Studies on the Effect of Neonatal Hypermetabolism on Hypothalamo-Pituitary-Thyroid Axis in Adult Rats

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Synopsis

Neonatal rats which had received a daily injection of 50 μg of 2,4-dinitrophenol (DNP) or 30 μg of L-thyroxine (T4) for 7 days beginning on the day of birth were compared as to the late effect of the hypothalamo-pituitary-thyroid axis with the neonatal saline control. Neo DNP rats and neo T4 rats revealed the retardation of growth compared with neo saline rats. The plasma level of TSH in both groups presented its low response following TRH administration. Furthermore, plasma TSH levels following the challenge of PTU were depressed in both neo DNP and neo T4 rats compared with neo saline control rats. A small dose of T4 injection, however, did not bring any difference on plasma TSH levels between neo T4 and neo saline control rats while neo DNP rats showed a little blunted response of pituitary compared with neo T4 and neo saline rats. Pituitary contents of TSH in neo T4 rats decreased, but not in neo DNP rats. These results suggest that neonatal hypermetabolism causes the hypofunction of pituitary-thyroid axis through adult life and that the alteration of hypothalamus may be more obvious in neo T4 rats than in neo DNP rats.

Recently it has been reported that the administration of a large dosis of sodium L-thyroxine (T4) to the neonatal rats produces the persistent alteration in hypothalamic centers regulating pituitary TSH secretion on adult rats (Bakke and Lawrence, 1966; Bakke et al., 1974; Kieffer et al., 1976). The neonatal animals treated with T4 exhibited retarded growth and produced a variety of endocrine abnormalities through the course of adult life (Gellert et al., 1971; Bakke et al., 1975; Kieffer et al., 1976). Thus this group of abnormalities has been called neo thyroxine syndrome. These neo thyroxine syndrome rats, however, were injected with 15-30 μg of T4 daily during the first 7 days of life (Bakke and Lawrence, 1966; Gellert et al., 1971; Bakke et al., 1974 and 1975; Kieffer et al., 1976). It is clear that such a large dosis of T4 administration produces severe hypermetabolic situations on neonatal animals as indicated by the increase of oxygen consumption, an elevation of body temperature, an increased basal metabolic rate and so much caloric consumption.

It has been recognized that the severe hyperthyroidism in neonatal animals produces persistent abnormalities in adult life so far. Although it is generally accepted that various metabolic disorders in the neonatal period modify the endocrine and central nervous system, disagreement exists whether these kinds of abnormalities are specific for neo T4 syndrome or not (Meites and Fiel, 1965; Winick and Noble, 1966;
Stephan et al., 1971; Muzzo and Brasel, 1973; Shambaugh and Wilber, 1974).

2, 4-Dinitrophenol (DNP) has been recognized as an agent of inducing hypermetabolism in various animals by virtue of its uncoupling effect on oxidative phosphorylation with the Krebs tricarboxylic acid cycle (Witter et al., 1953). It is also well known that administration of DNP decreases plasma PBI levels and produces T₄ displacement from serum protein (Witter et al., 1953; Wolff et al., 1961). DNP was employed in the present study to produce hypermetabolism on neonatal rats (neo DNP) with L-thyroxine treated neonatal rats (neo T₄) and both groups to saline treated neonatal rats (neo saline) to see the effect of neonatal hypermetabolism on the hypothalamo-pituitary-thyroid axis in the adult stage.

Materials and Methods

Sprague-Dawley pregnant rats, weighing 300-400 g, were used in the present study. After several days of breeding, 94 pups were obtained from mother rats on the same day. Just after delivery, all pups were separated from mothers. Pups were divided into three groups: 1) a saline injected group as the control 2) a T₄ injected group 3) a DNP injected group. These pups were maintained on mother’s milk and on purina laboratory chow and tap water ad libitum. They were kept under the conditions of controlled temperature of 24°C and controlled lighting of 12 hr of light and 12 hr of dark.

