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PLASMA CREATINE PHOSPHOKINASE STUDY IN THYROIDECTOMIZED RABBITS AND EFFECT OF THYROXINE AND ANALOGUES

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Abstract—Thyroidectomy was followed in eleven of fourteen surviving rabbits by an increase in plasma creatine phosphokinase activity, followed after some time by a spontaneous decrease. Normal CPK activity in control rabbits was 0·2–1·7 mU/ml (determination without addition of glutathione) and 2·5–11·2 mU/ml (with addition of glutathione). CPK activity in thyroidectomized rabbits was 0·4–31·6 mU/ml (determination without addition of glutathione) and 3·8–140 mU/ml (with addition of glutathione) but was in at least at one time higher than 20 mU/ml in eleven of fourteen animals. This increased plasma CPK activity can be rapidly normalized by subcutaneously injected 3:5:3′-triiodo-L-thyronine 3:5:3′:5′-tetraiodo-L-thyronine (= L-thyroxine) and by the thyroxine analogue 3:5:3′:5′-tetraiodothyropropionic acid (= desaminothyroxine) but 3:5:3′-triiodo-D-thyronine is much less effective than its L-form.

IN 1963, Graig and Ross[1] reported increased creatine phosphokinase (CPK) (ATP: creatine phosphotransferase EC 2.7.3.2) activity in the serum of myxoedematous patients. Griffith[2] corroborated this, and Saito *et al.*[3] found that serum CPK activity was also increased in cretinism. Their publications occasioned us to carry out a study of blood plasma CPK activity in normal and thyroidectomized rabbits.

METHOD AND MATERIALS

Thyroidectomy was performed under light ether anaesthesia in twenty-one adult female rabbits weighing 2·5–3 kg. Seven animals died within 24 hr of the operation as a result of lesions of the recurrent nerve. The effect of thyroidectomy was controlled by determinations of plasma cholesterol content. The control group had a cholesterol content in the range of 24–78 mg/100 ml (nine animals). In the thyroidectomized group the cholesterol content rose to 136–228 mg/100 ml and persisted at this level for the duration of the experiment (in untreated animals).

The plasma CPK activity in the fourteen survivors was determined by the spectrophotometric method described by Tanzer and Gilvarg,[4] making use of reagents from the Boehringer CPK Test Combination. When glutathione was used as activator, 1 mg was added to 1 ml plasma.

The precision of the method was established by ten replicate analogues on the same sample and is expressed as standard deviation (S.D.) and coefficient of variation (C.V. = ratio of standard deviation to the mean expressed as a percentage). At a level

of about 2·5 mU without glutathione: S.D. = 0·24; C.V. = 9·8 per cent. With the addition of glutathione at a level of about 16 mU: S.D. = 1·45; C.V. = 8·9 per cent.

The blood was always obtained by puncture from the ear vein, using heparinized syringes.

Once it was found that the plasma CPK activity in thyroidectomized rabbits increased considerably, four groups of three rabbits each were treated with solutions of:

(1) L-thyroxine = 3:5:3′:5′-tetraiodo-L-thyronine;
(2) 3:5:3′-triiodo-L-thyronine;
(3) 3:5:3′-triiodo-D-thyronine;
(4) 3:5:3′:5′-tetraiodothyropropionic acid (= desaminothyroxine).

All substances were injected subcutaneously. The solutions of L-thyroxine, of 3:5:3′-triiodo-L-thyronine and 3:5:3′-triiodo-D-thyronine were prepared freshly before use.

Normal nonthyroidectomized rabbits were used as controls.

Simulated injections of alcohol propylene glycol were given daily for a period of 2 weeks to three control rabbits and two thyroidectomized rabbits. This had no effect on CPK-activity. The control rabbits Nos. 35, 36 and 37 were thyroidectomized later.

RESULTS

Table 1 indicates that a control group of ten normal rabbits showed a plasma CPK activity (determined without addition of glutathione) ranging from 0·2 to

TABLE 1. CONTROL GROUP, CPK ACTIVITY IN BLOOD PLASMA (WITHOUT ADDITION OF GLUTATHIONE)

Registered No. of rabbit	CPK (mU/ml)	Registered No. of rabbit	CPK (mU/ml)
1	0·3	37	0·4
2	0·6	38	0·7
3	0·3	40	0·8–0·7–0·8–0·9–1·1*
35	0·3	42	1·7–1·5–1·4–1·6*
36	0·2	43	0·5–0·7*

* Repeated determination on the same sample.

1·7 mU/ml. This range does not exceed that reported by various authors in the literature for normal human values.

Table 2 indicates that a control group of twelve normal rabbits showed a plasma CPK activity (determined after addition of glutathione) ranging from 2·5 to 11·2 mU/ml.

Table 3 indicates that the group of thirteen thyroidectomized rabbits showed a greater range of plasma CPK activity (determined without glutathione addition). Distinctly increased values were found in eleven of the thirteen animals. A slight increase was seen in Nos. 31 and S9.

