Effect of Reserpine on the Increase in Myocardial Adenyl Cyclase Activity Produced by Thyroid Hormone (35578)

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(Introduced by W. J. Harrington)

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Hyperthyroidism is accompanied by an enhancement of the intrinsic level of myocardial contractility, a condition which is independent of the adrenergic system (1) and which appears to be due to a direct effect of thyroid hormone on the heart (1, 2). It is therefore of interest that reserpine and guanethidine, two potent antiadrenergic agents, are extremely effective in the treatment of the cardiac manifestations of hyperthyroidism (3, 4). Although their efficacy could be due to a reduction of the background level of adrenergic stimulation which might be playing a contributory role in producing the hyperdynamic circulatory state present in this disease, it is also possible that these drugs have specific antithyroid activity.

We recently demonstrated that in a particulate preparation of cat myocardium thyroid hormone acutely increases adenyl cyclase activity (5). Although a similar enhancement of adenyl cyclase activity has not as yet been found *in vivo* (6–8), these results raise the possibility that the direct effects of thyroid hormones on the heart may be mediated by the adenyl cyclase system. In an attempt to gain more insight into the possible biochemical mechanisms by which thyroid hormones and reserpine exert their cardiac effects in hyperthyroidism, we examined the effects of thyroxine and triiodothyronine on adenyl cyclase activity in a particulate preparation of myocardium when: (i) reserpine was present in the incubation mixture; and (ii) when animals had been pretreated with reserpine 24 hr prior to sacrifice.

Materials and Methods. Reserpine was obtained from Eli Lilly and Company, Indianapolis, Indiana; L-thyroxine, sodium salt, from Sigma Chemical Company, St. Louis, Missouri; αATP-^{32}P, 0.8–5.0 mCi/mmole from International Chemical and Nuclear Corporation, City of Industry, California; cyclic 3′,5′-AMP-^{3}H, 1 Ci/mmole from Schwarz Bioresearch, Orangeburg, N.Y.; Dowex 50W-X8, 100–200 mesh, from Calbiochem, Los Angeles, California.

Cats were injected intraperitoneally with 2.0 mg/kg of reserpine 24 hr prior to sacrifice. The cats were anesthetized with intraperitoneal pentobarbital (25–35 mg/kg) and the heart was quickly removed. A portion of the left ventricle was quickly frozen on dry ice and used for the assay of norepinephrine as measured spectrophotometrically by the trihydroxyindole method (9). The remainder of the left ventricle was dissected free of endocardium and epicardium and used for the assay of adenyl cyclase activity.

Adenyl cyclase assay. Paired determinations performed on a normal cat and a cat to which reserpine had been administered previously were used for each experiment. Approximately 220-250 mg of left ventricular muscle was homogenized in 4.5 ml of cold 0.25 *M* sucrose with a motor-driven homogenizer at 1°. The homogenate was centrifuged at 12,000*g* for 10 min at 4° and the supernatant fluid was decanted; the particles were washed with cold 0.25 *M* sucrose and resuspended and recentrifuged at 12,000*g* for 10 min. The washed particles were resuspended and homogenized in the cold 0.25 *M* sucrose. Protein was determined by the method of Lowry *et al.* (10). Adenyl cyclase was assayed by a recently developed method (11). The partic-

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ulate fraction containing 0.05–0.10 mg of protein in a total volume of 0.06 ml was incubated at 37° for 3 min with 1.6 m*M* adenosine triphosphate (ATP), ATP-^{32}P, 1.1–1.5 μCi; 8 m*M* theophylline; 2.0 m*M* $MgCl_2$; 21 m*M* Tris Cl (pH 7.7); human serum albumin, 0.8 mg/ml; and *l*-norepinephrine in concentrations ranging from 1×10^{-8} to 5×10^{-5} moles/liter, or thyroxine or triiodothyronine in concentrations ranging from 1×10^{-7} to 5×10^{-6} moles/liter. The incubations were started by adding the particulate fraction, which had been kept at 1°, to the other components which were at 23°. The hormones were added to the particles just before the incubations were initiated. The incubations were stopped by adding 0.1 ml of a solution containing 4 μmoles of ATP, 1.25 μmoles of cyclic 3′,5′-AMP, and 0.15 μCi of cyclic 3,′5′-AMP-^{3}H and boiled for 3 min. The cyclic 3′,5′-AMP-^{32}P accumulated was determined as previously described (5).

