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Research Article

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FLA-63 and α-MT did not significantly reduce pancreatic catecholamine concentrations, indicating that in the basal state, these agents predominantly deplete central catecholamine stores. Nevertheless, pancreatic TRH concentrations were markedly reduced by FLA-63, and this effect was significantly attenuated by the simultaneous intracerebroventricular (icv) administration of NE. In contrast to the effects of FLA-63, α-MT caused a significant increase in pancreatic TRH concentrations, and this effect was significantly lessened by icv DA. To determine whether the sympathetic nervous system might be one route by which these central effects are mediated, a chemical sympathectomy was induced with guanethidine. This treatment selectively reduced pancreatic concentrations of NE, and caused a marked increase in pancreatic TRH concentrations.

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ABSTRACT These studies have been undertaken to evaluate the role of the brain noradrenergic and dopaminergic pathways in the regulation of the secretion of thyrotropin-releasing hormone (TRH) in the central nervous system (CNS) and pancreas of the neonatal rat. When CNS stores of norepinephrine (NE) were selectively reduced by the subcutaneous administration of the dopamine-β-hydroxylase inhibitor FLA-63, TRH concentrations were significantly reduced throughout the brain. However, when CNS stores of both NE and dopamine (DA) were depleted by the subcutaneous administration of the tyrosine hydroxylase inhibitor α-methyl-*p*-tyrosine (α-MT), TRH concentrations in the brain were not significantly altered.

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From these observations, we conclude the following: (*a*) within the central nervous system, both NE and DA are involved in regulating brain TRH secretion or biosynthesis, and the direction of action of these two neurotransmitters appears to be opposite; (*b*) pancreatic TRH secretion or biosynthesis is also controlled by the brain noradrenergic and dopaminergic systems, and the net effects of each of these pathways appears to be opposite; (*c*) at least one route by which impulses from the brain may travel and modulate pancreatic TRH secretion or biosynthesis is by the sympathetic nervous system.

INTRODUCTION

Several lines of evidence have implicated the catecholamines norepinephrine (NE)[1] and dopamine (DA) in the regulation of thyrotropin-releasing hormone (TRH) secretion from the hypothalamus of the rat (1). The in vivo studies, which have monitored changes in pituitary thyrotropin (TSH) secretion, suggest that NE stimulates hypothalamic TRH release by an α-adrenergic receptor mechanism. This conclusion is based on the observations that TSH secretion is increased by the intracerebroventricular (icv) administration of NE or an α-adrenergic receptor agonist (2, 3), whereas TSH secretion is decreased when the synthesis of NE is blocked, and when α-adrenergic receptor antagonists are administered (3, 4). In addition, the increase in serum TSH, which occurs in response to cold ex-

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[1] *Abbreviations used in this paper*: α-MT, α-methyl-p-tyrosine; CNS, central nervous system; DA, dopamine; FLA-63, bis(4-methyl-1-homopiperazinyl-thiocarbamyl)disulfite; ICV, intracerebroventricular; NE, norepinephrine; PBS, phosphate-buffered saline; TRH, thyrotropin releasing hormone; TSH, thyrotropin.

posure, is attenuated or abolished by agents that deplete NE or antagonize α-adrenergic receptors (2–4).

The role of dopamine in the regulation of hypothalamic TRH secretion has been more difficult to define from studies of TSH secretion. Activation of the central dopaminergic system has been reported to inhibit the release of TSH (3, 5, 6), but there may be more than one mechanism involved in mediating this effect. DA stimulates the release of somatostatin (7, 8), which may in turn inhibit the release of TRH directly (9), and DA also inhibits the release of TSH by a direct action on the thyrotroph cell (10). In spite of these multiple sites of action, a number of investigators have shown that DA stimulates the release of TRH from hypothalamic fragments or synaptosomes (11–13).

Although the aforementioned studies have provided evidence for the involvement of the catecholamines in mediating hypothalamic TRH secretion, very little is known about the regulation of this peptide in other parts of the CNS or in extraneural tissues. Kardon et al. (14) were unable to demonstrate changes in the concentration of TRH in the hypothalamus or extrahypothalamic brain of the adult rat after the systemic administration of a variety of agents known to deplete brain amine stores. Although Winokur et al. (15) demonstrated that the intracisternal injection of the catecholamine neurotoxin 6-hydroxydopamine caused substantial increases in TRH concentrations in certain areas of the brain of the adult rat, the authors did not conclude that the changes in TRH concentrations were due to alterations in catecholamine biosynthesis.

In the studies reported below, the effects of various manipulations of catecholamine biosynthesis upon TRH concentrations in the CNS and pancreas have been examined in the neonatal rat. The experiments have been performed with animals of up to 21 d of age because at this time of life, marked ontogenetic changes in the TRH concentrations of the brain and pancreas are occurring (16–19). It was therefore anticipated that in these developing tissues, changes in TRH concentrations might be more readily demonstrated when the neurotransmitter environment was altered. Moreover, the presence of large amounts of TRH in the pancreas of the neonatal rat has permitted a simultaneous evaluation of the neurotransmitter control of TRH in this extraneural site. The studies suggest that within the brain, both NE and DA are involved in regulating the secretion or synthesis of TRH. Additionally, the results suggest that TRH metabolism in the pancreas is also modulated by the central catecholaminergic pathways, and that the sympathetic nervous system is involved in mediating this neural control.