The range was from 0·4 to 31·6 mU/ml. All these values were obtained before the animals received injections.

Table 4 shows that the plasma CPK activity (determined after glutathione addition) in the group of fourteen thyroidectomized rabbits ranged from 3·8 to 140 mU/ml. In two of the fourteen animals (Nos. 31 and S9), the values did not exceed those in the

control group of Table 2; in No. 13, too, the increase was inconsiderable (up to 16·4 mU/ml). In the remaining eleven animals, however, a CPK activity exceeding 20 mU/ml was found at least once. The rabbits Nos. 31 and S9 were killed with an overdosage of nembutal intraperitoneally and suspect pieces of tissue in the thyroideal region were examined. In rabbit S9 a thyroid gland residue was present; the thyroidectomy in that animal had been incomplete. No thyroid gland rests were found in rabbit No. 31.

TABLE 2. CONTROL GROUP, CPK ACTIVITY IN BLOOD PLASMA (WITH ADDITION OF GLUTATHIONE)

Registered No. of rabbit	CPK (mU/ml)	Registered No. of rabbit	CPK (mU/ml)
40	5·4	54	3·9–3·9*
42	11·2	55	3·9–7·1*
46	3·0	56	5·5
47	6·6	95	2·5
49	10·3	98	9·0
50	3·4	99	4·5

* Duplicate determination on the same sample.

TABLE 3. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS (WITHOUT ADDITION OF GLUTATHIONE)

Registered No. of rabbit	CPK (mU/ml)	Time after operation (days)	Registered No. of rabbit	CPK (mU/ml)	Time after operation (days)
13	0·4	67	30	8·2	23
	4·7	75		2·9	29
	4·8	82	31	1·8	16
	9·8–6·9*	95		2·6	22
	4·1	102	32	20·2	17
15	7·1	53		3·1	23
	2·8	59	33	8·0	10
	24·9	69		2·5	16
	18·0	74	35	0·6	6
	15·2–17·8*	80		7·0	13
	24·9–31·6*	87	36	2·6	7
27	2·9	15		1·1	13
	2·8	21	37	3·0	9
	6·5	30	39	2·5–2·8*	10
	3·2	36	S9	1·5–2·2*	29
	5·6–6·2*	58			
28	7·6	15			
	1·6	21			
	3·6	29			
	3·2	36			
	7·1–8·6*	58			

* Duplicate determination on the same sample.

All values presented in Table 4 pertain to thyroidectomized animals before they received injections.

Next, a study was made of the effect of thyroxine and a few of its analogues on increased plasma CPK activity in thyroidectomized rabbits.

Table 5 indicates that the increased CPK activity returned to normal within 9 days

of treatment by subcutaneous injections of 0·33 mg L-thyroxine; a new increase in CPK activity occurred 59 days after discontinuation of injections.

Table 6 shows that, in two rabbits within 6 days and in one rabbit within 12 days of treatment by subcutaneous injections of 16 μg 3:5:3′-triiodo-L-thyronine, the

TABLE 4. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS (WITH ADDITION OF GLUTATHIONE)

Registered No. of rabbit	CPK (mU/ml)	Time after operation (days)	Registered No. of rabbit	CPK (mU/ml)	Time after operation (days)
13	16·4	150	31	6·9	80
	8·4	218		11·8	95
				6·7	182
15	91·3	136	32	41·7	80
	140	140		38·6	81
	39·8	197			
27	5·6–6·0*	93	33	13·0	73
	25·2	109		25·2	74
	10·1	173	35	41·2	76
28	52·6	94	36	53·2	142
30	3·8	86	37	22·4	72
	34·7	102	39	24·1	72
	14·6	189	45	31·4	95
			S9	10·2	197

* Duplicate determinations on the same sample.

TABLE 5. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS BEFORE AND AFTER SUBCUTANEOUS INJECTIONS WITH 1/3 mg/day OF 3:5:3′:5′ TETRAIODO-L-THYRONINE (= L-THYROXINE)

Registered No. of rabbit*	CPK before treatment (mU/ml)	CPK after 9 days treatment (mU/ml)	CPK 59 days after last injection (mU/ml)
35	41·2	5·7	22·4
37	22·4	3·0	15·1
40 (control)	5·4	—	—
42 (control)	—	—	11·2

* One of the three thyroidectomized rabbits of this group died during the experiment of an intercurrent disease (pneumonia).
CPK determinations with addition of glutathione.

TABLE 6. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS BEFORE AND AFTER SUBCUTANEOUS INJECTIONS WITH 16 μg 3:5:3′-TRIIODO-L-THYRONINE/DAY

Registered No. of rabbit	CPK before treatment (mU/ml)	CPK (mU/ml)		CPK (mU/ml) 58 days after last injection
		6 days treatment	12 days treatment	
28	52·6	4·8	—	14·8
32	38·6	23·8	3·8	37·5
33	25·2	5·4	—	15·1
49 (control)	10·3			—
50 (control)				3·4

CPK determinations with addition of glutathione.