Results. Norepinephrine content of left ventricles in reserpine-pretreated animals. The norepinephrine content of left ventricles from normal cats was 1.25 ± 0.16 μg/g as compared to zero in the group which had received reserpine.

Effects of reserpine on the capacity of L-*thyroxine and* L-*triiodothyronine to increase myocardial adenyl cyclase activity.* The thyroid hormones at 5×10^{-6} *M* increased accumulation of cyclic 3′,5′-AMP-^{32}P by approximately 50–60%. Reserpine pretreatment did not alter this response nor the response of adenyl cyclase to a concentration of norepinephrine that produces a maximal stimulatory effect (8×10^{-5} *M*). In addition, the concentration–response curves of both L-thyroxine (Fig. 1) and L-norepinephrine (Fig. 2) were unchanged. Half-maximal activity for the thyroxine-mediated activation was approximately 4×10^{-7} *M* and for norepinephrine 8×10^{-6} *M* in both

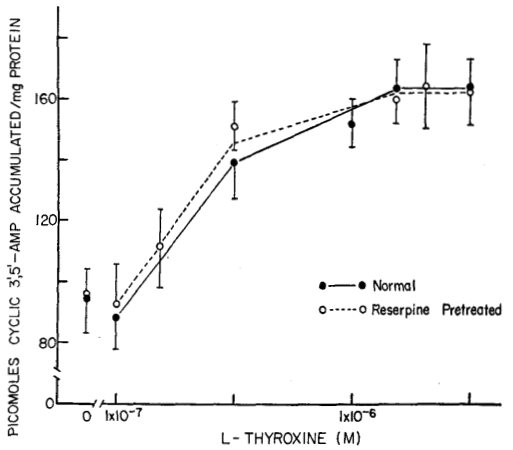

FIG. 1. Concentration–response curve demonstrating concentration related increase in myocardial adenyl cyclase activity produced by thyroxine in normal and reserpine-pretreated cats. Each point represents the mean ± SE of 8–12 samples from 4 cats.

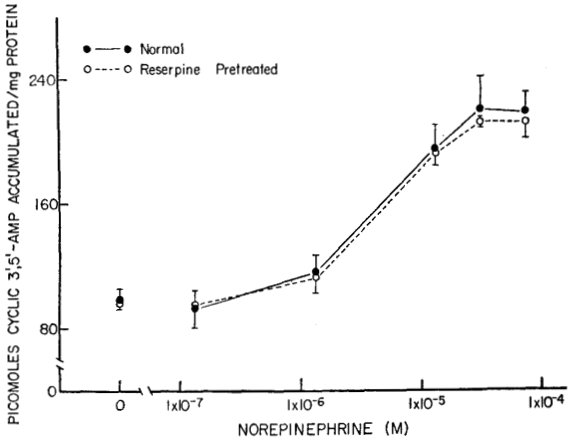

FIG. 2. Concentration–response curve demonstrating concentration related increase in myocardial adenyl cyclase activity produced by norepinephrine in normal and reserpine pretreated cats. Each point represents the mean ± SE of 7–11 samples from 5 cats.

the control and reserpine-pretreated groups.

Effect of reserpine added, in vitro. Reserpine, 1×10^{-5} *M,* added directly to particulate preparations of myocardium from normal cats, did not alter the increase in adenyl cyclase activity caused by thyroxine. Thyroxine, 5×10^{-6} *M,* increased cyclic 3′,5′-AMP accumulation from a mean control of 85 ± 10 pmoles/mg protein/3 min to 140 ± 12 pmoles in the absence of reserpine and from 78 ± 6 pmoles to 130 ± 8 pmoles in the presence of reserpine.