METHODS

Female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, MA, and matings were carried out in our laboratory. The animals were housed three to a cage and allowed free access to Charles River rat chow and tap water. The rooms were lit from 7 a.m. to 5 p.m., and the ambient temperature was maintained at 21±1°C. On day 19 or 20 of the pregnancy, the dams were housed independently; the time of delivery was noted, and the litters were equalized so that each dam nursed between 10 and 12 pups.

Alpha methyl-*p*-tyrosine methyl ester (α-MT), norepinephrine hydrochloride, and dopamine hydrochloride were obtained from Sigma Chemical Co., St. Louis, MO; bis(4-methyl-1-homopiperazinyl-thiocarbamyl) disulfite (FLA-63) was obtained from Aldrich Chemical Co. Milwaukee, WI, and guanethidine sulfate was kindly donated by Ciba-Geigy, Summit, NJ.

Experimental procedures. To minimize the ontogenetic variability in TRH concentrations, individual experiments have been performed with littermates from a single litter. The depletion experiments have been performed with rats of 6–12 d of age because at this time the brain is sufficiently developed to permit a regional dissection, and the pancreas contains large amounts of TRH that are readily detectable by radioimmunoassay. At allotted times, the neonates were removed from the dams, marked, and injected with the test substance or with vehicle. The pups were then returned to their mothers and at selected intervals were killed by instant decapitation. The brain was dissected into the following areas: cerebral cortex, cerebellum, hypothalamus, hippocampus, midbrain and pons, medulla and brain stem, amygdala and entorhinal cortex, and olfactory cortex. After the dissection of the nervous system, the pancreas was removed.

Measurement of TRH in neonatal rat tisues. The tissues were placed into preweighed vials containing 1 N acetic acid at 0°C. These vials were then reweighed and the tissue weights calculated. Each tissue was homogenized with a Polytron (Brinkmann Instruments, Inc., Westbury, NY) and heated in a boiling water bath for 10 min. The extracts were then centrifuged (3,000 rpm, 15 min) and the supernates decanted. The supernates were then snap frozen on dry ice and lyophilized. After lyophilization, the extracts were resuspended in 0.01 M phosphate-buffered, 0.15 M NaCl, pH 7.5, and used directly in the TRH radioimmunoassay (20). This method of extraction of tissues yielded a recovery of synthetic TRH of 90±3%. The TRH radioimmunoassay routinely detects 2 pg TRH per assay tube, and has an interassay coefficient of variation of 10%.

Measurement of tissue catecholamines. In separate experiments, the NE and DA concentrations in the tissues were estimated. Pups were decapitated and the brain and pancreas rapidly removed and placed on ice. The brain was dissected into various regions as described above, and each area was weighed and frozen at −70°C for 16 h. NE and DA were measured by a modified spectrofluorometric method (21). The catecholamines were extracted from the tissues into n-butanol, and then into 0.2 N acetic acid. An aliquot of the aqueous catecholamine-rich phase was oxidized with iodine and the fluorescent emission read (NE, excitation: 380 nm; emission: 480 nm, uncorrected spectra: DA, excitation: 320 nm; emission: 375 nm). This method specifically measures the naturally occurring catecholamines and does not detect the catecholamine metabolites. Both internal and external standards were run during each experiment, and the exci-

tation and emission spectra were adjusted with the natural standard on the day of the experiment. The recovery of each catecholamine was routinely between 80 and 90%.

Selective NE depletion. To induce a selective depletion of NE, pups were treated with four injections of FLA-63, 25 mg/kg s.c. This agent was dissolved in 100% ethanol and then mixed with 0.9% NaCl containing 2% Tween 20. The final concentration of ethanol in the mixture was 5%. Injections were commenced on the afternoon of day 8, and continued until the morning of day 10. Animals were killed 4 h after the last dose, and control animals that received diluent alone were handled in the same manner.

Combined depletion of NE and DA. A combined depletion of NE and DA was induced by treating the neonates with α-MT. This drug was dissolved in 0.9% NaCl and administered as a single dose of 400 mg/kg s.c. Control animals received 0.9% NaCl, and all animals were killed 16 h afterwards.

Induction of a chemical sympathectomy. A chemical sympathectomy was induced by treating the neonates with guanethidine sulfate. This agent was dissolved in 0.9% NaCl, and the pH was adjusted to 8.0 with 0.1 N HCl. Daily injections of 75 mg/kg s.c. were commenced on day 3 of life and continued for 1 wk. The animals were killed on day 11 of life, 24 h after the last injection. Control animals received daily injections of 0.9% NaCl.

Icv administration of catecholamines. In these experiments, rats of 21 d of age were used. The animals were lightly anesthetized with Ketaject 40 mg i.m. (Bristol Laboratories, Syracuse, NY) and ether, and were then placed in a stereotaxic apparatus. A coronal incision was made in the scalp, the galea aponeurotica was scraped off the skull, and the bregma was identified. The following coordinates were chosen and have been based on preliminary experiments in which the dye fast green was successfully introduced into the lateral ventricle: 1.0 mm lateral, 0.4 mm posterior, and 4.5 mm vertical to the bregma. Each catecholamine was dissolved in 0.1% ascorbic acid to prevent its degradation, and injected in a total volume of 10 μl using a 30-gauge needle. Control animals were injected with 0.1% ascorbic acid.