From the first day of birth, pups were injected daily with saline, T₄ and DNP subcutaneously for 7 days. Recrystallized dinitrophenol (DNP) and sodium L-thyroxine were prepared in solutions of alkaline saline as adjusted pH 7.2. Thyroxine treated pups were injected with 30 µg of L-T₄, which was a similar dose in the previous report (Bakke et al., 1975), per each animal per day. DNP rats were treated with 50 µg of DNP, this dose was considered to produce a hypermetabolism on rats, since about two times the human dose to elevate the basal metabolic rate (BMR) on human (Castor et al., 1956), per animal per day. As a preliminary experiment, three doses of DNP (100, 50, 10 µg) were injected into neonatal rats but unfortunately all the 100 µg of DNP injected group was not different from the neo saline group. Then 50 µg dose of DNP was used in the present study.

The total amount of injected T₄ and DNP for each animal was 210 µg and 350 µg respectively and injection was given in the morning and in the evening. Control animals were injected on the same schedule with alkaline saline as vehicle. Pups were weighed periodically. Following puberty, at 76 days of age, synthetic TRH (200 ng/100 g B.W.) was administered intravenously under light ether anesthesia and blood samples were obtained with venipuncture with skin incision. In the following experiment, at 112 days of age, all rats were fed with 0.15% propylthiouracil (PTU) for two weeks to examine the feedback mechanism and also PTU treated rats were injected, with exogenous T₄ (1.0 µg/100 g B.W.) to see its negative feedback mechanism. All blood samples were collected in heparinized syringes and isolated plasma was kept frozen until analysis of TSH, T₄ and T₃ by radioimmunoassay. Rat TSH RIA kit was kindly supplied from the NIAMDD-Rat pituitary hormone program. All of the samples from each experiment were measured in the same assay system. Coefficients of variation of intra and inter assay were less 12% for all assays. Statistical evaluation of the data was made with the one-way analysis of variance, Newman-Keuls multiple comparison, Student’s t-test.

Results

Changes of body weight in neo DNP, neo T₄ and neo saline rats

Both of neo DNP and neo T₄ rats showed an obvious retardation of growth in the initial term compared with the neo saline control group. Differences of body weight in each group are presented in Fig. 1. After 12 weeks of age, male neo DNP rats gained weight similar as neo saline rats did. Both of male and female neo T₄ rats remained in a retardation of growth after 12 weeks of age and also female rats in neo DNP group still revealed a low
growth curve but not so severely as neo T₄ rats.

Changes of endocrine organ weights and basal plasma TSH, T₄ and T₃ concentration

The neo T₄ rats remained significantly smaller in size after 161 days of birth and their pituitary or thyroid was less in weight than neo saline control rats while neo DNP rats showed no differences compared with neo saline rats (Table 1). The basal level of plasma TSH in neo T₄ male rats revealed considerably lower values than neo saline control rats and neo DNP rats (p<0.05). As for the basal level of plasma T₃ and T₄, there were no differences between three groups.

Changes in plasma TSH concentration following exogenous TRH administration

In order to test the pituitary responsiveness, intravenous administration of TRH (200 ng/100 g B.W.) was performed in each group. The blunted response of plasma TSH after TRH administration was observed in both sexes of neo T₄ rats and neo DNP rats. The maximal response of plasma TSH in male neo T₄ and neo DNP rats was 48.2% and 59.1% of that of neo saline control rats, respectively and in female neo T₄ and neo DNP rats, the maximal response of plasma TSH was 55.0% and 64.4% of that of the neo saline control, respectively. Basal levels of plasma TSH in neo DNP, neo T₄ and in neo saline rats were 104±14, 43±6 and 168±35 μu/ml in male, respectively and 62±14, 37±5 and 79±11 μu/ml in female, respectively. Basal levels of plasma TSH in both sexes of neo T₄ rats showed significant difference compared with those in neo saline control rats (male: p<0.02, female: p<0.01). The results were presented in Fig. 2.
Changes in plasma TSH concentration following PTU treatment

The feedback mechanism in hypothalamo-pituitary-thyroid axis was evaluated on these neo T₄ and neo DNP rats by comparing the values with those of neo saline control rats. When PTU was administered to neo T₄, neo DNP and neo saline rats for two weeks, plasma concentration of TSH was obviously changed as shown in Fig. 3. After thyroid hormone synthesis was blocked with PTU treatment, plasma concentration of TSH considerably increased in neo saline rats. In contrast, impaired elevation of plasma TSH was noted in both groups of neo T₄ and neo DNP rats. Although the blunted response of plasma TSH after PTU treatment was more prominent in neo T₄ rats than in neo DNP rats, the difference between two groups was not significant. The values were revealed as TSH (increment of plasma TSH).