TABLE 7. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS BEFORE AND AFTER SUBCUTANEOUS INJECTIONS WITH 3:5:3′-TRIIODO-D-THYRONINE

Registered No. of rabbit	CPK before treatment (mU/ml)	CPK after treatment 500 μg–6 days (mU/ml)	CPK after treatment 100 μg–5 days (mU/ml)	CPK after treatment 100 μg–5 days (mU/ml)	CPK after treatment 100 μg–4 days (mU/ml)	CPK n-days after last injection (mU/ml)
32	37·5	1·7	—	—	—	44 (n = 67)
35	22·4	—*	19·0	12·9	12·3	33 (n = 47)
37	15·1	—*	10·1	5·0	—	8 (n = 61)
50 (control)	3·4					
42 (control)		11·2				
54 (control)			3·9			
99 (control)				4·5		
55 (control)					3·9	

CPK determinations with addition of glutathione.
* Not injected.

increased plasma CPK activity returned to normal. A new increase in CPK activity occurred 58 days after discontinuation of injections. The daily dose required to produce this effect is strikingly small.

Table 7 shows that it was much more difficult to cause a decrease in CPK activity by subcutaneous injections of 3:5:3′-triiodo-D-thyronine. The daily dose required was much larger and the duration of treatment in two of the three animals much longer than with 3:5:3′-triiodo-L-thyronine. In one animal, the upper limit of normal activity of the control group was not yet fully attained after 14 days. In two of the three animals, a distinct increase in CPK activity following discontinuation of injections was observed. As Table 8 shows, rapid diminution of CPK activity was achieved with

TABLE 8. CPK ACTIVITY IN BLOOD PLASMA OF THYROIDECTOMIZED RABBITS BEFORE AND AFTER SUBCUTANEOUS INJECTIONS WITH 3:5:3′:5′-TETRAIODO-THYROPROPIONIC ACID (= DESAMINO THYROXINE)

Registered No. of rabbit	CPK before treatment (mU/ml)	CPK after treatment 0·5 mg–5 days (mU/ml)	CPK after treatment 0·5 mg–4 days (mU/ml)	CPK 46 days after last injection (mU/ml)
15	39·8	23·5	10·1	33·6
33	15·1	10·6	4·5	10·3
36	53·2	45·9	4·5	27
50 (control)	3·4	—	—	—
99 (control)		4·5	—	—
55 (control)			3·9	—
55 (control)				7·1

CPK determinations with addition of glutathione.

the aid of 3:5:3′:5′-tetraiodothyropropionic acid (desaminothyroxine). For 5 days, 0·5 mg of this substance was subcutaneously injected in an aqueous solution at pH 10.

After starting this experiment we found that Stasilli *et al.*[5] already described a breakdown of desaminothyroxine in alkaline solution. In view of this we switched to solutions of this substance in alcoholpropylene glycol. Subcutaneous injections of this solution caused a rapid decrease in CPK activity after 4 days. The CPK activity was distinctly increased again 46 days after discontinuation of these injections.

DISCUSSION

Thyroidectomy is found to be followed in rabbits by an increase in plasma CPK activity. A follow-up on the CPK activity discloses a somewhat irregular course (Table 3). In Nos. 13 and 27 the initial increase is followed by a decrease in CPK activity to normal and in No. 30 to nearly normal. This course has its parallel in human myopathies such as dystrophia myotonica and Duchenne's pseudohypertrophic form of muscular dystrophy (Thomas *et al.*[6] and Heyck and Laudahn[7]). As expected, L-thyroxine and 3:5:3′-triiodo-L-thyronine were capable of causing rapid normalization of the activity. It is of interest that desaminothyroxine (3:5:3′:5′-tetraidothyropropionic acid) is likewise effective in this respect. The effect of this substance on the BMR is only one-tenth of the effect of L-thyroxine. The effect of 3:5:3′-triiodo-D-thyronine was clearly less pronounced than that of the L-form.

REFERENCES

1. F. A. GRAIG and G. ROSS, *Metabolism* **12**, 57 (1963).
2. P. D. GRIFFITH, *Lancet* **I**, 894 (1963).
3. M. SAITO, I. HIBI, M. KAWAZULA and Y. FUKUYAMA, *Lancet* **II**, 252 (1963).
4. M. L. TANZER and C. GILVARG, *J. biol. Chem.* **234**, 3201 (1959).
5. N. R. STASILLI, R. L. KROC and R. I. MELTZER, *Endocrinology* **64**, 62 (1959).
6. W. H. S. THOMSON, P. LEYBURN and J. N. WALTON, *Br. med. J.* **II**, 1276 (1960).
7. H. HEYCK and G. LAUDAHN, *Klin. Wschr.* **41**, 905 (1963).