EFFECT OF THE ANTIOXIDANT DIBUNOL ON ADRENOCORTICAL, THYROID, AND ADENOHYPOPYSEAL FUNCTION IN ADULT AND OLD RATS

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Dibunol (4-methyl-2,6-di-tert-butylphenol; D), an antioxidant of the phenolic group, with a varied degree of stereochemical screening of the hydroxyl group [8], has become widely used in experimental and clinical practices [1-5]. Usually all the effects of D are reduced to its influence on lipid peroxidation (LPO) in the tissues. Following research conducted by our team into the role of neuroendocrine changes in the mechanisms of aging [6,7], the writer studied the effect of D on function of the adrenal cortex, thyroid gland, and adenhypophysis, which produces trophic hormones for the other two glands.

EXPERIMENTAL METHOD

Experiments were carried out on adult (4-6 months) and old (24-26 months) male Wistar rats. D, synthesized at the Institute of Chemical Physics, Academy of Sciences of the USSR, was injected intraperitoneally in a single dose of 100 mg/kg body weight. At different times after injection of D (3, 6, 9, 12, 24, and 48 h) concentrations of corticosterone (CS), triiodothyronine (T_3), ACTH, and thyrotrophin (TSH) in the blood plasma and the CS concentration in tissue of the adenohypophysis were determined. The dose of D and times of assay of the hormones were chosen on the grounds of data relating to the dynamics of action of D on the antioxidative activity of the tissues [2]. T_3, ACTH, and TSH were determined by radioimmunoassay: T_3 was determined by RIA-Gnost-T_3 kits (West Germany) and expressed in nanomoles/liter; ACTH and TSH were determined by ACTHK and TSHK-PR kits from CEA-Sorin (France) and expressed in nanograms/liter and microunits (μU)/m respectively. The CS concentration in the plasma and adenohypophyseal tissue was determined by a fluorometric method [9] and expressed in nanomoles/liter of blood plasma or nanomoles/gram wet weight of adenohypophyseal tissue.

EXPERIMENTAL RESULTS

In the control no significant age differences were found in the CS concentration either in blood plasma or in adenohypophyseal tissue. Injection of D caused biphasic changes in the CS concentration in both tissues studied in adult and old animals (Table 1).

It will be clear from Table 1 that during the first few hours after injection of D marked activation of steroid production was observed: the CS concentration in the blood plasma and adrenal gland rose sharply. After 24 h, an abrupt fall of the CS concentration below the initial level was observed, followed by recovery to the initial level after 48 h. The plasma ACTH concentration in intact old rats was significantly higher than in adult animals. Changes in the plasma ACTH concentration in rats of the two age groups under the influence of D were biphasic in character, similar to the biphasic changes in CS concentration in the blood plasma adenohypophyseal tissue. Marked activation of the corticotrophic function of the adenohypophysis was observed during the first hours of action of D, with a fall in the ACTH concentration in the blood plasma after 24 h (statistically significant in adult animals) and subsequent return to the initial values 48 h after injection of D.

Comparison of the time course of steroid production and the character of changes in adenohypophyseal corticotrophic function under the influence of a single injection of D demonstrates the undoubted connection between these processes: changes in CS concentration are clearly followed by changes in adrenocorticotrophic function of the adenohypophysis. The parallel between changes in the plasma ACTH concentration and the CS concentration in the plasms and adrenocortical tissue suggests that the primary point of application for the action of antioxidant D in the pituitary—adrenocortical system is its effect on the adrenocorticotrophic

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TABLE 1. CS Concentration (A) in Blood Plasma and Adenohypohyseal Tissue and ACTH Concentration (B) in Plasma of Adult and Old Male Rats at Different Times after Single Intraperitoneal Injection of Dibunol in a Dose of 100 mg/kg (M±m)

Hormone	Group of rats	Tissue	Control	Time after injection of dibunol, h					
				3	6	9	12	24	48
A. CS	Adult	Blood	661±31	1096±70	1436±209	1107±138	898±49	117±34	693±146
	P			<0,001*	<0,01*	<0,01*	<0,001*	<0,001*	>0,05*
		Adenohypophysis	66,4±5,7	89,6±15,5	147,6±10,0	109,5±17,9	86,7±12,3	20,1±5,0	55,1±6,5
	P			>0,05*	<0,001*	0,05*	>0,05*	<0,001*	>0,05*
	Old	Blood	551±59	876±116	1040±142	865±42	857±61	202±35	558±53
	P		>0,05**	<0,05*	<0,02*	<0,01*	<0,01*	<0,001*	>0,05*
		Adenohypophysis	60,7±4,1	88,9±20,3	132,5±7,8	74,2±12,4	69,0±4,7	37,6±5,8	64,6±10,0
	P		>0,05**	>0,05*	<0,001*	>0,05*	>0,05*	<0,02*	>0,05*
B. ACTH	Adult	Blood	79,1±10,9	233,7±29,4	397,2±43,5	372,2±98,7	380,0±92,3	23,2±10,8	45,5±18,7
	P			<0,01*	<0,001*	<0,05*	<0,01*	<0,01*	>0,05*
	Old		126,2±18,0	327,6±82,9	374,2±106,2	392,7±111,0	278,7±89,0	93,7±49,3	91,2±23,8
	P		<0,05**	<0,05*	<0,05*	<0,05*	>0,05*	>0,05*	>0,05*

Legend. Here and in Table 2: *) differences from control significant; **) differences between adult and old animals are significant.

