rats between 100 and 135 mmHg, when measured about 2 weeks before the experiments. Some of the rats were adapted to +30 °C for 7 days before use. The cold-exposure was used to elevate serum TSH levels (+4 °C for 30 min). The TSH cold-response is known to be mediated via increased TRH-release in the hypothalamus[10,11], and this test is therefore used to study changes in the activity of the hypotalamic TRH neurons. The warmth-adaptation has been a routine because a high cold-response

Table 1. Thyroid weight (mg/100 g b.wt), serum thyroxine (T_4, ng/ml) and tri-iodothyronine (T_3, ng/ml) concentrations, pituitary weight (mg/100 g b.wt), pituitary TSH (μg/mg of wet tissue weight) and medial basal hypothalamic TRH concentration (pg/mg of tissue wet weight) in Wistar/Kyoto control rats and spontaneously hypertensive rats. Some of the rats were kept at +20 °C (room temperature); the rest were kept at +30 °C for 7 days before the experiments

Parameter	Temperature and treatment	Control rats	Hypertensive rats
Thyroid weight (mg/100 g)	At +20 °C	6.9±0.84 (9)	8.5±0.34* (21)
	At +30 °C	4.9±0.53 (10)	5.8±0.30 (16)
Serum T_4 (ng/ml)	At +20 °C	29±3 (12)	42±2** (11)
	At +30 °C	31±2 (7)	26±3 (10)
Serum T_3 (ng/ml)	At +20 °C	0.76±0.09 (12)	0.80±0.06 (10)
	At +30 °C	0.60±0.08 (7)	0.41±0.03** (10)
Pituitary weight (mg/100 g)	At +20 °C	3.9±0.23 (15)	5.3±0.15*** (13)
	At +30 °C	3.2±0.11 (45)	3.2±0.10 (39)
Pituitary TSH concentration (μg/mg)	At +20 °C		
	Saline injection	77±2 (5)	85±3* (10)
	TRH injection, 125 ng	88±9 (5)	99±6* (10)
	At +30 °C		
	Saline injection	60±9 (12)	86±18 (14)
	Cold exposure	95±19 (7)	136±22 (9)
	TRH injection, 50 ng	43±5 (13)	44±10 (7)
	TRH injection, 125 ng	34±4 (12)	34±4 (7)
Medial basal hypothalamic TRH concentration (pg/mg)	Untreated, +30 °C	129±15 (12)	109±13 (20)
	Cold exposure	162±14 (7)	146±14 (11)
	Saline injection, +20 °C	85±7 (5)	94±12 (5)
	TRH injection, 125 ng	88±8 (10)	114±13 (8)

Mean±SEM. Number of animals in parenthesis. Statistical significance of the differences between the control rats and hypertensive rats are given as follows: * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

is a rule[12]. Also one-half of the TRH-induced TSH responses were performed in the adapted rats. In this test either 50 ng/100 g or 125 ng/100 g of TRH was injected i.p., and the rats were killed by guillotine 30 min later, when the TSH levels had passed the peak and were declining. The blood of the trunk was collected and serum TSH[13], T_3[14], T_4[14] concentrations were measured with radioimmunoassays. The tissues were dissected and weighed. Anterior pituitaries were homogenized in 1.0 ml of 0.05 M phosphate buffer, 1% bovine serum albumin, pH 7.6, diluted 1:200 and analyzed for TSH. The medial basal hypothalamus (MBH) fragments were extracted in 2.0 ml of methanol, and assayed for TRH[15]. Arithmetic means, SEMs and SDs were calculated. Student's t-test was used for comparison of 2 means. In the case of 3 or more means 1-way analysis of variance was first applied.

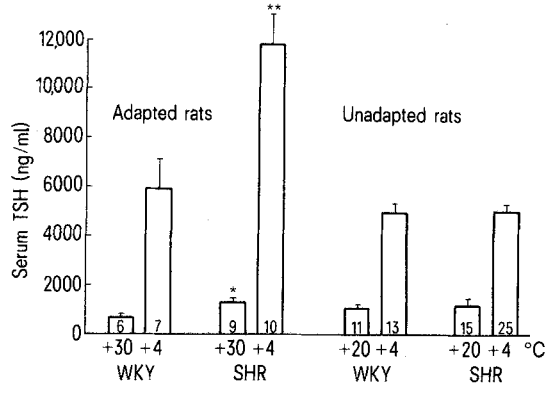
Results. The SHR kept at +20 °C had small goitres ($p < 0.05$) and hypertrophied anterior pituitaries ($p < 0.001$) which contained significantly more TSH than those of the WKY control rats. Serum T_4 levels were increased in the SHR kept at +20 °C ($p < 0.01$). T_3 levels were decreased in the SHR kept at +30 °C ($p < 0.01$). Medial basal hypothalamic TRH levels were similar in the SHR and WKY control rats (table 1). The SHR and WKY rats had similar TSH responses to exogenous TRH at both +20 °C and +30 °C (table 2). The TSH cold-response of the rats adapted to +30 °C was highly significantly greater in the SHR than in the WKY control rats. Also the basal TSH levels were higher in the SHR. Such differences could not be seen in the rats kept at +20 °C (fig. 1, table 2).

