Thyroid hormone-catecholamine interrelationships during exposure to cold

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Abstract. Basal plasma levels for adrenalin (A), noradrenalin (NA), l-triiodothyronine (T₃), and l-thyroxine (T₄) were determined in rats with a chronically inserted catheter. The experiments described in this report were started 3 days after the surgical procedure when T₃ and T₄ levels had returned to normal. Basal levels for the catecholamines were reached already 4 h after the operation. The T₃/T₄ ratio in plasma was significantly increased after 3, 7, and 14 days in rats kept at 4°C and the same holds for the iodide in the 24-h urine after 7 and 14 days at 4°C. The venous NA plasma concentration was increased 6- to 12-fold during the same period of exposure to cold, whereas the A concentration remained at the basal level.

During infusion of NA at 23°C the T₃/T₄ ratio in plasma was significantly increased after 7 days compared to pair-fed controls, and the same holds for the iodide excretion in the 24-h urine. This paper presents further evidence for a role of the sympathetic nervous system on T₄ metabolism in rats at resting conditions.

In a previous publication we showed that after one week of exposure to cold the T₃ concentration is elevated in rats (van Hardeveld et al. 1979a). We postulated that enhanced sympathetic activity in the cold might contribute to this elevation. Evidence for this was provided by the retardation of the T₃ increase under cold conditions after chemical sympathectomy by 6-hydroxydopamine. However, we did not measure catecholamine levels in these experiments. A further attempt to investigate the role of catecholamines in thyroid hormone metabolism is described here. A radio-enzymatic assay was used for noradrenalin and adrenalin, as a means to monitor alterations in catecholamine plasma levels during exposure to cold.

To obtain meaningful resting values a method was applied which utilizes a chronically inserted jugular or carotid catheter from which sequential blood samples can be collected without immobilizing or disturbing the animal.

Materials and Methods

Materials
Dithiotreitol (DTT) was purchased from Calbiochem, San Diego, California, USA, EGT A (ethyleneglycol-bis beta-aminoethyl ether-N,N'-tetraacetic acid) from SERVA, Heidelberg, W Germany, polyvinylpyrrolidone (PVP), noradrenalin-HCl, and DL-propranolol-HCl from Sigma Chemical Company, St. Louis, Mo., USA, Pyopen® (carbenicillin) from Beecham Research Laboratories, S A, Heppignies, Belgium, and calparine (calciumheparinate) from Laboratoire CHOAY, Paris, France.

Animals
Male rats of a Wistar strain were used. At the start of the experiments the rats were 13 weeks old and weighed about 300 g. Throughout all experiments the animals were housed individually in metabolism cages, which were placed in a room with a light and darkness cycle of 12 h (light on at 6 a.m) and a temperature of 23 ± 1°C. The rats were fed a normal laboratory chow (Hope Farms BV, Linschoten, The Netherlands) which, according to the manufacturer's information, contained 350 µg iodine/kg.
Surgical procedure, sample collections, and resting conditions

The right carotid artery or jugular vein was cannulated with PVC tubing (ID 0.5 mm, OD 1.0 mm, Talas, Ommen, The Netherlands) under ether anaesthesia. The cannula was inserted into the carotid artery to a depth of 2 cm, or into the jugular vein to a depth of 4 cm, just into the right atrium. The cannula was filled with a 5% solution of PVP in saline containing 500 IU/ml calparine and the antibiotic pyopen (0.01 g/ml), which was replaced by a fresh solution daily.

The free end of the cannula was drawn under the skin and exteriorized on the neck; it extended out of the cage (total length 50 cm) and was protected with thicker PVC tubing attached to the skin on the neck and counter-balanced outside the cage. The rats were placed in the metabolism cages after receiving an im injection with pyopen (0.1 g in 0.2 ml saline). The operation lasted 20 min.

