Acta Physiologica Hungarica, Volume 76 (2), pp. 137–141 (1990)

INFLUENCE OF EXPERIMENTALLY INDUCED ENDOTOXEMIAS ON THE THYROID FUNCTION OF RATS

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Received November 15, 1989
Accepted November 21, 1989

The general membrane-damaging effect of endotoxins (LPS) may be also demonstrated on the follicular cells of thyroid gland. Serum T_4 level significantly decreased and the response of thyroid gland to exogenous THS was markedly inhibited in experimental endotoxin and other so-called enteroendotoxemic shocks (e.g. intestinal ischemia, tourniquet shock, intestinal syndrome of radiation disease). A single subtoxic dose of LPS given to newborn rats decreased the T_4 level, the response of thyroid to TSH in adulthood and caused a somatic retardation. The radio-detoxified endotoxin (TOLERIN®) did not inhibit the thyroid response to TSH. TOLERIN® pretreatment protected the rats against LPS and other enteroendotoxemic shocks.

Keywords: endotoxin (LPS); radiodetoxified endotoxin (TOLERIN®); TSH; T_4 (thyroxin); thyroid gland; endotoxemia; intestinal syndrome of radiation disease

It is well-known that bacterial endotoxins have a membrane damaging effect [2, 7]. Because the TSH receptor is a membrane receptor we suppose that this effect of LPS is important in the mechanism of the decrease of T_4 level. Low serum T_3 and T_4 levels are common following infections and LPS induced fever of rabbits [8, 22].

It seems probable that in infectious diseases early suppression of TSH-release and subsequent decrease of T_4 secretion occurs [21]. At the same time it is well-known that experimental LPS shock diminishes plasma TSH level [13]. All these data indicate that the membrane-damaging effect of LPS, entering the circulation [2], may play a role in the change of the function of the thyroid gland. It seemed worthy to investigate how the thyroid responses to TSH were altered in *adult* rats during LPS shock and whether the administration of LPS in neonatal period of life might also perturb the follicular cell

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Presented at the Membrane Transport Symposium held in Sümeg, May 10–13, 1989, Hungary.

membranes influencing the development of animals, decreasing the T_4 level and how it moderated the response to exogenous TSH in adulthood.

The pathogenesis of certain experimental shock conditions may involve absorption of bacterial LPS [2, 14, 19]. It has been investigated how the basal T_4 serum level changed concerning the response of thyroid gland to exogenous TSH treatment. For these experiments two models were selected: the *superior mesenteric artery* occlusion (SMAO) and the *tourniquet induced limb ischemia.*

It has also been recognized that whole body irradiation produced short term changes in the function of the thyroid gland which were not caused by the direct effect of the radiation on the gland [1, 12].

On the other hand, it was demonstrated in our earlier experiments using lead acetate induced endotoxin hypersensitivity [20] that the high dose whole-body irradiation induced intestinal syndrome was an enteroendotoxemia [4]. This result was confirmed by other authors [9]. An improtant role of bacterial flora of the intestinal tract in the pathogenesis of intestinal syndrome is also supported by other experiments, in germfree mice [15]. Recently some authors suggested that sepsis and endotoxemia did not play a significant role in the intestinal radiation syndrome [11]. These data indicate the necessity of studying the effect of high dose irradiation on serum T_4 level and on the response of the thyroid gland to exogenous TSH. Finally we wanted to investigate the effect of radio-detoxified LPS (TOLERIN) and endotoxin tolerance on the thyroid function of rats.

Material and methods

Rats: Adult and newborn male and female $RLEF_1$ hybrid (LATI) and CFY/LATI outbreed rats were used. Animals were housed in type II plastic cages using soft wood shavings and commercial chow (LATI, Gödöllő, Hungary), having access to food and tap water ad libitum.

LPS: Escherichia coli 089 LPS was extracted by the phenol-water method [23].

The LD_{50} value of this LPS was 1.96 mg/kg. Radiodetoxified-LPS was prepared from the above-mentioned LPS by ionizing radiation (^{60}Co-gamma, 150 kGy, in Noratom Gamma 350 C) [3, 10]. The LD_{50} value of this preparation (TOLERIN®: Institute Human for Serobacteriological Production and Research, Budapest, Hungary) was 21.25 mg/kg [5].

