

Regulation of Episodic Growth Hormone Secretion by the Central Epinephrine System

STUDIES IN THE CHRONICALLY CANNULATED RAT

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ABSTRACT Catecholamines are postulated to regulate growth hormone (GH) secretion by their influence on the release of two hypothalamic substances, somatostatin, which inhibits GH release, and GH-releasing factor, as yet unidentified. Extensive pharmacologic studies in man and animals indicate a stimulatory effect of central norepinephrine and dopamine on GH, but the function of epinephrine (EPI) is uncertain. Furthermore, many of the agents used to study the role of catecholamines in GH regulation are not selective in that they affect adrenergic as well as noradrenergic and/or dopaminergic neurotransmission. In the present investigation, central nervous system (CNS) EPI biosynthesis was selectively interrupted with the specific norepinephrine *N*-methyltransferase inhibitors, SK&F 64139 (Smith, Kline & French Laboratories) and LY 78335, (Eli Lilly & Co. Research Laboratories) and the effects of central EPI depletion on episodic GH secretion in the chronically cannulated rat model were determined. Inhibition of CNS EPI synthesis with SK&F 64139 caused complete suppression of episodic GH secretion and concomitantly reduced the EPI level in the hypothalamus without affecting dopamine or norepinephrine. Administration of LY 78335 produced similar effects on pulsatile GH. Morphine-induced, but not clonidine-induced, GH release also was blocked by SK&F 64139. These results indicate that (a) the central EPI system has a major stimulatory function in episodic GH release, (b) morphine-induced GH release is mediated by the central EPI system, and (c) clonidine stimulates GH release by activation of postsynaptic α -adrenergic receptors.

Drugs that affect CNS adrenergic systems have a potential role in the diagnosis and treatment of disorders of GH secretion.

INTRODUCTION

Growth hormone (GH)¹ secretion by the pituitary gland appears to be regulated by two hypothalamic hormones, one stimulatory (GH-releasing factor) and the other inhibitory (somatostatin). Although there is substantial physiological and biochemical evidence for the existence of GH-releasing factor, its structure has not been determined (1). Somatostatin has been characterized as a tetradecapeptide (2). Extensive studies in man and experimental animals suggest that the release of somatostatin and GH-releasing factor is regulated by catecholamine-containing neurons in the central nervous system (CNS) (1, 3).

Catecholamines were first implicated in GH regulation in man by the demonstration that insulin- and vasopressin-induced GH secretion was partially inhibited by the α -adrenergic blocker, phentolamine (4). It was subsequently shown that L-dopa stimulates GH secretion in man (5, 6). Because L-dopa is a metabolic precursor of dopamine (DA), norepinephrine (NE), and epinephrine (EPI), it became essential to examine the effects of more selective dopaminergic and noradrenergic drugs on GH release. For example, CNS α -adrenergic receptor stimulation with clonidine elicits GH release in man (7) and a number of lower species (8-11). Evidence concerning the role of brain DA in GH control is less consistent. In man, CNS DA receptor

L. C. Terry is the recipient of a Clinical Investigator Award from the Veterans Administration.

Received for publication 27 March 1981 and in revised form 9 September 1981.

¹ Abbreviations used in this paper: CNS, central nervous system; DA, dopamine; EPI, epinephrine; GH, growth hormone; NE, norepinephrine; NMT, norepinephrine *N*-methyltransferase; PRL, prolactin.

stimulation with apomorphine causes GH release (12). However, activation or blockade of DA receptors in experimental animals has little effect on GH (8–10, 13), indicating that its effects may be specific to man.

Serial measurements of plasma GH levels in man and experimental animals show abrupt, spontaneous fluctuations (1, 14, 15). For these oscillations to occur, the hypothalamic-pituitary connections must be intact (16). Because episodic GH secretion is particularly prominent in the male rat, this animal has provided a useful experimental model for the study of the adrenergic mechanisms involved in its regulation. In rats, a variety of noradrenergic synthesis inhibitors and receptor antagonists block the pulsatile secretion of GH (3). Moreover, noradrenergic antagonists prevent the increase in GH induced by morphine (17).