During blood collection the rats were allowed to move freely in their individual cages and appeared to be unaware of the procedure. The method of sampling was standardized as much as possible; even a small amount of agitation of the rats, immediately before or during sampling, can change the plasma levels of A and NA significantly (Kvetnansky et al. 1978; Depocas & Behrens 1977; Bühlcr et al. 1978). Samples were usually taken at 11.00 a.m. Arterial blood samples were collected directly in iced ml plastic tubes containing heparin, whereas venous blood samples were collected with a syringe and transferred immediately to iced ml plastic heparinized tubes. When adrenalin and noradrenalin were to be determined, the blood was immediately stabilized in the tubes or syringes with 10 μl of a solution containing 1.3 mmol DTT and 0.26 mmol EGTA per ml double-distilled water, per ml of blood (Sole & Hussain 1977).

Blood samples for the measurement of catecholamines and thyroid hormones were collected separately. The plasma was separated as soon as possible by centrifugation and was frozen at −75°C. The erythrocytes were returned to the rats via the cannula in the same volume of saline.

For 24-h urine collections, clean funnels were placed under the cages every 24 h. To cause the animals empty their bladders before and at the end of a collection period, a piece of wadding sprinkled with ether was brought into the cages. Collecting urine or starting a collection period always occurred after blood sampling.

In this experimental setting we determined the time-point at which stress- and operation-induced alterations in catecholamine and thyroid hormone levels have tapered off.

Shortly after the operation, the arterial A and NA levels were increased 5- and 3-fold, respectively, compared to the basal value, which was reached after 4 h. This basal level was 1.1 ± 0.7 pmol/ml for A and 2.0 ± 0.6 pmol/ml for NA (n = 21). In the first hour after the operation, the T₃ and T₄ levels showed large individual fluctuations. The decreased food intake in the first 2 days after the operation did not significantly influence the T₃ levels, but induced a 50% decrease of the T₄ levels after 2 days. The values at 4 and 12 h, i.e., the period in which the basal catecholamine levels were reached and the decreased food intake did not exert its influence, were taken as basal levels for T₃ and T₄. The basal values for T₃ and T₄ were 1.12 ± 0.26 and 44.8 ± 11.8 pmol/ml plasma (n = 12), respectively. After 3 days, when the food consumption was normalized, the T₄ concentration too had returned to its normal level. No significant difference was found between venous and arterial thyroid hormone levels. This is in contrast to the catecholamine levels in arterial and venous blood. The basal venous levels of A and NA (0.5 ± 0.2 and 1.3 ± 0.7 pmol/ml (n = 12), respectively), were significantly lower than the basal arterial levels (A: P < 0.001, NA: P < 0.01).

The experiments described in this paper were started at least 3 days after the surgical procedure when T₃ and T₄ levels had returned to normal.

Analyses

A highly sensitive radio-enzymatic assay was used for the separate determination of adrenalin, noradrenalin, and dopamine. In these experiments we did not determine dopamine. For the first part of the assay we used a slightly modified version of the method developed by Sole & Hussain (1977). The second extraction step in their method was omitted. The plates used for thin-layer chromatography were developed for 2 h in tertiary amylalcohol:methylamine:benezene (30:15:10). From this point on we used a modified version of the method developed by Passon & Peuler (1973). All steps after chromatography were performed in the scintillation vial. After conversion to vanillin of the methylated products only one extraction step was used. A sample of pooled plasma extract was also determined (external standard) in each assay. The sensitivity of the assay was 10 pg A or NA per ml plasma and the inter-assay variability 9% for A and 12% for NA.

For T₃ and T₄ determinations, a radioimmunoassay (RIA) method was used (Janssen et al. 1979). Iodide in the urine was measured with an Auto-technical auto-analyzer technique based on the wet ashing digestion method of Zak et al. (1952).

Experiments

Experiment A. Effects of exposure to cold.

The jugular vein of 11 rats was cannulated. Three days later, when the animals had recovered, samples were taken for the determination of catecholamines and thyroid hormones. Next, the rats, in the same metabolism cages, were placed in a cold room kept at 4 ± 1°C, and with a light and darkness cycle of 12 h (light on at 6 a.m.).

Samples (1 ml) were taken after 3, 7, and 14 days in the
cold. The haemoglobin concentration was also measured before the exposure and after 3 and 7 days in the cold.

The 24-h urine was collected after 7 and 14 days in the cold and the iodide content was determined. The animals had free access to food and water, and food consumption was measured daily.