Light microscopy of the lumbar sympathetic ganglia. These experiments were performed in neonates of 11 d of age. One guanethidine-treated and one control rat were anesthetized with pentobarbital, the thorax opened, and a 21-gauge butterfly needle inserted into the left cardiac ventricle. The animals were then perfused with 0.1 M phosphate buffer, pH 7.4, followed by 3.6% glutaraldehyde in 0.1 M phosphate for 20 min. 30 min later, the abdominal sympathetic chain was removed. The sympathetic ganglia at L4 level were identified and fixed in 3.6% glutaraldehyde for 3 d, postfixed in 2% osmium tetroxide in PBS, dehydrated in graded ethanol solutions, and embedded into Spurr epoxy resin. Semithin sections were cut with a glass knife and stained with 0.1% toluidine blue in 1% sodium borate.

Statistics. Statistical evaluation of the results was performed with Student's *t* test for paired and unpaired data (22).

RESULTS

TRH, NE, and DA concentrations in the brain and pancreas. In the 10-d-old rat, concentrations of TRH (picogram per milligram wet wt) in the CNS and pancreas were: hypothalamus, 61±3; amygdala and entorhinal cortex, 2.1±0.5; olfactory cortex, 5.6±0.5; medulla oblongata and brain stem, 10.1±1.4; pons and midbrain, 3.2±0.9; hippocampus, 0.34±0.07; pancreas, 72±7. TRH concentrations in the cerebral cortex and cerebellum were below the limit of detection (<0.2 pg/mg).

Concentrations of NE (microgram per gram tissue) in these various areas were: hypothalamus, 1.31±0.14; medulla oblongata and brain stem, 0.92±0.03; pons and midbrain, 0.26±0.05; hippocampus, 0.46±0.06; pancreas, 0.40±0.02.

The concentrations of DA (micrograms per gram tissue) in these sites were: hypothalamus, 0.42±0.11; medulla oblongata and brain stem, 0.24±0.05; pons and midbrain, 1.5±0.14; hippocampus, 0.61±0.02; pancreas, 0.66±0.04. Because of the variation of TRH and catecholamine concentrations in the brain and pancreas, the changes induced by the pharmacological agents have been expressed as a percentage of control values, which have been assigned a value of 100.

Effect of FLA-63 treatment on TRH concentrations. These experiments were undertaken to determine the effect of a selective depletion of NE on TRH concentrations. When one dose of FLA-63 was given, (25 mg/kg s.c.), pancreatic concentrations of TRH were reduced to 45% of control values (Fig. 1, $P < 0.001$). CNS concentrations of TRH tended to be reduced in most areas, but this reduction only reached statistical significance in the amygdala and entorhinal cortex (Fig. 1). However, when four doses of FLA-63 were used, TRH concentrations became significantly decreased throughout the CNS as well as in the pancreas (Fig. 2).

Effect of FLA-63 treatment on NE and DA concentrations (Table I). When four doses were administered, FLA-63 caused a significant depletion of NE in the hypothalamus, and in all extrahypothalamic brain areas examined. DA concentrations were unchanged in all areas except the midbrain and pons where a significant increase was noted. However, concentrations of pancreatic NE and DA were not significantly altered by the FLA-63 treatment.

Effect of icv NE on the reduction in pancreatic TRH concentrations induced by FLA-63. The experiments described above have demonstrated that FLA-63 markedly reduced pancreatic concentrations of TRH without altering catecholamine concentrations in that organ. Therefore, to test the hypothesis that the fall in pancreatic TRH concentrations was due to the central effect of FLA-63 on brain noradrenergic pathways, animals treated with FLA-63 were also given NE by the icv route. Animals of 21 d of age were used. A control group (A, $n = 5$) received 0.9% NaCl s.c. A second group (B, $n = 5$) received FLA-63 25 mg/kg s.c. and icv 0.1% ascorbic acid; a third group (C, $n = 6$) received FLA-63 25 mg/kg s.c. and icv NE (0.5

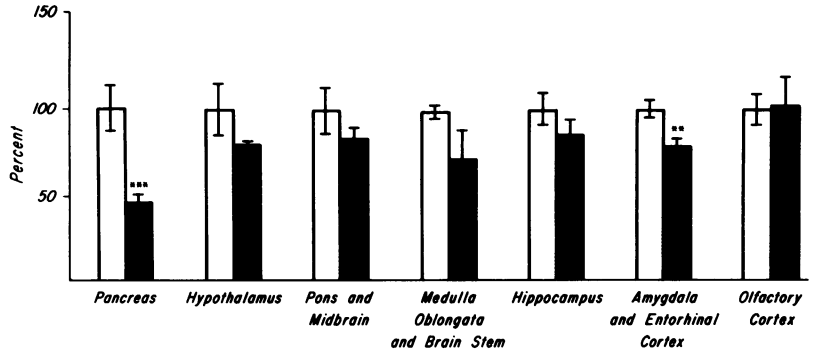

FIGURE 1 The acute effect of FLA-63 on TRH concentrations in the pancreas and CNS. Animals of 8 d of age were injected with FLA-63, 25 mg/kg s.c. (hatched bars) or vehicle (open bars) and killed 8 h afterwards. In this and all the subsequent figures, the bars represent the mean±SE of six animals per group. $^{**}P < 0.01$; $^{***}P < 0.001$.