TABLE 2. Concentrations of T_3 (A) and TSH (B) in Plasma of Adult and Old Male Rats at Various Times after Single Intraperitoneal Injection of Dibunol in a Dose of 100 mg/kg (M±m)

Hormone	Group of rats	Control	Time after injection of dibunol, h					
			3	6	9	12	24	48
A. T_3	Adult	0,68±0,05	0,13±0,02	0,07±0,04	0,19±0,07	0,18±0,05	0,47±0,10	0,56±0,04
	P		<0,001*	<0,001*	<0,001*	<0,001*	>0,05*	>0,05*
	Old	0,43±0,07	0,11±0,06	0,07±0,03	0,05±0,02	0,05±0,01	0,25±0,07	0,50±0,09
	P	<0,05**	<0,001*	<0,001*	<0,001*	<0,001*	>0,05*	>0,05*
B. TSH	Adult	3,68±0,24	1,12±0,50	1,59±0,55	3,93±0,64	4,59±0,44	7,45±0,42	6,55±0,42
	P		<0,001*	<0,001*	>0,05*	>0,05*	<0,001*	<0,001*
	Old	5,72±0,45	4,39±0,53	2,39±0,86	4,04±0,43	4,95±0,34	6,77±0,32	6,25±0,57
	P	<0,01**	>0,05*	<0,01*	<0,05*	>0,05*	>0,05*	>0,05*

function of the adenohypophysis.

The T_3 concentration in the rats' blood plasma fell with age. Injection of D had a significant effect on thyroid function in animals of both age groups (Table 2A). Only 3 h after injection of D the plasma T_3 concentration had fallen sharply, and it remained low until 12 h after the injection, and did not return to its initial values before the end of the first day (Table 2).

It will be clear from Table 2 that the TSH concentration in blood plasma of intact rats aged 24-26 months was higher than in adult rats. Under the influence of D marked changes took place in the thyrotrophic function of the adenohypophysis. In adult animals a sharp fall was observed in the plasma TSH concentration 3 and 6 h after injection of D, after 9 h the TSH concentration was back at its initial level, and after 24 and 48 h it was significantly raised. In old animals a significant fall in the plasma TSH concentration was observed after 6-9 h, followed by restoration of the initial values with effect from 12 h after injection of D.

Comparison of changes in the T_3 and TSH concentrations in the blood plasma under the influence of D shows that their direction was the same during the first hours of action of the antioxidant: depression of the thyrotrophic function of the adenohypophysis was accompanied by corresponding changes in T_3 production. However, normalization of the TSH concentration in the blood plasma took place after 9 h (adults) and 13 h (old rats) whereas the T_3 concentration as before remained below the initial values. Not until later (after 24 and 48 h), when TSH production in the adult animals was significantly higher than initially, was the normal T_2 concentration in the blood plasma restored. In old animals, recovery of thyroid hormone production 24 h after injection of D was not accompanied by any statistically significant activation of the thyrotophic function of the adenophypophysis.

Analysis of the time course of changes in the plasma TSH and T_3 concentrations after a single injection of D, in the same way as examination of the pituitary–adrenocortical system, leads to the conclusion that a decisive role in the mechanism of action of D on the system of

thyroid regulation must also be played by its primary action on the specific function of the adenohypophysis. Under those circumstances, however, a definite disturbance of integrity of the feedback mechanisms in the regulation of thyroid function by the trophic hormone after injection of D can be detected; some delay is found in the changes in the plasma T_3 concentration compared with changes in the thyrotrophic function of the adenohypophysis.

A single injection of the antioxidant D in a dose widely used in experimental research [2,4,5], close to the therapeutic dose for clinical use [3], thus has a marked effect on function of the endocrine glands studied.

Analysis of changes observed in production of adenohypophyseal trophic hormones (ACTH and TSH) and hormones of peripheral target glands (CS and T_3) under the influence of D suggests that the hypophyseal (possibly the hypothalamo-hypophyseal) region is particularly sensitive to the action of the antioxidant. Under the influence of D significant changes take place in the endocrine status of the body. Changes in concentrations of ACTH and CS, and of TSH and T_3, as we know, can lead to significant metabolic and functional changes in the body. That is why, with the action of D on the body is assessed in different situations, not only its direct effects on LPO processes, but also its effects mediated through the pituitary–endocrine glands system and the particular features of the resulting readjustment of endocrine regulation of the functions of the body arising under these circumstances must be taken into consideration.

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