The relationship between food consumption and iodide excreted into the urine was determined by regression analysis from the values measured 3 to 6 days after the operation, when the rats used in all experiments had recovered, but had not undergone further experimental handling. The following equation states the relationship:

\[ \mu g I^\dagger/24 h = 0.17 \times (g \text{ food})/24 h + 0.77, \]

at room temperature (\( n = 44, r = 0.84 \)).

**Experiment B.** Effects of noradrenalin at 23°C.

A NA infusion was given to 6 rats via the right jugular vein with a MHRE/2 Watson Marlow pump at a constant rate of 24 ml/day. The NA dose was gradually increased from 0.059 to 0.59 nmol NA/min/g\(^{0.74} \) body weight over a period of 4 days. Infusion of the highest dose was continued for another 3 days.

The infusate had the following composition: 0.0156–0.156 \( \mu \text{mol} \) NA-HCl/ml, 0.75 \( \mu \text{mol} \) vitamin C/ml as a preservative to prevent degradation of NA, 8 IU heparin/ml, and \( 10^4 \) g pyopen/ml in saline. The solution was prepared daily and shielded from light.

Six control rats were infused with the same solvent, under otherwise identical conditions. The experimental scheme of the controls lagged one day behind that of the NA rats, to permit pair-feeding of the controls.

Venous samples for \( T_3 \) and \( T_4 \) determinations were taken shortly before the infusion was started and on the 4th and 7th day of infusion.

The 24-h urine was collected on the day before infusion and on the 5th and 7th day of infusion. The distribution of food and the collection of 24-h urine took place at fixed times, and the food consumption and water intake were measured daily.

**Statistical analysis**

For the calculation of the significance of differences between groups, Student's \( t \)-test was used (paired or unpaired).

**Results**

**Experiment A**

The purpose of this experiment was in the first place to evaluate in this new experimental setting the observations made by van Hardeveld et al.

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**Table 1.**

Venous NA, \( T_3 \), and \( T_4 \) plasma levels, \( T_3/T_4 \) ratio in plasma, and iodide content of 24-h urine during exposure to cold.

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>NA (pmol/ml)</strong></td>
<td></td>
</tr>
<tr>
<td>( 5 )</td>
<td>1.1 ± 0.3</td>
</tr>
<tr>
<td><strong>T3 (pmol/ml)</strong></td>
<td></td>
</tr>
<tr>
<td>( 11 )</td>
<td>1.03 ± 0.12</td>
</tr>
<tr>
<td><strong>T4 (pmol/ml)</strong></td>
<td></td>
</tr>
<tr>
<td>( 11 )</td>
<td>45.7 ± 10.1</td>
</tr>
<tr>
<td>( T_3/T_4 ) ( 10^3 )</td>
<td></td>
</tr>
<tr>
<td>( 11 )</td>
<td>23 ± 4</td>
</tr>
<tr>
<td><strong>Iodide (( \mu g/24 h ))</strong></td>
<td></td>
</tr>
<tr>
<td>( 5 )</td>
<td>3.46 ± 0.74</td>
</tr>
</tbody>
</table>

Rats with a cannula in the jugular vein were exposed to cold (4 ± 1°C) for up to 14 days. Blood was sampled before the exposure (day 0) and after 3, 7, and 14 days in the cold. The iodide content of the 24-h urine was determined after 7 and 14 days in the cold and before exposure. Values are means ± SD for the number of rats indicated in parentheses.

* \( P < 0.005 \) vs. day 0. ** \( P < 0.001 \) vs. day 0. *** \( P < 0.01 \) vs. day 0. **** \( P < 0.02 \) vs. day 0.
was significantly increased ($P < 0.001$) after 3, 7, and 14 days in the cold. At the same time-points the venous NA level was increased 6-, 8-, and 12-fold, respectively, whereas the A concentration remained on the basal level (Table 1 and Fig. 1). The iodide content of the 24-h urine after 7 and 14 days in the cold was increased about 2-fold (Table 1) ($P < 0.001$), and the food intake was significantly increased after 3 days ($P < 0.01$), 7 days ($P < 0.005$), and 14 days ($P < 0.001$), i.e., from 20 ± 3 to 24 ± 1, 25 ± 5 and 34 ± 2 g/day, respectively ($n = 5, 6, 6$, and 5, respectively).