Treatments: LPS and TOLERIN were injected intravenously or intraperitoneally at a dose of 1.0 mg/rat (adults) or 1 μg/rat (newborns). TSH (AMBINON, Organon OSS, The Netherlands) 3 I. U. was given intraperitoneally 3 h after the injection of LPS or TOLERIN [5].

T_4 level determination: One hour after the injection of TSH, rats were exsanguinated and dissected. Serum was prepared from their blood and kept at −20 °C till analysis. T_4 determinations were performed using ^{125}I-T_4 radioimmuno-assay (RIA kit, Institute of Isotope of the Hungarian Academy of Sciences, Budapest, Hungary) [6, 17, 18].

Irradiation: Rats were irradiated with THX-280 type X-ray apparatus (200 kV, 20 mA, 0.5 mm Cu filter, 60 cm body-middle-distance). $LD_{100/30}$ whole body irradiation dose (8 Gy) was used. The animals were subdivided to 4 groups.

First group: unirradiated control, 2. group: irradiated control, 3. group: irradiated body without thyroid gland (shielded), 4. group: irradiated thyroid gland without body (shielded) [6].

Operations: For the production of experimental intestinal ischemia the occlusion (ligature) of the superior (cranial) mesenteric artery (SMAO) was performed in nembutal narcosis and kept for 150 min. In case of sham-operation after laparatomy, the SMAO was identified but no ligature was made. The ischemia ("tourniquet shock") was induced by strangulation of the hind limb with a rubber ring (3 cm in diameter) under nembutal anaesthesia [19].

Results and discussion

The results of experiments are summarized in the Fig. 1. In normal adult rats the TSH significantly increased the serum level of T_4. However, in animals treated with LPS in neonatal age the basal level of T_4 was decreased

