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Role of the Sympathetic Nervous System in the Control of the Goitrogenic Response in the Rat*

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ABSTRACT. The role of the sympathetic nervous system in the control of the goitrogenic response was examined in adult male rats subjected to superior cervical ganglionectomy (SCGx) 7 days earlier. In the first experiment, superior cervical ganglionectomized (SCGx) or sham-operated animals were treated with the goitrogenic agent methylmercaptoimidazole (MMI) for 4 days, and their thyroid weights and plasma TSH levels were measured. After MMI administration, the increase in thyroid weight was significantly greater in SCGx than in sham-operated rats. The plasma TSH increases after MMI were similar in both groups. To rule out the possible involvement of the pineal gland, MMI treatment was carried out in rats subjected to pinealectomy or sham pinealectomy 7 days earlier. Thyroid weights and plasma TSH levels after pinealectomy were not different from controls in vehicle- or MMI-treated rats. In a third experiment, the goitrogenic response to TSH was assessed in SCGx or sham-operated rats; the thyroid weight of SCGx animals increased by 72% after TSH treatment compared to a 35% increase in the controls ($P < 0.01$). Seven days after SCGx [^{3}H]uridine incorporation into thyroid RNA increased significantly by 64%, and 4 weeks after SCGx, spontaneous goiter developed. Blood flow, estimated from the uptake of a tracer dose of ^{86}Rb, decreased significantly in the thyroid and pineal gland after SCGx. Norepinephrine and epinephrine levels in the thyroid gland were depressed 70–80% within 24 h after SCGx, whereas dopamine levels remained unaltered. An increase in the number of α-adrenoceptor sites, assessed by [^{3}H]dihydroergocryptine binding to thyroid membranes, was found in SCGx rats, while the β-adrenoceptors (assessed by [^{3}H]dihydroalprenolol binding) did not change. The results suggest that the cervical sympathetics normally exert a negative influence on thyroid function in the rat. (*Endocrinology* 109: 2202, 1981)

MANY authors have studied the possible involvement of the sympathetic nervous system in the regulation of thyroid function with contradictory results. Depending upon the animal species tested as well as the experimental approach used, exogenously injected catecholamines have been found to stimulate (1–6), to inhibit (7–9), or not to influence (10–13) thyroid function. Comparatively less attention has been paid to changes in thyroid activity after surgical or chemical removal of the thyroid gland's sympathetic innervation. Since this sympathetic innervation is known to originate in the superior cervical ganglia (SCG), it is possible to manipulate the neural input in a number of situations by surgically excising the ganglia or suppressing their afferent preganglionic fibers. The present study describes the effects of superior cervical ganglionectomy (SCGx) on various aspects of thyroid function, including the goitrogenic response to methylmercaptoimidazole (MMI), TSH, RNA synthesis, α- and β-adrenoceptors, and catecholamine content. The results obtained suggest that the sympathetic nervous system exerts an inhibitory influence on thyroid activity in the rat.

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Materials and Methods

Animals

Adult male Wistar rats (180–260 g) were kept in a lighted environment between 0700–2100 h daily and were given access to Purina chow and water *ad libitum*. SCGx, pinealectomy, or sham operations were performed under light ether anesthesia, as described previously (14, 15). Animals regained activity within 10 or 20 min after surgery, and there was no mortality or brain damage (as inspected macroscopically when the animals were killed) produced by the pinealectomy procedure.

Goiter production

Rats were given four consecutive daily ip injections of 4 mg MMI diluted in 0.5 ml saline. Injections were started at different times post operation. Twenty-four hours after the last MMI injection, the animals were killed, and the thyroid glands were

carefully dissected and weighed.

In another series of experiments, the rats were injected sc with 100 mU/day TSH (NIH-B9) for 4 days.

^{86}Rb uptake

Two minutes after iv injection in the jugular vein of 1 μCi ^{86}Rb dissolved in 0.25 ml saline, radioisotope uptake was determined as an estimation of the percent change in blood flow caused by SCGx (16). Thyroid and pineal glands as well as a blood sample were rapidly obtained, and their radioactivity was measured in an automatic γ-spectrometer. Results were expressed as thyroid to blood or pineal to blood radioactivity ratios. In a pilot experiment, a plateau in ^{86}Rb uptake was reached 1 min after injection which persisted for at least 1 min.