The percentage of haemoglobin did not alter significantly after 3 and 7 days in the cold compared to the value before the exposure (before: 12.2 ± 0.5 g/100 ml (6); 3 days: 12.2 ± 1.4 g/100 ml (6); 7 days: 12.7 ± 0.5 g/100 ml (4)).

**Experiment B**

The administration of noradrenalin to rats at room temperature via an iv infusion made it possible to determine whether the elevated noradrenalin levels observed during exposure to cold play a role in the mechanism underlying the increased $T_4$ metabolism in the cold.

The influence of the administered NA could be observed clearly: both heart rate and respiration increased. Food consumption decreased initially, but on the last day of the infusion the 6 rats were eating normally (Table 2). Water intake and urine volume increased markedly compared to controls: after 7 days of infusion the rats consumed 45 ± 5 ml water per day and the controls 16 ± 6 ml ($P < 0.001$); the volume of the urine was 52 ± 7 vs. 23 ± 3 ml/day for the controls ($P < 0.001$).

The $T_3/T_4$ ratio in venous plasma was higher in the NA animals than that in the controls, but the difference only became significant after 7 days ($P < 0.01$). The $T_3/T_4$ ratio of the controls did not alter significantly (Fig. 2). The $T_3$ concentration before infusion and after 4 and 7 days of infusion did not alter significantly, as was the case for the controls. The $T_3$ levels of NA rats did not differ significantly from those of the controls on the three sampling days (Fig. 2). The $T_4$ concentration after 4 and 7 days infusion was considerably lower than that of the controls ($P < 0.05$). The $T_4$ concentration of the controls did not alter significantly during infusion (Fig. 2).

The iodide content of the 24-h urine of NA-infused animals was significantly higher than that of the pair-fed controls after 7 days of infusion.
Table 2.
Iodide content of 24-h urine and food intake during NA infusion.

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA I (µg/24 h)</td>
<td>2.62 ± 0.51</td>
<td>2.19 ± 0.73</td>
<td>3.95 ± 0.49*</td>
</tr>
<tr>
<td>Controls I (µg/24 h)</td>
<td>2.52 ± 0.94</td>
<td>2.09 ± 0.67</td>
<td>3.24 ± 0.35**</td>
</tr>
<tr>
<td>Food intake (g/24 h) of NA rats</td>
<td>11 ± 3</td>
<td>6 ± 2</td>
<td>16 ± 2</td>
</tr>
</tbody>
</table>

NA was infused into 6 rats via the jugular vein for 7 days at room temperature at a dose of 0.059–0.59 nmol NA/min/g0.74 body weight and at a rate of 24 ml/day (for further details, see legend to Fig. 2). The controls were infused with the same solution but without NA, and were pair-fed. The iodide content of the 24-h urine was determined before infusion (day 0) and after 5 and 7 days. Values are means ± SD (n = 6).

* P < 0.01 vs. controls, P < 0.001 vs. day 0, P < 0.005 vs. day 5.
** NS vs. day 0, P < 0.01 vs. day 5.

(P < 0.01, Table 2), and was increased significantly compared to day 0 and day 5 (P < 0.001 and P < 0.005, respectively). The iodide content of the 24-h urine of the controls was not significantly different from that on day 0, and significantly higher than that on day 5 (P < 0.01).

Discussion

Meaningful resting values of catecholamines cannot be obtained in situations of stress due to anaesthetics, handling, or surgery (Bühler et al. 1978; Depocas & Behrens 1977). To solve this...
problem for a study in rats, a method was applied which utilizes a chronically inserted jugular or carotid catheter from which sequential blood samples can be collected without immobilizing or disturbing the animal.

It was shown that basal catecholamine levels were reached 4 h after the operation. Our arterial values lie in the same range as the arterial resting values found by other investigators (Depocas & Behrens 1977; Kvetansky et al. 1978). From our introductory experiments it can be concluded that in a system with chronically implanted catheters it is advisable to wait 3 days before starting this type of experiments on thyroid hormone metabolism, because shortly after the operation stress factors give rise to large individual differences in thyroid hormone concentration and after 12 h the effects of lowered food intake become observable.