μmol). Intracerebroventricular injections were performed 2 h after the s.c. injections, and all the animals were killed 8 h after the s.c. injection. As expected, FLA-63 significantly reduced pancreatic TRH concentrations (B vs. A: 6.6±0.4 vs. 13.3±1.3 pg/mg, $P < 0.001$), and this fall was significantly lessened by icv NE (C vs. B: 9.8±0.7 vs. 6.6±0.5 pg/mg, $P < 0.01$). Pancreatic TRH concentrations in group C animals were significantly lower than the control animals (C vs. A, $P < 0.02$).

Effect of α-MT on TRH concentrations (Fig. 3). These experiments were conducted to ascertain the effect of reducing NE and DA concentrations on TRH concentrations in the CNS and pancreas. 16 h after a single dose of α-MT (400 mg/kg s.c.), TRH concentrations in the CNS remained unaltered. However, pancreatic TRH concentrations were increased by 250% compared with control animals ($P < 0.01$).

Effect of α-MT on NE and DA concentrations (Table I). The administration of α-MT led to a significant decrease in NE and DA concentrations in the hypothalamus and all extrahypothalamic brain areas examined. However, pancreatic concentrations of these catecholamines were not significantly altered.

Effect of icv DA on the increase in pancreatic TRH concentrations induced by α-MT. The above experiments have shown that α-MT caused alterations in pancreatic TRH concentrations without changing catecholamine concentrations in that organ. Therefore, to test the hypothesis that the observed increase in pancreatic TRH concentrations by α-MT was due to an effect of this agent on central catecholaminergic pathways, the effect of icv DA was assessed in α-MT-treated animals. Rats of 21 d of age were used and were divided into three groups: a control group (A, $n = 6$) that received 0.9% NaCl s.c.; a second group (B, $n = 5$) received α-MT 400 mg/kg s.c. and icv 0.1% ascorbic acid; and a third group (C, $n = 5$) received α-MT 400 mg/kg s.c. and icv DA (5 μmol). Intracerebroventricular injections were performed 2 and 16

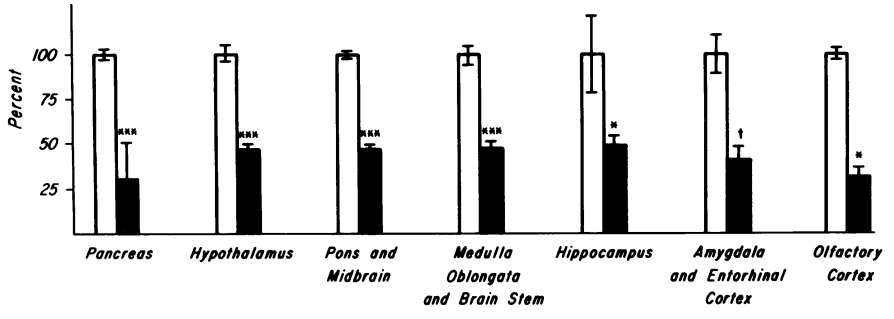

FIGURE 2 The effect of the chronic administration of FLA-63 on TRH concentrations in the pancreas and CNS. Animals were given four injections of FLA-63, 25 mg/kg s.c. (hatched bars) at 12-h intervals commencing on the afternoon of day 8 of life. Control animals (open bars) received vehicle alone; all animals were killed 4 h after the last injection. $^{*}P < 0.05$; $^{***}P < 0.001$; $\dagger P < 0.02$.

TABLE I

*Catecholamine Concentrations in the CNS and Pancreas**

Drug	Catechol-amine	Pancreas	Hypothala-mus	Pons and midbrain	Medulla ob-longata and brain stem	Hippocam-pus
		%	%	%	%	%
FLA-63	NE	85±8*	81±6‖	61±7¶	80±1¶	82±4‖
	DA	86±4*	106±27*	255±44‖	89±11*	67±12*
α-methyl-tyrosine	NE	78±9*	45±10‖	46±10‡	58±7¶	76±6‡
	DA	99±14*	57±12§	14±3¶	20±8¶	17±4¶
Guanethidine	NE	48±16¶	122±11*	100±8*	113±5*	68±29*
	DA	86±6*	114±11*	93±4*	91±6*	97±17*

* After the administration of FLA-63, α-methyl-tyrosine, and guanethidine. Results have been expressed as percentage of controls and are the mean±SE of five animals per group.

* P = NS.

‡ $P < 0.05$.

§ $P < 0.02$.

‖ $P < 0.01$.

¶ $P < 0.001$.

h after the s.c. injection. As expected, α-MT induced a significant increase in pancreatic TRH concentrations (B vs. A, 56±4.4 vs. 9.7±1.3 pg/mg, $P < 0.001$), and this increase was significantly attenuated in animals also given icv DA (C vs. B, 36±6.7 vs. 56±4.4 pg/mg, $P < 0.05$). Pancreatic TRH concentrations in group C animals were still significantly higher than the control animals (C vs. A, $P < 0.01$).

Effect of chemical sympathectomy on TRH concentrations. The above experiments have demonstrated that alterations in central catecholamine stores cause alterations in pancreatic TRH concentrations. One way by which these alterations in the brain might be perceived at the level of the pancreas is by transmission through the sympathetic nervous system. To test the hypothesis that an alteration in the activity of the sympathetic nervous system might cause an alteration of pancreatic TRH secretion, a chemical sympathectomy was induced wth guanethidine. When neonates were treated with this agent, TRH concentrations in the pancreas were increased to 275% of control values (Fig. 4, $P < 0.02$), but remained unchanged in all areas of the CNS.