[^{3}H]Uridine incorporation into RNA

Thyroid RNA synthesis was estimated from [^{3}H]uridine incorporation into RNA. Each lobe was sliced with a razor blade and incubated in Krebs-Ringer bicarbonate-glucose at 37 C under 95% O_2-5% CO_2. After 30 min, a tracer dose of [^{3}H]uridine ([5-^{3}H]uridine; 28.3 Ci/mmol) was added (usually 3 μCi/ml), and the incubation was continued for 60 min. The glands were carefully washed with cold saline and homogenized in water with an all-glass motor-driven Potter-Elvehjem-type apparatus. RNA was extracted according to the method of Munro and Fleck (17), as described for the thyroid (18).

Catecholamine assays

The norepinephrine, epinephrine, and dopamine contents of the thyroid glands were measured by a radioenzymatic procedure (19). Briefly, the thyroids were homogenized in 0.3 N perchloric acid, the homogenates were centrifuged, and aliquots of the supernatants were incubated at 37 C for 60 min with a mixture containing Tris-HCl-EGTA buffer, pH 9.6; $MgCl_2$; partially purified catechol-O-methyltransferase, and S-[^{3}H]adenosylmethionine. The tritiated O-methyl catecholamine derivatives were extracted into toluene-isoamyl alcohol (3:2, vol/vol) and subjected to thin layer chromatography using the solvent system chloroform-ethanol-ethylamine (16:3:2). Radioactivity was measured by liquid scintillation spectrometry after scraping off the corresponding chromatogram zones into vials. Metanephrine and normetanephrine were oxidized with sodium periodate to [^{3}H]vanillin and extracted into toluene-Omnifluor before counting.

Adrenoceptor-binding assays

Binding studies on thyroid adrenoceptors were carried out according to standard procedures (20). α- and β-adrenoceptors were assayed using [^{3}H]dihydroergocryptine ([^{3}H]DHE; 25.7 Ci/mmol) and [^{3}H]dihydroalprenolol hydrochloride ([^{3}H]DHA; 51.1 Ci/mmol) binding to crude P_2 pellets, respectively. The final pellet was resuspended in 75 mM Tris-HCl buffer, pH 7.4, containing 25 mM $MgCl_2$. Phentolamine and propranolol were used as competitors for α- and β-adrenoceptor binding, respectively. In each experiment, a parallel series of blank preparations was run as controls. Incubations were carried out under optimal conditions (30 min at 25 C for α-adrenoceptors; 10 min at 37 C for β-adrenoceptors). Separation of free from bound ligand was achieved by rapid filtration through Whatman GFB glass fiber filters (Whatman, Inc., Clifton, NJ), which in the case of [^{3}H]DHE binding studies were presoaked in cold buffer containing 5 mM dopamine, 1 mM pyrocatechol, 1 mM pyrogallol, 0.1% ascorbic acid, and 0.1% albumin. After washing with 10 ml ice-cold buffer, the radioactivity left on the filter was measured by liquid scintillation spectrometry. In all of the experiments, the difference between the total radioligand bound and the total radioligand bound in the presence of 10 μM competitor (nonspecific binding) was taken as the amount of specific radioligand binding. Equilibrium binding constants were calculated by Scatchard analysis after the transformation of data (20).

Determination of plasma TSH level

Rat plasma TSH concentrations were measured by RIA using a double antibody procedure and reagents provided by the Rat Pituitary Hormone Distribution Program (NIAMDD). Results are expressed as nanograms of TSH-RP-1 per ml plasma. The assay sensitivity was 10–20 ng.

Statistical analysis of the data

Results from the experiments on the effects of SCGx or pinealectomy on the goitrogenic response were analyzed by a factorial analysis of variance, followed by a modified *t* test (21). Line slope and intercepts of Scatchard analysis of equilibrium binding constants were calculated by regression analysis, followed by an analysis of covariance (21). Results derived from the experiments on ^{86}Rb uptake, catecholamine levels, and RNA synthesis were analyzed by Student's *t* test.

Results

Effects of SCGx on the goitrogenic response to MMI

Rats subjected to SCGx or sham operation 7 days earlier were treated with MMI for 4 days, and their thyroid weights and plasma TSH levels were measured (Table 1). SCGx did not affect any of the parameters studied in saline-injected animals. Factorial analysis (SCGx × MMI) revealed a significant interaction ($P < 0.01$) between MMI and SCGx, *i.e.* after MMI administration, the increase in thyroid weight was significantly greater in superior cervical ganglionectomized (SCGx) animals than in sham-operated rats. The plasma TSH responses to MMI treatment were essentially similar in both experimental groups, as revealed by the absence of the interaction surgery × MMI in the factorial analysis (Table 1).

When MMI treatment was started 2 or 4 days after SCGx, a tendency for thyroid weight to be greater than that in controls was detectable; the differences, however, did not attain significance (data not shown).