Effect of T₄ i.v. injection on plasma TSH concentration following PTU treatment

As shown in Table 2, there were no significant differences in suppression of plasma TSH concentration by i.v. injection of a small dose of T₄ between neo saline rats and both of neo T₄ and neo DNP rats. Male neo DNP rats however, showed a significant difference in suppression of plasma TSH concentration against neo T₄ rats (p < 0.05 at 2, 6 hr) but not in female rats. Apparently the initial concentrations of plasma TSH in neo T₄ rats were significantly reduced following PTU treatment compared with neo saline rats (male: p < 0.05, female: p < 0.02) but not in neo DNP rats.

Comparisons of pituitary TSH contents in neo DNP, neo T₄ and neo saline rats

Fig. 4 shows the pituitary contents of TSH in neo DNP, neo T₄ and neo saline rats. In both sexes of neo T₄ rats it was
Fig. 2. Plasma TSH responses after synthetic TRH (200 ng/100 g B.W.) i.v injection into neo saline, neo DNP and neo T4 rats. Left panel shows male rats and right panel shows female rats. All values are mean±S.E.M. The number in parentheses represents the number of animals in each group. Statistical differences (vs. neo saline) are show by asterisks.

*: p<0.05, **p<0.02, ***p<0.01

Fig. 3. Comparisons of plasma TSH following PTU challenge for two weeks on neo DNP, neo T4 and neo saline rats. Left panel shows male rats and right panel shows female rats. The number in parentheses represents the number of animals in each group. Mean±S.E.M. are shown. Statistical differences (vs. neo saline group) are shown by asterisks.

*: p<0.05, **p<0.02, ***p<0.01
Table 2. Changes in plasma TSH concentrations after the i.v. injection of a small dose of T4 (1.0 µg/100 g B.W.) into PTU treated neo saline, neo T4 and neo DNP rats.

<table>
<thead>
<tr>
<th>Group rat</th>
<th>Sex</th>
<th>N</th>
<th>Initial TSH value (µU/ML)</th>
<th>0 h</th>
<th>Percent of initial value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neo saline</td>
<td>M</td>
<td>9</td>
<td>593±44</td>
<td>100</td>
<td>22.0±2.7</td>
</tr>
<tr>
<td>Neo DNP</td>
<td>M</td>
<td>7</td>
<td>510±42</td>
<td>100</td>
<td>31.8±3.5*</td>
</tr>
<tr>
<td>Neo T4</td>
<td>M</td>
<td>6</td>
<td>406±52</td>
<td>100</td>
<td>21.3±2.2</td>
</tr>
<tr>
<td>Neo saline</td>
<td>F</td>
<td>6</td>
<td>996±127</td>
<td>100</td>
<td>22.3±3.4</td>
</tr>
<tr>
<td>Neo DNP</td>
<td>F</td>
<td>5</td>
<td>645±103</td>
<td>100</td>
<td>29.2±6.0</td>
</tr>
<tr>
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<td>F</td>
<td>6</td>
<td>687±84</td>
<td>100</td>
<td>27.6±3.7</td>
</tr>
</tbody>
</table>

The values are given as mean±S.E.M. Statistical differences (vs. neo saline group—α and vs. neo T4 group—β) are shown by asterisks. *p<0.05, **p<0.02

Fig. 4. Comparisons of pituitary TSH contents between neo saline, neo DNP and neo T4 rats. Left panel shows male rats and right panel shows female rats. The number in parentheses refers to the number of animals in each group. All values are mean±S.E.M., Statistical differences (vs. neo saline) are shown by asterisks. *p<0.05, **p<0.02, ***p<0.01

observed that pituitary contents of TSH were significantly decreased compared with those in saline neo rats (male: 192±32 vs. 300±25 µu/pit., p<0.05 female: 204±29 vs. 389±68 µu/pit., p<0.05). On the other hand, female and male rats in the DNP group did not show a significant difference compared with neo saline rats. It is obvious that pituitary contents of TSH were decreased in male neo T4 rats compared with those in male neo DNP rats (192±32 vs. 316±38 µu/pit., p<0.05) but the difference was not observed in female rats.