Effect of guanethidine treatment on the histological appearances of the thoraco-lumbar sympathetic ganglia. The sympathetic ganglion chain was examined by light microscopy to confirm that the dose of guanethidine used in the foregoing experiments was sufficient to cause a sympathectomy. The ganglia from the guanethidine-treated animals showed a marked

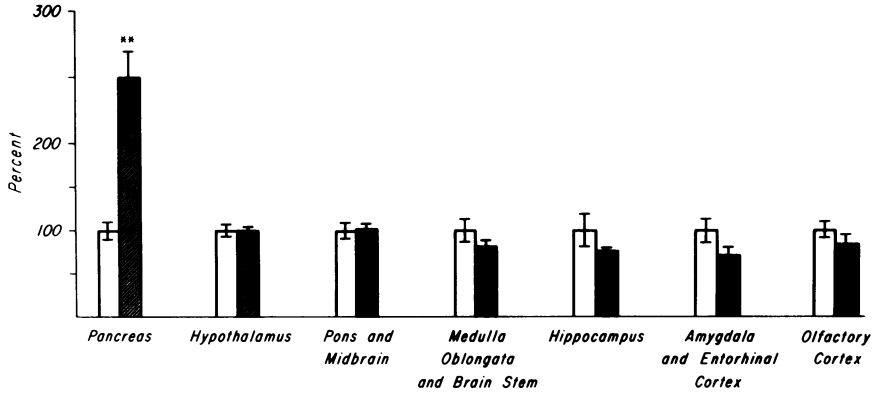

FIGURE 3 The effect of the administration of α-MT on TRH concentrations in the pancreas and CNS. 8-d-old rats were injected with either α-MT 400 mg/kg s.c. (hatched bars) or 0.9% NaCl (open bars) and killed 16 h later. **$P < 0.01$.

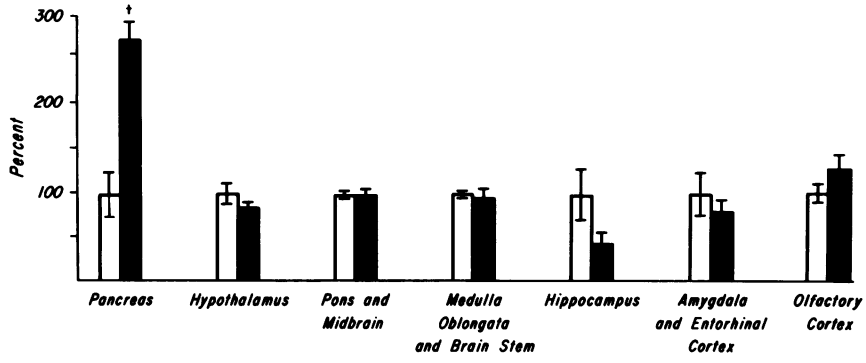

FIGURE 4 The effect of guanethidine treatment on TRH concentrations in the pancreas and CNS. Guanethidine, 75 mg/kg (hatched bars), was injected from day 3 to day 10 of life and the pups were killed 24 h after the last injection. Control animals (open bars) received 0.9% NaCl s.c. †$P < 0.02$.

reduction in size, an almost complete disappearance of ganglion cells, and an increase in the number of satellite cells. (Fig. 5a and b).

Effect of chemical sympathectomy on NE and DA concentrations (Table I). The guanethidine treatment led to a significant reduction in pancreatic NE concentrations, but DA concentrations were not significantly altered. The concentrations of these amines within the CNS were unchanged by the guanethidine treatment.

DISCUSSION

The studies reported here demonstrate that TRH concentrations in the CNS and pancreas are altered when catecholamine concentrations are changed in these areas. When brain NE concentrations were selectively reduced by FLA-63, TRH concentrations were significantly diminished in the hypothalamus and in all extrahypothalamic brain areas. These findings indicate that NE controls the metabolism of TRH not only in the hypothalamus, but throughout the CNS. These studies have not excluded the possibility that the observed reductions in TRH concentrations are also due to a reduction in brain epinephrine concentrations induced by the FLA-63 treatment. Because the content of TRH is determined by its rate of biosynthesis, secretion, and degradation, these studies do not elucidate the subcellular mechanisms by which NE controls TRH metabolism. Nevertheless, it does appear that NE acts on sites of TRH metabolism that are distinct from the release mechanism. Most studies have suggested that NE stimulates the release of TRH from the hypothalamus (1–4, 9, 23). However, were the secretion of TRH the only step influenced by NE, one would have expected to see an increase in hypothalamic TRH concentrations in vivo in the presence of reduced concentrations of NE. The observed decline in TRH concentrations in this setting suggests that NE may also affect other aspects of TRH metabolism such as biosynthesis, degradation, or both of these steps.

In addition to a regulatory role for NE, these studies also suggest that DA is involved in regulating the metabolism of TRH throughout the CNS. This conclusion is based on the results of the experiments with α-MT. This agent inhibits the enzyme tyrosine hydroxylase, which is the rate-limiting enzyme of the catecholamine biosynthetic pathway, thus causing a depletion of both NE and DA throughout the CNS (24). In the presence of this combined depletion of catecholamines, concentrations of TRH in the hypothalamus and extrahypothalamic brain remained unaltered, suggesting that the net effects of DA on the metabolism of TRH are opposite to those of NE, and that the concentration of TRH in a given brain area is determined by the relative concentrations of NE and DA in that area at a particular time. Support for this suggestion is provided by the studies of Spindel et al. (25), which have shown that treatments enhancing central dopaminergic transmission reduce the content of TRH in the striatum. It is conceivable that DA may act on several steps in the metabolism of TRH. Both in vivo (3–6) and in vitro (11–13) studies have suggested that DA affects cellular events responsible for TRH release, although the studies by Marcano de Cotte et al. (26) have demonstrated that DA does not alter the degradation of TRH in hypothalamic synaptosomes. Whether DA may modulate the biosynthesis of TRH in a manner analogous to its action on prolactin biosynthesis (27) is a subject for future investigation.

