Heart Muscle Catecholamines and Enzymes in Experimental Thyroid Diseases

Shoichi Yamagata, M.D., Rikuro Sasaki, M.D., Sadao Ohira, M.D., Tatsuro Hira, M.D., Akiyuki Hosh, M.D., Hisao Otsuka, M.D., Kenji Suzuki, M.D., Tetsumaru Kikuchi, M.D., Shunichi Sat, M.D., Yasuo Watabe, M.D., Mamoru Chiba, M.D., and Kiyoshi Ito, M.D.

Heart muscle catecholamine content, cholinesterase activity, and glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities were measured in experimental thyroid rats. A significant decrease of adrenaline content was found in hyperthyroid group. This finding appears to signify in the explanation of increased responsiveness of the cardiovascular system to adrenaline in hyperthyroidism. Cholinesterase activity was increased in hyperthyroid group and decreased in hypothyroid group. This might be related to complicated functions of the autonomic nervous system. Activities of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were decreased in hyperthyroid as well as hypothyroid group. It is likely that muscular involvements in thyroid diseases are significant enough to cause these changes.

The exact mechanism of action of the thyroid hormone is completely unknown. Yet the thyroid hormone stimulates the myocardium to increase its rate and force of contraction; the opposite effects are noted in thyroid hormone deficiency. This has been considered to be an appropriate adjustment to the peripheral need for oxygen. Recent concept, however, is that the action of thyroid hormone is closely related to the effects of catecholamines, based on the fact that the cardiovascular effects in hyperthyroid patients are exaggerated to administration of catecholamines. No direct correlations between heart muscle catecholamines and thyroid hormone have been established, though a number of studies have been made on role of catecholamines in thyroid diseases.

Serum cholinesterase activity has been found to be related to thyroid diseases, but its significance has been poorly mentioned. Furthermore, this enzyme activity in heart muscle has never been studied in thyroid diseases.

From Medical Department of Prof. S. Yamagata, Tohoku University School of Medicine, Sendai.
Literally hundreds of studies have been conducted on the effects of thyroid hormone on various enzyme systems, but no concerns have been centered on glutamic oxaloacetic transaminase and glutamic pyruvic transaminase. This study was undertaken to find further correlations between the effects of thyroid hormone and catecholamines, pseudocholinesterase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase in the heart muscle in experimental thyroid rats.

**EXPERIMENTAL MATERIAL AND METHODS**

Mature male rats, Wistar strain, weighing 120-130 Gm. were used. Animal was divided into 3 groups. (1) hyperthyroid group: 0.2 ml. of thyronine solution (5 mg. of 3, 5, 3' triiodothyronine was solved in 0.5 ml. of 0.1 N NaOH and then diluted into 300 ml. of destilled water) was intraperitoneally injected daily for 7 days. The each dose contains 33 μg. of thyronine, corresponding 15-30 times of therapeutic dose in man. (2) hypothyroid group: 1 mg. of 1-methyl-2-mercaptoimidazole* (product of Chugai Pharmaceut. Co. commercial preparation: Mercazole) dissolved in destilled water was intraperitoneally given daily for 7 days. The single dose corresponds similarly 15-30 times of therapeutic dose in man. (3) control group: untreated. All rats were fed with same animal foods. The oxygen consumption and pulse rate through electrocardiogram were measured to find hyper- and hypofunction of the thyroid gland, compared with control group, after 7 daily consecutive administration of the drugs. An apparatus, glass tube with a slender glass tube at the one end, was devised for measurement of oxygen consumption (Fig. 1). After general anesthesia by intraperitoneal administration of 30-40 mg. of methylhexabital natrium, rats were placed inside of the apparatus with small packages of soda lime. Then the inside of apparatus is completely replaced by the oxygen and the slender glass tube is sealed by a few drops of water, which moves toward the inside with consumption of the oxygen to maintain the barometric equilibrium. The oxygen consumption was measured, as shown in Fig. 2. The pulse rate measured through electrocardiogram is shown in Fig. 3. After satisfactory hyper- and hypofunction of the thyroid gland was confirmed, rats were killed and hearts were removed for the following measurements.

Catecholamines were eluated by the method of Euler and Orwen,5) using both ventricular muscle homogenized with a small amount of acid alcohol (pre-

* Courteously supplied from Chugai Pharmaceut. Co.
Pseudocholinesterase was measured, using both atrial muscle homogenized with bicarbonate buffer, through Warburg manometric method according to Ammon's method modified by Tamai. Tamai states that the final concentration of optimal acetylcholine as substrate is 0.025 mol and the measurement was made according to this concentration.

Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were measured by Sigma-Frankel method, using 10% ventricular muscle homogenate prepared with 0.25 mol sucrose.