Discussion. Hyperthyroidism is associated with marked changes in cardiac function including increases in heart rate and cardiac output, and enhancement of myocardial contractility (1, 12). It had been suggested that these findings were secondary to an increased sensitivity of the heart to sympathetic stimulation (13). However, several studies have been reported which demonstrate that the catecholamines produce similar increases in heart rate (14) and myocardial contractility (1, 15) in euthyroid and hyperthyroid animals. Moreover, we have demonstrated recently that hyperthyroidism does not alter the norepinephrine-mediated activation of myocardial adenyl cyclase (7), the enzyme believed responsible for mediating the effects of catecholamines on the heart (16).

That thyroid hormone itself has direct positive inotropic and chronotropic effects and that this activity might be responsible, at least in part, for the hyperdynamic circulatory state present in hyperthyroidism was suggested by the observations that triiodothyronine added to isolated heart cells of a 24-hr chick embryo culture produces an immediate increase in the rate of pulsation (2), and that the intrinsic contractile state of isolated papillary muscles obtained from hyperthyroid cats is augmented, even when myocardial norepinephrine stores are depleted by pretreatment of the animals with reserpine (1).

In an investigation designed to elucidate the possible biochemical mechanisms responsible for mediating the inotropic actions of thyroid hormone we found that like the catecholamines, L-triiodothyronine and L-thyroxine are capable of increasing the activity of adenyl cyclase in particulate preparations of cat heart (5). Although the physiological significance of this effect is unclear, the role of adenyl cyclase in mediating the positive inotropic effects of the catecholamines suggests that such a mechanism might also be operative when hearts are chronically exposed to excessive amounts of thyroid hormone.

To date, the evidence of assigning such a role to the adenyl cyclase system is not firm. Nevertheless, the hypothesis that the direct inotropic action of thyroid hormones on the heart is mediated by adenyl cyclase provided us with the opportunity to test another hypothesis: that the beneficial circulatory effects of reserpine in patients with hyperthyroidism occur by specifically interfering with the interaction between thyroid hormone and its biochemical mediator. The data of the present investigation demonstrate that neither pretreatment of cats with reserpine nor direct addition of reserpine *in vitro* alters the thyroxine or triiodothyronine-mediated activation of adenyl cyclase in particulate fractions from cat left ventricles. Thus, if further study substantiates the hypothesis that adenyl cyclase plays an important role in mediating the direct cardiac effects of thyroid hormones, these results would indicate that the efficacy of reserpine and guanethidine in the treatment of hyperthyroid patients is not due to a specific antithyroid effect, but more likely to their ability to impair the sympathetic component of the hyperdynamic circulatory state present in hyperthyroidism.

It is important to emphasize that there is no conclusive evidence that the *in vitro* addition of thyroxin acts in a similar manner or at the same site as the hormone *in vivo*. For example, there is a significant delay between the administration of thyroxine *in vivo* and the resultant biochemical and physiologic changes. This suggests that factors such as diffusion and metabolism of the hormone may be responsible for the lag period necessary for a sufficient concentration of hormone to appear at the active site. Broken cell systems as utilized in this investigation might provide ready access to the site of action of the hormone enabling acute effects to be observed.

Summary. Thyroid hormone activates adenyl cyclase present in a particulate prepa-

ration of cat myocardium, an action that might contribute to the hyperdynamic circulatory state present in hyperthyroidism. Although the marked improvement in the cardiac manifestations of hyperthyroidism produced by reserpine is thought to be due to depletion of the norepinephrine stores of adrenergic nerve terminals, such improvement might be partly caused by an inhibitory effect of reserpine on the thyroid hormone-mediated activation of adenyl cyclase. We therefore examined the effects of reserpine on the capacity of thyroid hormone to activate adenyl cyclase in particulate preparations of cat myocardium. The results demonstrated that reserpine did not impair activation of adenyl cyclase by thyroxine and triiodothyroxine when: (i) reserpine was present in the incubation mixture; and (ii) when cats had been pretreated with reserpine 24 hr prior to sacrifice. Half maximal activity in both the reserpine pretreated and normal cats was 4×10^{-7} M. Thus, it would appear that the salutary circulatory effects of reserpine in hyperthyroidism are not mediated by a direct effect on the activation of adenyl cyclase by thyroid hormone.

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