Pancreatic catecholamine concentrations were not significantly altered by FLA-63 and α-MT, indicating that these compounds have a predominantly central site of action. These observations are in accord with

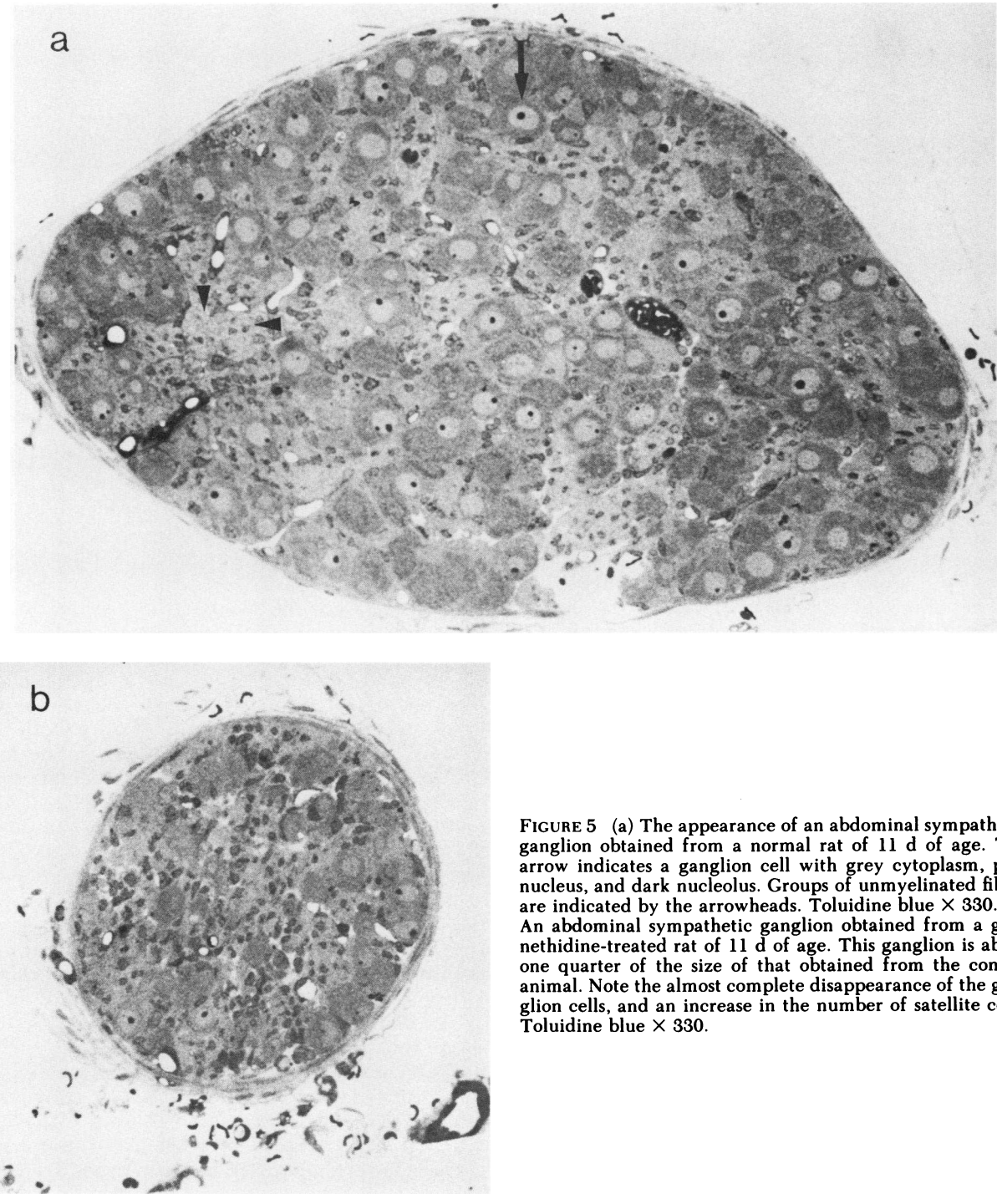

FIGURE 5 (a) The appearance of an abdominal sympathetic ganglion obtained from a normal rat of 11 d of age. The arrow indicates a ganglion cell with grey cytoplasm, pale nucleus, and dark nucleolus. Groups of unmyelinated fibers are indicated by the arrowheads. Toluidine blue × 330. (b) An abdominal sympathetic ganglion obtained from a guanethidine-treated rat of 11 d of age. This ganglion is about one quarter of the size of that obtained from the control animal. Note the almost complete disappearance of the ganglion cells, and an increase in the number of satellite cells. Toluidine blue × 330.

those of other workers (28–30). However, the administration of both these agents produced changes in pancreatic TRH concentrations that were opposite in direction, and these observations are discussed below.