TABLE 1. Effect of SCGx (Exp 1) or pinealectomy (Px) (Exp 2) on the goitrogenic response to MMI in rats

	Treatment	n	Thyroid wt, (mg/100 g BW)	Plasma TSH (ng/ml)
Exp 1				
SCGx	Saline	7	5.78 ± 0.74	501 ± 82
	MMI	8	10.07 ± 1.38	1317 ± 306
Sham operation	Saline	8	5.69 ± 0.56	492 ± 68
	MMI	8	7.88 ± 0.86*	1124 ± 299
Exp 2				
Px	Saline	6	7.73 ± 0.69	625 ± 180
	MMI	6	9.67 ± 0.66	1475 ± 382
Sham operation	Saline	7	6.89 ± 0.69	685 ± 92
	MMI	6	9.67 ± 0.66	1775 ± 584

Rats subjected to surgery 7 days earlier were injected with MMI (4 mg/day for 4 days). Results are expressed as the mean ± SEM. Statistical analysis was performed by a factorial analysis of variance, followed by a modified *t* test (21).

* Significantly different from SCGx rats injected with MMI ($P < 0.01$).

TABLE 2. Effect of SCGx on the goitrogenic response to TSH in rats

Group	n	Thyroid wt (mg/100 g BW)
SCGx	8	10.1 ± 1.6
Sham operation	8	7.7 ± 0.5

Rats subjected to SCGx or sham operation 7 days earlier were injected with TSH (100 mU/day for 4 days). Results are expressed as the mean ± SEM. The difference between the groups is significant ($P < 0.01$, by Student's *t* test).

Effect of pinealectomy on the goitrogenic response to MMI

Rats subjected to pinealectomy or sham operation 7 days earlier were treated with MMI for 4 days, and their thyroid weights and plasma TSH levels were measured (Table 1). Pinealectomy did not modify any of the parameters examined in saline-injected rats; their responses to MMI treatment were not affected either, as revealed by absence of the interaction surgery × MMI in the factorial analysis.

Effect of SCGx on the goitrogenic response to TSH

SCGx rats were more sensitive to TSH treatment than their sham-operated controls (Table 2). After the injection of 100 mU TSH/day for 4 days, thyroid weight increased by 78% in SCGx rats and by 35% in controls; the differences between the two groups was significant ($P < 0.01$).

Effect of SCGx on thyroid RNA synthesis

Although SCGx did not affect thyroid weight when the animals were killed 7 days after surgery (Table 1), a significant increase in [^{3}H]uridine incorporation into thyroid RNA was detected at this time (Table 3). Spontaneous goiter was apparent in another group of SCGx rats subjected to surgery 4 weeks earlier (8.07 ± 1.22 and 5.28 ± 0.99 mg/100 g BW for SCGx and sham-operated controls, respectively; $P < 0.01$).

Effect of SCGx on ^{86}Rb uptake by thyroid and pineal glands

^{86}Rb uptake was measured as an estimation of the organ's blood flow (16). After SCGx, a significant decrease in tissue to blood radioactivity ratio was detectable in the thyroid and pineal glands (Fig. 1).

Effect of SCGx on the content of norepinephrine, epinephrine, and dopamine in the thyroid gland

A time-course experiment on the effect of SCGx on the thyroid catecholamine content is shown in Fig. 2. Both norepinephrine and epinephrine reached minimal values 24 h after SCGx and remained low for at least 7 days. An effect of surgical stress on the thyroid dopamine content was observed.

Effect of SCGx on thyroidal α- and β-adrenoceptors

The effect of SCGx on α- and β-adrenoceptors is shown in Fig. 3. The binding studies indicated that the specific

TABLE 3. Effect of SCGx on [^{3}H]uridine incorporation into thyroid RNA of rats subjected to surgery 7 days earlier

Group	n	RNA synthesis (dpm/μg RNA)
SCGx	7	16.0 ± 4.2
Sham operation	6	9.7 ± 2.7*

* Significantly different from SCGx rats ($P < 0.01$, by Student's *t* test).

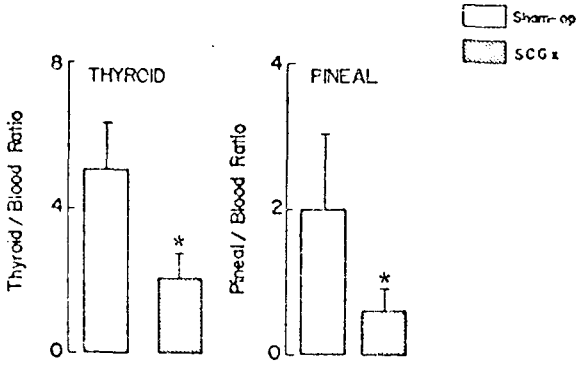

FIG. 1. ^{86}Rb uptake by the thyroid and pineal glands of rats subjected to SCGx 7 days earlier. Results are expressed as tissue to blood radioactivity ratios (mean ± SEM; n = 6 in each group). *, $P < 0.05$, by Student's *t* test.