**RESULTS**

Free catecholamine contents were shown in Fig. 4. Average adrenaline content was $0.04 \pm 0.04 \mu g./Gm.$ in hyperthyroid group, $0.18 \pm 0.09$
μg./Gm. in hypothyroid group and 0.20±0.10 μg./Gm. in control group. There was very significant decrease of adrenaline content in hyperthyroid group compared with hypothyroid and control group (p<0.1%, respectively). Average noradrenaline content was 0.29±0.20 μg./Gm. in hyperthyroid group, 0.12±0.09 μg./Gm. in hypothyroid group and 0.30±0.28 μg./Gm. in control group. There was a tendency of decrease in hypothyroid group, but were no significant differences between 3 groups (2.5%<p<5.0% between hyperthyroid and hypothyroid group).

Cholinesterase activities were shown in Fig. 5. Average cholinesterase activity was 137.5±12.5 μl./100 mg. in hyperthyroid group, 110.6±9.8 μl./100 mg. in control group and 101.8±11.0 μl./100 mg. in hypothyroid group. There was very significant increase in hyperthyroid group compared with control and hypothyroid group (p<0.1%, respectively). There was a significant decrease in hypothyroid group compared with control group (0.5%<p<1.0%). Therefore, there were significant differences between 3 groups.

Glutamic oxaloacetic transaminase activities were shown in Fig. 6. Average glutamic oxaloacetic transaminase activity was 98.1±7.9U/mg. in hyperthyroid group, 99.6±12.6U/mg. in hypothyroid group and 127.1±10.6U/mg. in control group. There was very significant decrease in hyperthyroid group and a significant decrease in hypothyroid group compared with control group (p<0.1% and p<1.0%, respectively). Glutamic pyruvic transaminase activities were shown in Fig. 7. Average activity was 8.7±1.3 U/mg. in control group, 6.3±0.8 U/mg. in hyperthyroid group, and 6.1±1.0 U/mg. in hypothyroid group. There was a significant decrease in hyperthyroid and hypothyroid group compared with control group (p<1.0%, respectively).
DISCUSSION

That effects of adrenaline to the cardiovascular system and on the oxygen consumption are similar to those of thyroid hormone is well known. It has been also found that the myocardial and vascular effects of catecholamines are greatly enhanced by thyroid hormone. Brewster and his associates have shown as a result of experiment in dogs that thyroxine per se has no effect either on the basal metabolic rate or on ventricular dynamics in the absence of the sympathetic nervous system and its pressor amines, and discussed that the signs and symptoms of hyperthyroidism might be interpreted as a result of increased catecholamine activities caused by thyroxine. Murray and Kelly found that response to adrenaline in thyroid patients was of diagnostic value as well as the basal metabolic rate, protein bound iodine or I uptake. Although there have been convincing facts that the cardiovascular system is more sensitive to catecholamines in hyperthyroid patients than in normal individuals, the mechanism has been incompletely understood. Several hypotheses have been proposed: (1) increased catecholamines in the blood, (2) decreased monoamine oxidase activity, and (3) decreased contents of catecholamines in organs. As to the first hypothesis, Diller and Kilpatrik reported that the excretion of adrenaline in hyperthyroid patients was increased in parallel with its severity using biological assay technique. Goldfien and co-workers found an increased adrenaline in plasma in hyperthyroid patients measuring the ethylenediamine condensation method. Euler, however, states that any definite relationships between catecholamine excretion and thyroid diseases were found. Ishida recently published his work on the metabolism of catecholamines in thyroid patients and reported that no any distinct changes in the urinary excretion of catecholamines and their metabolites were found in thyroid patients. The main metabolic pathway of catecholamine, as generally accepted, is catecholamine—methoxy catecholamine (or dihydroxy mandelic acid)—vanil mandelic acid. So if there is an increased endogenous production of catecholamines in a certain pathological condition, an increased urinary excretion of catecholamines, methoxy catecholamine and vanil mandelic acid should occur. Then Ishida concludes that the endogenous production of catecholamines is in the normal range in hyperthyroid patients.

As to the second hypothesis, Spinks has found a decreased monoamine oxidase activity in the wall of the aorta in rabbits by administration of thyroxine. Burn supported this hypothesis with many experiments. Furthermore, Spinks and Burn, Trendelenburg, and Zile and Lardy found a decreased monoamine oxidase activity in the homogenate...
of liver in rats or rabbits administered pulverized thyroid gland. They reported that a monoamine oxidase activity is increased in the liver homogenate of thyroidectomized rats. If there are alterations in these catecholamine inactivating enzymes in thyroid diseases, the metabolism of catecholamines should be modified. Ishida has shown that urinary excretion of the metabolites of catecholamines or their ratio (urinary catecholamines/methoxy catecholamines/vanil mandelic acid) are ranging from 1/11/120 to 1/16/44 in normal subjects as well as hyperthyroid patients before and after administration of monoamine oxidase inhibitors. He also states that there are no differences in that ratio in 2-24 hour urine specimens after infusion of noradrenaline between hyperthyroid patients and controls. He concluded that activities of monoamine oxidase and O-methyl transferase are normal in thyroid diseases. But there is still a possibility, proposed by Burn in the explaining the increased sensitivity to catecholamines in hyperthyroid animals, that the enzyme activity is altered only in the vascular wall leaving the other tissues intact.