Pancreatic TRH concentrations were markedly reduced by FLA-63, and this effect was seen as early as 8 h after a single dose, at a time when significant changes in brain TRH were not readily apparent. The simultaneous icv administration of NE significantly lessened the fall in pancreatic TRH concentrations induced by FLA-63, suggesting that FLA-63 produced this effect by an action on central noradrenergic path-

ways. These findings therefore suggest that activation of central noradrenergic pathways either inhibits pancreatic TRH release or stimulates its rate of biosynthesis. In contrast to the effect of FLA-63, α-MT produced a marked increase in pancreatic TRH concentrations. This increase was significantly attenuated by the icv administration of DA, strongly suggesting that α-MT induced the increase in pancreatic TRH concentrations by modifying central dopaminergic transmission. These findings therefore suggest that activation of brain dopaminergic pathways stimulates pancreatic TRH release or inhibits its rate of biosynthesis.

The experiments described above have suggested that pancreatic TRH secretion or biosynthesis is controlled by central catecholaminergic pathways, and that the noradrenergic system is stimulatory, and the dopaminergic system is inhibitory. Neural signals are transmitted to the pancreas by sympathetic and parasympathetic nerve fibers (31). Therefore, to explore whether the sympathetic nervous system might be one route by which neural signals may alter pancreatic TRH secretion or biosynthesis, a chemical sympathectomy was induced with guanethidine. When administered to newborn rats, this agent causes a selective destruction of sympathetic ganglia without affecting the parasympathetic nervous system (32). In contrast to 6-hydroxydopamine, which also destroys central catecholaminergic cell bodies, these structures are unaffected by guanethidine, thereby further enhancing the specificity of the lesion produced (33). When examined by light microscopy, the histological appearances of the lumbar sympathetic ganglia confirmed that the dose of guanethidine used in these experiments was sufficient to produce an almost complete sympathectomy (34).

CNS stores of NE and DA were unaltered by the guanethidine treatment. However, pancreatic concentrations of NE were significantly reduced, but DA concentrations were not significantly altered. This selective reduction in pancreatic NE by guanethidine can be accounted for by the anatomical distribution of the catecholamines within the pancreas. NE is predominantly found within the adrenergic nerves that innervate the pancreas (35), and the destruction of the ganglia supplying these nerves would be expected to be accompanied by a lowering of NE concentrations. On the other hand, the predominant location of DA is within the islets themselves, where presumably it would escape the effects of guanethidine.

Pancreatic TRH concentrations were markedly increased in the sympathectomized animals demonstrating that pancreatic TRH release or biosynthesis is dependent on the functional status of the sympathetic nervous system. These findings lend support to the view that centrally mediated alterations in sympathetic outflow could modify pancreatic TRH secretion or biosynthesis. The changes in TRH concentrations induced by the chemical sympathectomy were similar in direction and magnitude to those caused by the α-MT treatment, and both these procedures would tend to produce a functional disconnection of the pancreas from the central catecholaminergic neurons.

The results of the sympathectomy also suggest that the responses of the TRH-producing cell to NE may be fundamentally different in the pancreas from those in the CNS. This conclusion is based on the observations that selective depletion of brain NE by FLA-63 causes a reduction in brain TRH concentrations, whereas selective depletion of pancreatic NE by guanethidine increases pancreatic TRH concentrations. These observations are paralleled by the work of other investigators, and provide a further illustration that the response of a particular peptide-producing cell to a given stimulus may vary in different regions of the body. Schaeffer et al. (12) have demonstrated that DA releases TRH from synaptosomes from the hypothalamus but not from those of the septum. Similarly, norepinephrine stimulates the release of somatostatin from fragments of the medium eminence of the rat (7) but inhibits the release of this peptide from rat pancreatic islets (36). At present, the physiological basis underlying these heterogeneous responses is far from clear, but may pertain to the different functions that these cells serve in different anatomical sites.

When the anatomical localization of TRH in the rat pancreas is taken into account, the suggestion that its metabolism may be modulated by the CNS is not surprising. The radioimmunological studies of Martino et al. (37) have demonstrated that TRH is located predominantly, if not exclusively, within the islets of Langerhans, and the immunohistochemical studies of Koivusalo et al. (38) have localized TRH-positive cells to the outer margin of the islet. The pancreatic islets are innervated by fibers derived from the parasympathetic and sympathetic nervous systems. The parasympathetic fibers are cholinergic and are derived from the vagal trunks, whereas the sympathetic fibers are adrenergic and are derived from the greater and middle splanchnic nerves (31).

Evidence of a role for the autonomic nervous system in the control of insulin, glucagon, and somatostatin secretion from the pancreas is based on studies in which either the vagus or splanchnic nerves have been stimulated or sectioned, or in which adrenergic receptor agonists and antagonists have been used (39, 40). A postulated role for the brain in regulating the secretion of pancreatic islet hormones is derived from studies using stimulation or ablation of various areas of the hypothalamus. Electrical stimulation of the ventromedial nucleus of the hypothalamus increases plasma

glucagon and inhibits insulin secretion (41). On the other hand, electrical lesions of the ventromedial hypothalamus induce acute hyperinsulinemia (42) and hyperresponsiveness of the A and D cells to secretagogues (43). The hyperinsulinemia, hyperphagia, and obesity, which are consequences of the lesion, are abolished by vagotomy (44, 45). However, the mechanisms by which lesions of the ventromedial hypothalamus produce this parasympathetic overactivity are unclear, and it has been proposed that the syndrome is only seen when the lesion damages the nearby ventral noradrenergic bundle (46), suggesting that alterations in central catecholamine pathways may produce functional alterations of the autonomic nervous system.