Discussion. Our results confirm the earlier findings[1-5,7-9] that there is a small thyrotrophic overactivity in the SHR kept at room temperature. A high basal TSH level is not a constant finding but the manifestations are seen as small goitres, large anterior pituitaries which contain much TSH, and increased serum T_4 levels. There is no extra reserve for enhanced TSH secretion after TRH or cold-exposure in the SHR. However, when the rats are kept at +30 °C, where they need not to use any extra energy to maintain the body

Table 2. TRH-induced (50 ng/100 g or 125 ng/100 g b.wt, i.p.) TSH-response in the control rats and spontaneously hypertensive rats. The blood sample was taken 30 min after the TRH-injection

	Serum TSH, ng/ml	
	Control rats	Hypertensive rats
Rats kept at +20 °C		
Saline	1064±116 (11)	1183±168 (15)
TRH, 50 ng/100 g	1724±300 (5)	2355±336 (10)
TRH, 125 ng/100 g	2776±363 (8)	2447±392 (7)
Rats adapted to +30 °C		
Saline	566± 85 (6)	876±131 (6)
TRH, 50 ng/100 g	1741±424 (7)	2135±424 (7)
TRH, 125 ng/100 g	1371±159 (7)	2330±444 (7)

Mean±SEM. Number of animals in parenthesis.



Basal and cold-induced (+4 °C, 30 min) TSH levels in the hypertensive rats (SHR) and Wistar-Kyoto (WKY) control rats. Some of the rats were adapted at +30 °C for 7 days (left) and the rest were kept at room temperature (+20 °C, unadapted, right). Mean ± SEM. Number of animals is given at the bottom of the columns. Statistics: *$p < 0.05$, **$p < 0.01$ vs the corresponding WKY control group.

temperature, a similar incidence of thyrotrophic imbalance in the SHR cannot be observed. Serum T_3 levels are low and serum TSH levels elevated. Under these circumstances it was possible to demonstrate a highly significant increase in the TSH cold-response in the SHR as compared with the WKY control rats. On the other hand, the TRH-induced TSH secretion is not altered. These results demonstrate that the reason for the thyroidal disturbance in the SHR evidently lies in the hypothalamic TRH neurons rather than in the anterior pituitary. It is noteworthy that we do not see any alteration in the MBH TRH levels. Similarly, it has also been impossible earlier to detect a measurable change in the hypothalamic TRH content after practically any physiological or pharmacological treatment[16].

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Identification of JH III as the principal juvenile hormone in *Locusta migratoria*

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Summary. JH titers in the hemolymph of nymphal and adult female *Locusta migratoria migratorioides* (R. and F.) were determined using a selective mass spectroscopic detection technique. Only JH III could be found in either stage, with no detectable JH I (or II). Titers observed were 10–1000-fold lower than those found via a recently reported radioimmunoassay procedure.

Many species of insects representing several orders have been investigated in recent years to ascertain the structural nature of their juvenile hormones (JH). Among the Orthoptera only JH III (C_{16}JH) has been identified to date[1,2], in an admittedly small sampling of species. However, Baehr et al.[3] recently reported the existence of a JH I (C_{18}JH) 'immunoreactive substance' in nymphal hemolymph of *Locusta migratoria migratorioides* (R. and F.), identified and quantified by means of a radioimmunoassay (RIA) technique. These researchers, moreover, interpret this result to be a representation of authentic JH I titer in *Locusta*. Simultaneously, workers at the University of Utrecht, using gas chromatography-electron capture detection (GC-ECD) have found what is claimed to be JH III in the same species at comparable developmental stages[4,5]. As we have maintained a long-standing interest in JH identification and titer determinations[6,7], we in turn have examined hemolymph extracts from *Locusta* nymphs and female adults by a recently developed technique[8] using combined gas chromatography/mass spectroscopy (GC/MS) with selected ion monitoring (SIM), and report our findings on JH titers thereform.

Materials and methods. Insects were reared on grass and bran in a 15L-9D photoregime at 32 °C; under these conditions the IVth and Vth larval instars last 4–5 and 7 days respectively. The animals were staged for sampling

Comparative *Locusta* JH titer data obtained by GC/MS (A), GC-ECD (B[5]), and RIA (C[3])

Age/stage	Analytical method	JH[a,b]($\bar{X}_n$) I	II	III	n
24–48-h IVth instar nymphs	A	≤0.02	≤0.02	0.93	3
	B	<0.3	<0.1	1.1	2
	C	25.5	ND	ND	15
0–24-h Vth instar nymphs	A	≤0.02	≤0.02	0.35	2
	B	<1.7	<0.8	≤1.8	2
	C	30.0	ND	ND	21
10–11(18)-day female adults	A	≤0.03	≤0.02	41.3	3
	B	(<1.7)	(<2.0)	(48.0)	4
	C	ND	ND	ND	-

[a] Titers are expressed in ng/ml (A, C) or ng/g (B) of hemolymph [b] ND = not determined.