Increased NA levels in plasma during short exposure to cold (2 h) were found by Depocas & Behrens (1978), who used a radio-enzymatic method based on methylation of NA by phenyl-ethanolamine N-methyltransferase. Our results confirm and extend their data and show a considerable NA increase of 6-, 8-, and 12-fold in venous plasma after 3, 7, and 14 days of exposure to cold, respectively. In view of the fact that the adrenal concentration remains low, it may be concluded that the stimulus exerted by cold does not evoke an ordinary stress response but rather that the sympathetic nervous system is selectively stimulated. Since only a small fraction of the released NA escapes into the plasma, huge increases of the tissue levels can be expected. The T3/T4 ratio in venous plasma was increased in the cold, as was also reported by Bernal & Escobar del Rey (1975a,b) and by Albright et al. (1965) for plasma and several kinds of tissue homogenate (in these two studies isotopic equilibration methods were used). There is much evidence that the increased T3/T4 ratio is attributable, at least partially, to increased extrathyroidal conversion of T4 to T3 (Bernal & Escobar del Rey 1975a,b). The iodide level in the 24-h urine was increased after 7 and 14 days at 4°C, which was found by Albright et al. (1965) after 15 days and by Bernal & Escobar del Rey (1975a) during the first 4 days of exposure to cold.

Although food intake is an important determinative factor for the amount of iodide excreted into the urine, as was also shown in a quantitative way by determining the relationship between these two parameters (see Methods), we found in our study that the fractional increase of iodide excreted into the urine is larger than the fractional increase of food consumption after 7 and 14 days in the cold. When we actually used the formula by filling in the values of the food consumption, we found that after 7 days of exposure to cold the calculated mean iodide value was significantly lower than the measured value (P < 0.02). After 14 days no significant difference was observed. However, one should take into account that these figures were calculated by extrapolating from the values measured at room temperature.

Galton & Nisula (1969) found that the thyroidal uptake of iodide was increased up to 3-fold in the cold-adapted rat. Since total glandular iodide was unchanged, they presumed the thyroid secretion rate to be similarly increased. Therefore we assumed that the amount of iodide in the urine was not influenced by altered thyroidal uptake or secretion. Bernal & Escobar del Rey (1975b) reported that the increased excretion of iodide into the urine cannot be explained solely by the increased consumption of food, and must be attributed at least partially to an increased extrathyroidal deiodination of T4 and T3.

The finding of simultaneously increased NA levels and an elevated T3/T4 ratio in plasma under cold conditions, combined with our earlier observation of the absence of a T3 increase during a week at 4°C after sympathectomy (van Hardeveld et al. 1979b), points to a role of NA in the process of increased T3 generation. Our experiments with NA infusions at 23°C have provided further evidence for an effect of the sympathetic nervous system on T3 metabolism. The increased T3/T4 ratio in venous plasma and iodide excretion in the 24-h urine found in our experiments with NA-infused animals are consonant with the observation of Galton (1965), who showed that in mice treatment with adrenalin or noradrenalin in vivo can increase the deiodinating activity of liver homogenates, which was decreased by reserpine, and the finding of Hillier (1968), who showed that noradrenalin injections raise the urinary radio-iodide excretion of thyroidectomized rats given daily injections of radioactive thyroxine. The possibility that exposure to cold and noradrenalin treatment affected the renal clearance of iodide is unlikely, as was shown by Hillier (1968) and Galton (1965), respectively. The amount of NA we infused was very high. However, as pointed out by Cryer (1976),
there is a steep NA gradient from the synaptic cleft at the sympathetic nerve endings to plasma, as shown by the fact that the largest part of the excreted NA is taken up again by the axon terminal and is metabolized locally. Hillier (1968) also emphasized the fact that large doses are necessary to elicit responses. It may therefore be expected that if NA, introduced directly into the circulation, is to produce increased tissue concentrations, very high plasma NA concentrations are required. Although their food consumption was decreased during the first half of the infusion period, our rats showed normal food consumption at the end of the experimental period, i.e., the time at which significant NA effects were found.

Acknowledgments

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References


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