From the results of the studies presented here and from the foregoing review, we postulate that the alterations in central catecholamine stores produced by FLA-63 and α-MT also change the functional state of the autonomic nervous system by altering the synaptic input at the level of the intermediolateral column of the spinal cord. The alteration in autonomic nervous system activity may in turn result in changes in pancreatic TRH concentrations. This study has provided a demonstration that the content of a neuropeptide in a peripheral organ may be altered by simply manipulating catecholamine concentrations within the CNS. Moreover, the findings demonstrate that the concentration of a pancreatic peptide may be critically dependent upon a dynamic interaction involving the brain catecholaminergic pathways. These studies have not excluded the possibility that the alterations in pan-

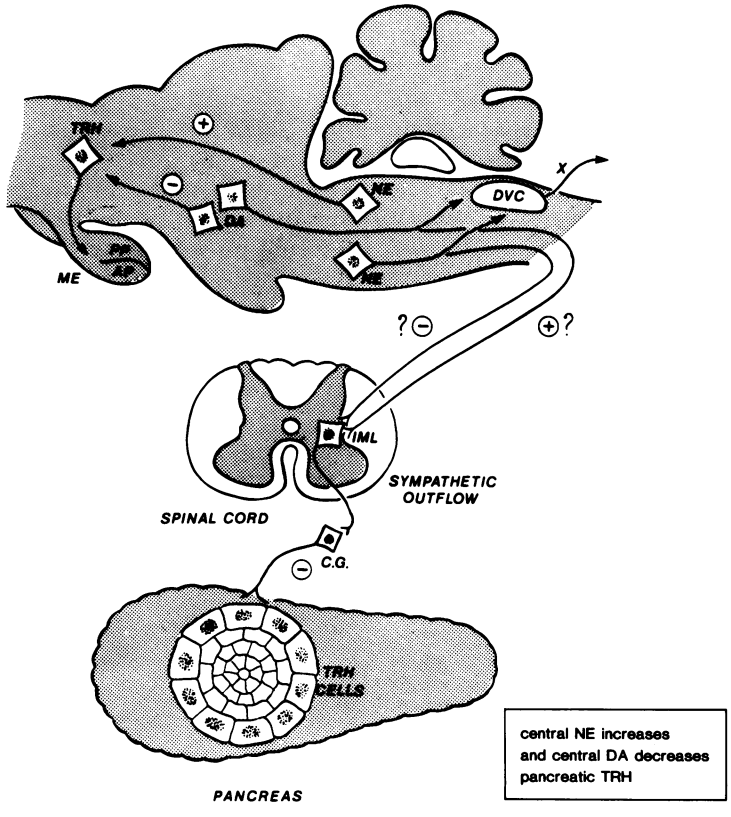

FIGURE 6 Schematic representation of the catecholaminergic regulation of TRH in the brain and pancreas of the rat. The experiments suggest that within the CNS, NE stimulates and DA inhibits brain TRH. Because sympathectomy leads to an increase in pancreatic TRH, the sympathetic outflow is inhibitory to pancreatic TRH. Although the precise way in which the descending catecholaminergic fibers regulate pancreatic TRH is not known, one possible mechanism is by noradrenergic fibers exerting inhibitory and DA fibers exerting stimulatory effects on preganglionic sympathetic neurons in the spinal cord (50). However, the central catecholaminergic pathways could also modulate pancreatic TRH by an interaction with the dorsal vagal complex, one of the sites of origin of the parasympathetic innervation of the pancreas (51, 52). The marginal location of the TRH cells in the pancreatic islets is based on the work of Koivusalo et al. (17). AP, anterior pituitary; PP, posterior pituitary; ME, median eminence; DVC, dorsal vagal complex; X, vagus nerve; IML, intermediolateral column; CG, celiac ganglion.

creatic TRH concentrations may have been partly due to changes in the secretion of epinephrine. This adrenomedullary catecholamine has been shown to be an important modulator of insulin, glucagon, and somatostatin secretion (40, 41).

It is of interest that other interrelationships between TRH and the autonomic nervous system have recently been demonstrated. Brown et al. (47) have reported that the icv administration of TRH causes an adrenal medullary-dependent hyperglycemia by an action on the central sympathetic outflow. Additionally, Holaday et al. (48) have shown that systemically injected TRH raises the blood pressure by an action on the sympathetic nervous system. It would therefore appear that the sympathetic nervous system can modify the metabolism of TRH, and that TRH in turn is capable of modulating the function of the sympathetic nervous system. We have recently shown that pancreatic secretion is an important source of TRH in the systemic circulation of the neonatal rat (19). It is therefore possible that alterations in sympathetic nervous system tone could alter the concentration of circulating TRH by an effect at the level of the pancreatic islets. Whether circulating TRH could in turn modulate the activity of the sympathetic nervous system as has been shown for circulating insulin remains to be determined (49).

We have summarized the results of these experiments diagrammatically in Fig. 6. These studies have pointed to a local role for both brain norepinephrine and DA in controlling brain TRH secretion or synthesis, and have suggested that the directions of influence of these neurotransmitters are opposite. Additionally, they have demonstrated that these brain amines exert a dual, opposing influence on pancreatic TRH secretion or synthesis, and that the sympathetic nervous system is involved in mediating this action.

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