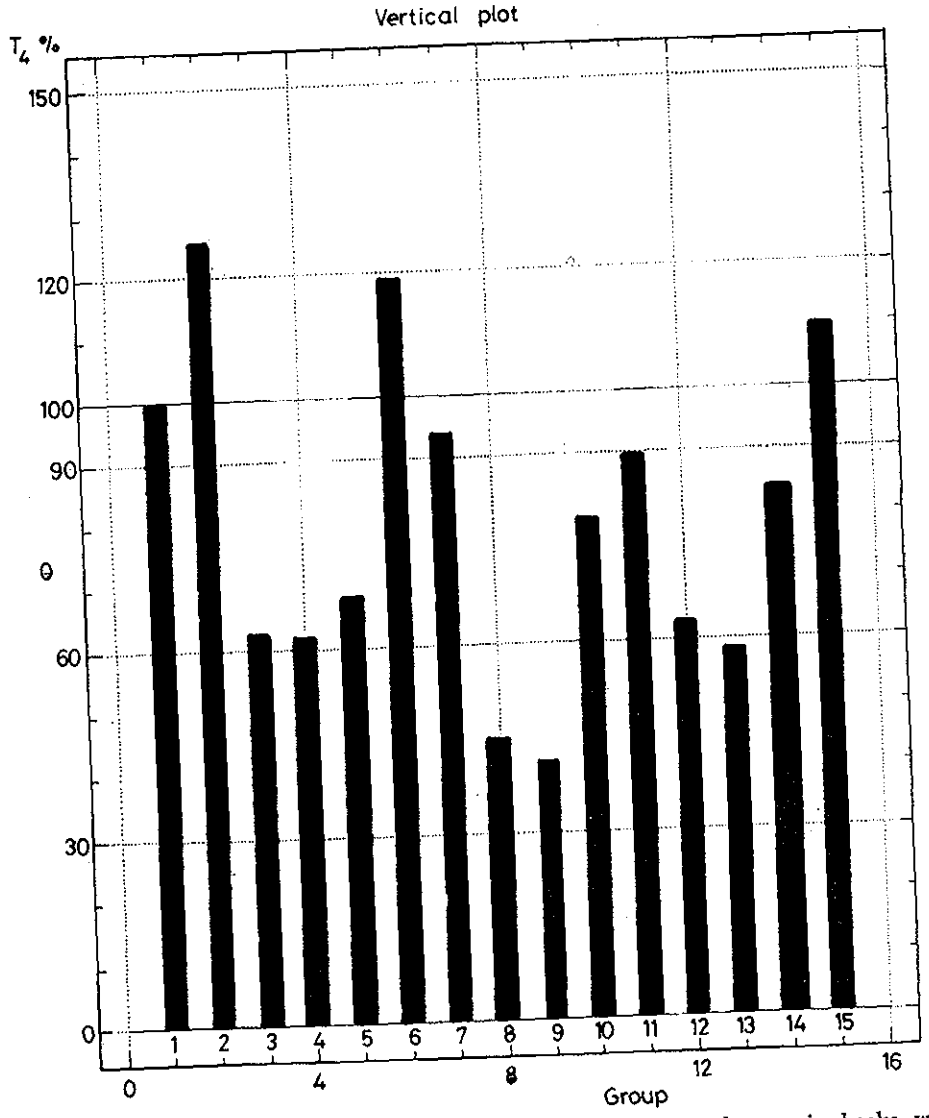

Fig. 1. Effect of LPS treatment (in neonatal or adult age), enteroendotoxemic shocks, whole body irradiation on thyroid function of rats. Explanation: 1. Control; 2. THS; 3. LPS; 4. LPS + TSH; 5. RD-LPS; 6. RD-LPS + TSH; 7. Sham-"SMAO"; 8. SMAO; 9. SMAO + TSH; 10. Tourniquet; 11. Tourniquet + TSH; 12. Whole body irradiation; 13. Whole body irradiation + TSH; 14. LPS in neonatal age; 15. LPS in neonatal age + TSH in adulthood

while the response to TSH in adulthood increased, the value did not reach the control level [18]. Thus one might conclude that endotoxin treatment in neonatal age had damaged the thyroid follicular membrane (including TSH receptors during the period of their maturation) which might persist and could be demonstrated also in adulthood. The development of animals treated with LPS in neonatal age were retarded. It is well-known that during the first part of the ontogenesis the thyroid hormones have a morphogenetic effects and play important role in the normal development.

During the LPS shock in adult rats the plasma T_4 level decreased markedly and was not compensated by administration of exogenous TSH [17]. However, the radiodetoxified LPS (TOLERIN®) treatment did not inhibit the response to exogenous TSH and decreased serum T_4 level to a lesser extent than unirradiated LPS. Our results indicated that radio-detoxified LPS lost its membrane-perturbing effect. It is attractive to consider that radio-detoxified LPS might be similar to the parent (toxic) LPS in some respects. However, in contrast to parent LPS, the radio-detoxified LPS is unable to initiate the processes leading to irreversible membrane damage. Concerning the general membrane damaging effect of LPS and its interactions with other hormone receptors we supposed that the membranes of follicular cells changed (or the number of TSH receptors decreased, too).

In the SMAO induced shock (intestinal ischemia) the T_4 level significantly decreased. TSH treatment did not influence the serum T_4 level compared to the controls. In the tourniquet shock the changes of T_4 level were similar but more moderate. Due to ischemia biogen amines are being formed in large amount in the tissues after the release of the ligature and they enter the circulation producing an enteroendotoxemic shock, too.

The irradiated animals on day 7 of the irradiation showed even more severe clinical symptoms of radiation disease. The high dose whole-body irradiation significantly decreased the function of rat thyroid gland and even inhibited the T_4 response to exogenous TSH. We suppose that this effect is induced by the LPS which is absorbed from the intestinal tract.

TOLERIN® pretreatment can provide protection against the shock induced by LPS challenge and does not disturbe the T_4 level and thyroid response to the TSH treatment.

These observations call the attention to the fact that differently induced experimental shock models (e.g. endotoxin shock, intestinal ischemic or tourniquet shock, so-called intestinal syndrome of the acute radiation disease) can produce similar changes in the function of the thyroid gland. Our results apart from the interest in hormone reception, may contribute to the clarification of the radiation sensitivity of the thyroid gland and also to a better understanding of the pathogenesis of radiation disease.

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