Discussion

Following the first observation by Eayrs and Holmes (1964) that neonatal rats injected with T3 showed permanent impairment of pituitary-thyroidal system. Bakke and Laurence (1966) postulated that the administration of large doses of sodium L-thyroxine (T4) to the neonatal rats resulted in late and persistent impairment of the pituitary-thyroidal system and body growth. Administration of large doses of
T₄ in their experiment, however, could produce metabolic disturbance as well as hypermetabolic situation on neonatal animals since hypermetabolism caused relative caloric deprivation during the neonatal period. Such caloric deprivation in the neonatal stage or undernutrition in the early stage were reported to result in a pituitary-thyroidal dysfunction (Shambaugh and Wilber, 1974). As indicated in the work of Shambaugh and Wilber (1974), the starvation in the early neonatal stage resulted of hypothyroidism, presumably, mediated by a deficiency of hypothalamic TRH. Thus caloric deprivation could be a cause of the dysfunction in the regulation mechanism of TSH secretion through hypothalamus as well as pituitary-thyroid axis. These observations suggest that such metabolic disorders could produce similar abnormalities in neo T₄ syndrome. As previously reported, administration of androgens to neonatal female rats revealed persistent alteration in hypothalamic regulatory centers of pituitary gonadotropine secretion (Barraclough, 1961; Harris, 1964). Exogenous T₄ administration might be produced specific dysfunction of thyrotrope regulating center in the hypothalamus as observed in neo androgen treated female rats. However, studies on hypothalamic TRH content have led to contradictory results in neo T₄ rats. In some reports, there is evidence that neo T₄ rats have shown elevated hypothalamic contents of TRH and the others, the investigators showed the contradict result (Bakke et al., 1975; Azizi et al., 1972). Their reports agreed with the view that neo T₄ rats have a dysfunction of hypothalamic TSH regulating center.

As previously described, DNP has been well known to elevate free T₄ concentration by its replacement effect on thyroxine binding protein (TBP) (Witter et al., 1953; Wolff et al., 1961). Unfortunately, concentration of free T₄ was not determined in our present study, and it could be speculated that plasma T₄ levels in neo T₄ rats are much higher than in neo DNP rat. Since the amount of TBP and plasma T₄, T₃ in the neonatal stage is so small (Dussault, 1975), the amount of T₄ from TBP by DNP in neo DNP rats is extremely low compared with the free T₄ level induced by exogenous T₄ administration in neo T₄ rats. In the previous paper (Wolff and Austen, 1958), it was pointed out that thyroxine (T₄) and 2, 4-dinitrophenol (DNP) evoked many similar responses in man or in the experimental animal. The similarities indicate that both agents increase the metabolic rate, deplete the glycogen of the liver and uncouple oxidative phosphorylation. The principal differences of DNP from T₄ were a failure to induce metamorphosis in the tad pole and inability to act as therapeutic agent in myxedema (Wolff and Austen, 1958). Moreover it has been noted that DNP interferes with mitochondrial generation of high energy and intermediates and stimulates mitochondrial adenosine triphosphatase activity (Tapley et al., 1967).

Since pituitary TSH secretion induced by TRH administration was blocked by DNP without the presence of plasma in the in vitro experiment (Wilber and Utiger, 1968), DNP probably caused direct effect on intracellular metabolism. It is particularly of considerable importance to note that DNP is not a hormone like T₄ or T₃. Our present study has revealed that hypermetabolism induced by DNP in neonatal rats produced a similar phenomenon as neo T₄ syndrome. Neo T₄ syndrome, however, demonstrated a more prominent impairment of hypothalamo-pituitary-thyroid axis than DNP treated hypermetabolic animals. Although the reason for the difference between neo DNP and neo T₄ rats is hard to explain, a variety of mechanisms will be proposed to explain this difference between two models, including the difference of hypothalamic TRH content or the difference of pituitary TSH synthesis and secretion.
rate. Further studies will be required to clarify this question.

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References