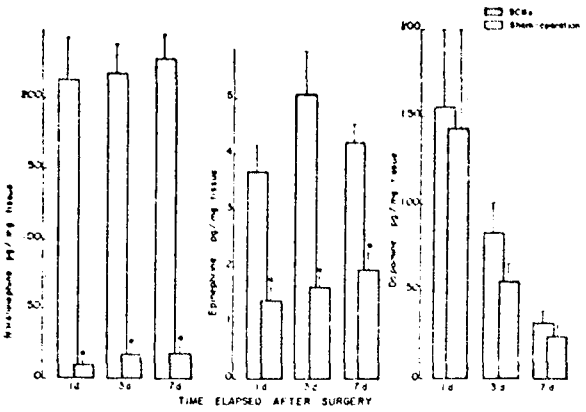

FIG. 2. Time course for thyroid catecholamine depletion after SCGx in rats. Results are expressed as the mean ± SEM (n = 6 in each group). *, $P < 0.01$, by Student's t test.

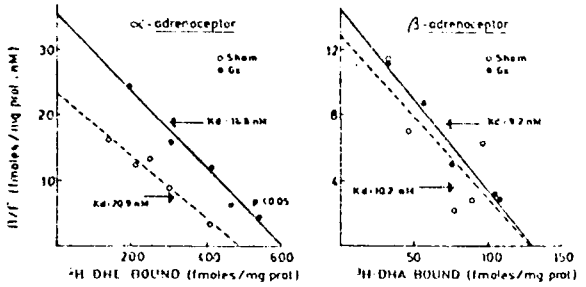

FIG. 3. Effect of SCGx (Gx), performed 7 days before the animals were killed, on the thyroid glands of adult male rats. α- and β-adrenoceptors were assayed by [³H]DHE and [³H]DHA binding, respectively, as described in the text. Scatchard plots of the data are shown; slopes and intercepts were calculated by regression analysis. The maximal number of α-adrenoceptors sites in SCGx rats (598 fmol/mg protein) differed significantly from that in sham-operated controls (430 fmol/mg protein; *$P < 0.05$, by analysis of covariance).

[³H]DHE and [³H]DHA binding were saturable in thyroid membranes from both SCGx and sham-operated rats. Scatchard analysis of the saturation curves indicated an apparent single population of binding sites with dissociation constants (K_d) of about 21 nM for [³H]DHE and 10 nM for [³H]DHA. In the case of α-adrenoceptors, K_d values remained approximately unchanged after SCGx, although the maximal number of binding sites (B_{max}) increased significantly by 21% ($P < 0.05$, by analysis of covariance). No changes in affinity or number of thyroid β-adrenoceptor sites were observed after SCGx (Fig. 3).

Discussion

The major findings of the present work are: 1) the goiter induced by MMI or TSH treatment is larger in rats subjected to SCGx 7 days earlier that that in sham-operated controls; 2) TSH levels in MMI-treated rats increased to the same extent as those in vehicle-injected animals regardless of whether the rats had the SCG intact or excised; 3) pinealectomy did not affect MMI-induced goiter or plasma TSH levels; 4) RNA synthesis increased in the thyroid glands of rats which had been subjected to SCGx 7 days earlier, and 4 weeks after SCGx, spontaneous goiter was observed; 5) SCGx depressed thyroid catecholamine levels, increased α-adrenoceptor sites, and depressed ^{86}Rb uptake.

The role of the sympathetic nervous system in the control of thyroid function has been examined both *in vivo* and *in vitro*. Acute iv injection of epinephrine or norepinephrine to dogs (12) or sheep (13) affected thyroid blood flow without changing labeled hormone secretion. In rabbits, a continuous infusion of epinephrine caused thyroid vasoconstriction as well as depression of the radioiodine uptake (22). A similar decrease in radioiodine uptake was found in rats after systemic epinephrine administration (7). In humans, both stimulatory and inhibitory effects of epinephrine were reported; an acute injection increased ^{131}I uptake, whereas a contiauous infusion depressed it (23). *In vitro* and *in vivo* experiments have shown that epinephrine and norepinephrine can stimulate (1-6), inhibit (7-9), or be devoid of activity on thyroid function (10-13). For example, 6 μM norepinephrine added to the incubation medium of thyroid glands inhibited TSH-induced secretion of thyroid hormones (9).