As to the third hypothesis, Burn has proposed his concept that response of an organ to adrenaline or noradrenaline is increased with the decrease in the content of catecholamine in that organ. He deduced this conclusion from the observation that the spiral strips of rabbit's aorta, in which the tissue catecholamine was previously depleted by reserpine, responded more vigorously than untreated controls. There, the exaggerated sensitivity in hyperthyroidism can be easily explained if the catecholamine content in the cardiovascular system is decreased.

In summary, the patterns of urinary excretion of catecholamines and their metabolites are unchanged in hyperthyroid patients and the circulating catecholamines in the blood are presumably in the normal limits. The enzyme system involved in the metabolism of catecholamines is perfectly normal in hyperthyroid patients. Increased responsiveness of organs to catecholamines has been suggested to be most likely.

Our study showed very significant decrease of adrenaline content in the heart muscle of hyperthyroid rats. This appears to prove Burn's concept is correct. Then it is considered that various cardiovascular signs and symptoms in hyperthyroidism are due to relative hyperadrenalemia based upon the decrease of adrenaline content in the heart muscle. Further elucidation, however, is necessary to find in what process the decrease of adrenaline content in the heart muscle occurs. Noradrenaline content in the heart muscle was unchanged in hyperthyroid group. It is unknown why only adrenaline content is decreased and noradrenaline content is unchanged, although a decrease of noradrenaline content is seemingly to occur. Then possibilities may be proposed that N-methylation process of noradrenaline to adrenaline is blocked in the heart muscle or an uptake
of adrenaline into the muscle is blocked in hyperthyroidism.

The catecholamine contents in the heart muscle in hypothyroid group were not different from of control group. As far as the heart muscle catecholamines are concerned, the opposite effects were not found in hypothyroid group, though the exact mechanism remains again unknown.

Since some correlations between pseudocholinesterase and thyroid disease were found in 1937,2 the literatures have been scarce,3,4 and its significance has never been considered. It has been reported that pseudocholinesterase is significantly elevated in sera of hyperthyroid patients. Increased activity of cholinesterase returns toward the normal level with adequate treatment. Our study showed that pseudocholinesterase activity in the heart muscle is parallel to previous findings in sera of thyroid patients. Cholinesterase hydrolyses acetylcholine, which stimulates the adrenal medulla to excrete catecholamines. On the other hand, cholinesterase has been shown to be inhibited by adrenaline.24,25 These facts suggest that there must be intricate autonomic nervous system involved in the mutual interrelation between cholinesterase activity and catecholamines. Although the current knowledge is lacking in this point of view and the interrelation between tissue cholinesterase and serum cholinesterase is unknown, it could be speculated that the increased cholinesterase activity in the heart muscle may be due to the decreased adrenaline content in hyperthyroid condition and then acetylcholine may be further degraded to augment sympathetic adrenergic action.

No attention has been made on the activities of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in relation to thyroid

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**Fig. 8.** Electron microscopic picture of heart muscle obtained from hyperthyroid rat. Arrangement of myofilaments is somewhat coarse. Mitochondriae are swollen and arrangement of cristae mitochondriales are irregular. (×10,000)

**Fig. 9.** Electron microscopic picture of heart muscle obtained from hypothyroid rat. No appreciable changes in the myofilaments are seen but fat drops are occasionally seen between the myofilaments. Cristae mitochondriales are somewhat irregular and unclear. Arrow indicates a small fat drop. (×10,000)
diseases, though numerous studies have been performed on many enzyme systems. Significant decrease of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities in the heart muscle in hyperthyroid as well as hypothyroid rats are interesting informations, which appear in the literature for the first time. Muscular thinning or swelling is a known finding in thyroid diseases. As shown in Fig. 8 and 9, electron microscopic specimens of heart muscle were also made in this study. Although no remarkable destruction in the fine structure of muscle was noted, the muscular involvement is significant enough to make glutamic oxaloacetic transaminase and glutamic pyruvic transaminase liberated into the blood, which has not been confirmed, however. This will be evaluated in the near future.

**SUMMARY**

(1) Heart muscle catecholamine content, pseudocholinesterase activity, and glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities were measured in rats after administration of 3, 5, 3'-triiodothyronine and 1-methyl-2-mercaptoimidazole in order to find further correlations between these and the effects of thyroid hormones. Heart muscle adrenaline content significantly decreased in hyperthyroid group compared with hypothyroid and control group. The significance of this finding in explaining hypersensitivity of adrenaline in hyperthyroidism was discussed. The metabolism of catecholamines in relation to this finding was also discussed.

(2) Pseudocholinesterase activity in the heart muscle significantly increased in hyperthyroid group and decreased in hypothyroid group compared with control group. Its possible significance in thyroid diseases was discussed.

(3) Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activities in the heart muscle significantly decreased in hyperthyroid as well as hypothyroid group compared with control group. It is likely that muscular involvements in thyroid diseases are significant enough to cause possible liberation of these enzymes.

**REFERENCES**

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