Extensive studies by Melander and coworkers (1, 2) in mice have shown that epinephrine and norepinephrine increase the number of colloid droplets and the release of radioiodine in prelabeled T_4-treated animals. In mice not treated with T_4, epinephrine and norepinephrine either decreased thyroid hormone release (1) or were ineffective (11). Unilateral stimulation of the SCG in T_4-treated mice caused a significant increase in the number of colloid droplets in the ipsilateral thyroid lobe and an increase in blood ^{131}I radioactivity (6). Injection of 6-hydroxydopamine increased colloid droplet formation shortly after treatment, an effect which disappeared 24 h later. Chronic SCGx did not alter the slope of ^{131}I release from the gland under similar experimental conditions (24).

The present results suggest that the SCG of the rat normally provides an inhibitory input for thyroid activity. The injection of the goitrogenic agent MMI to SCGx animals caused significant increases in thyroid weight compared to controls. Besides innervating the thyroid gland, the SCG supplies sympathetic innervation to cephalic structures like the pineal and submaxillary glands (25), provides vasoconstrictor control to brain and pituitary blood vessels (26), and controls secretion by the

choroid plexus (27). Since under certain circumstances, the pineal gland may affect thyroid function via a negative influence exerted either centrally or peripherally at the level of the gland (28, 29), these results could be subjected theoretically to any of the following interpretations: 1) SCGx removes a direct inhibitory signal exerted at the thyroid level; 2) SCGx acts indirectly via a functional pinealectomy (or any other centrally originated phenomenon affecting TSH release); or 3) SCGx modifies the sensitivity of the thyroid gland to MMI.

A subsequent experiment, in which the goiter induced by MMI in pinealectomized rats was indistinguishable from that found in sham-operated animals, suggests that the pineal gland was not involved in the observed phenomenon. A possible central nervous system mechanism affecting TSH release could be also ruled out, since plasma TSH levels were the same in MMI-treated, SCGx, or MMI-treated control rats as well as in MMI-treated, pinealectomized, or MMI-treated and sham-pinealectomized rats. In addition, SCGx appeared to exert its progoitrogenic effect even in the absence of MMI, because TSH treatment of denervated rats also resulted in greater than normal thyroid weight compared to similarly treated controls. Seven days after SCG removal, thyroid RNA synthesis increased by 65%, without significant changes in thyroid weight. Eventually spontaneous goiter developed in SCGx rats 4 weeks after surgery.

How may the sympathetic nervous system affect thyroid function locally? An intuitive answer to this question is that the removal of the SCG results in changes in blood flow, namely vasodilatation, thereby enhancing the response of the thyroid gland to normal circulating levels of TSH. To assess whether SCGx affects thyroid blood flow, we used an iv tracer dose of ^{86}Rb, an accepted method for blood flow estimation (16). To our surprise, SCGx did not increase thyroid blood flow, but decreased it significantly, not only in the thyroid gland but also in another sympathetically innervated structure, the pineal gland. A similar decrease in ^{86}Rb uptake by the pineal gland of SCGx rats was reported by Goldman (16). Therefore, our results tend to rule out the conclusion that the progoitrogenic effect of SCGx is due to an increase in blow flow; rather, they support a direct link of innervation with acini function, probably in the same way as in other neuroendocrine transducer cells (30).

Twenty-four hours after SCGx, the endogenous norepinephrine and epinephrine stores of the thyroid gland became depleted. Eventually this led, as in other adrenergically innervated organs (20, 31), to an increase in the number of thyroid α-adrenoceptor sites; in the present experiments, the number of β-adrenoceptor sites remained unchanged in the thyroid glands of SCGx rats. The mediation by α-adrenoceptors of the action of catecholamines on the thyroid should be stressed (1, 3, 4, 6, 9). Depending upon the experimental design and parameter used, catecholamine activity is synergistic (1, 3, 4, 6) or antagonistic (8, 9) with TSH. Incidentally, previous results from this laboratory have shown that the acute stimulation by TSH of RNA synthesis in calf thyroid slices is linked to an α-adrenergic mechanism (4). Further experiments are needed to assess whether an α-adrenergic supersensitivity develops in the thyroid gland after SCG ablation.

Summarizing, our results support the conclusion that the norepinephrine released from the thyroid sympathetic nerves impairs the thyroid response to TSH and that after removal of these postganglionic sympathetic fibers, stimulation of thyroid growth ensues. How this mechanism is linked to the physiological regulation of thyroid function awaits further investigation.

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