In all the previous studies, the noradrenergic agonists and antagonists used may also have affected transmission in EPI (adrenergic) systems. Results of earlier experiments suggest that EPI stimulates GH in primates (18) and rodents (19, 20) but not in man (21–24). The availability of several selective EPI synthesis inhibitors, developed for possible use as antihypertensive agents in man (25–28), have made it possible to alter the synthesis of EPI without affecting NE or DA. These compounds have been used in the present investigation to assess the involvement of brain EPI systems in regulation of episodic and morphine-induced GH secretion in the rat. Results of this study indicate that the CNS adrenergic system has a major function in the regulation of GH secretion.

METHODS

Animals

Male Sprague-Dawley rats (300–350 g, Harlan Industries Inc., Indianapolis, Ind.) were housed in temperature- and humidity-controlled laboratories with a 12–12-h light-dark cycle (lights on at 0600). The animals were given free access to laboratory chow and water.

Drugs

The selective EPI synthesis inhibitors, SK&F 64139 and SK&F 29661 (7,8-dichloro-1,2,3,4-tetrahydroisoquinoline and 1,2,3,4-tetrahydroisoquinoline-7-sulfonamide, respectively; Smith, Kline & French Laboratories, Philadelphia, Pa.), which act by inhibiting norepinephrine *N*-methyltransferase (NMT, EC 2.1.1.28), were dissolved in 0.15 M NaCl (pH 7.0) to yield a final concentration of 25 mg/ml and administered intraperitoneally in doses of 10, 25, and 50 mg/kg body wt. A third NMT inhibitor, LY 78335 (2,3-dichloro- α -methylbenzylamine; Eli Lilly & Company Research Laboratories, Indianapolis, Ind.) was dissolved in the same vehicle and administered intraperitoneally in doses of 25 and 50 mg/kg body wt. SK&F 64139 and LY 78335 cross the blood-brain barrier and inhibit central and peripheral EPI synthesis (26, 28). SK&F 29661 inhibits only peripheral EPI synthesis, be-

cause it does not cross the blood-brain barrier (27). The α -agonist, clonidine (Boehringer Laboratories, Montreal, Quebec, Canada) was given intravenously in a dose of 150 μ g/kg. Morphine sulfate (Elkins-Sinn, Inc., Cherry Hill, N. J.) was diluted in 0.15 M NaCl to a final concentration of 0.5 mg/ml and administered intravenously in a dose of 3 mg/kg.

Experimental procedure

Experiment 1. Chronic indwelling cannulae were placed in the right atrium via the right external jugular vein and adapted to isolation test chambers by methods described previously (29). Sampling began after recovery of preoperative body weight (usually 1 wk postoperatively). Blood samples ($4 \times 50 \mu$ l) were withdrawn every 15 min and immediately centrifuged. The plasma was frozen at -90°C until radioimmunoassay of GH and prolactin (PRL), and erythrocytes suspended in normal saline were returned to animals at the time of the next sample. Individual rats were usually used as their own control, receiving control injections on one experimental day and the drug(s) to be tested on a subsequent day. Animals (eight per group) received either the centrally active EPI synthesis inhibitors, SK&F 64139, and LY 78335, the peripherally active, SK&F 29661, or saline vehicle at 0930 and were sampled for 3 or 6 h beginning at 1000 to encompass one or two GH secretory episodes. The animal's behavior was monitored through one-way observation ports in the cubicles.

In a parallel experiment, male rats (eight per group) were killed by cervical dislocation 1.5, 2.5, and 3.5 h after receiving either SK&F 64139, SK&F 29661, or normal saline at 0930. Their brains were removed immediately, and hypothalami were dissected, weighted, snap-frozen on dry ice, and stored at -90°C for subsequent extraction and determination of DA, NE, and EPI. The hypothalamic dissection was limited rostrally by the optic chiasm, caudally by the mammillary bodies, and laterally by the hypothalamic sulci, and the dorsal cut was ~ 2 mm from the base of the brain.

Experiment 2. Clonidine or vehicle was given to SK&F 64139-pretreated animals at 1105 by the methods described for Experiment 1.

Experiment 3. To determine whether blockade of central EPI synthesis affected morphine-stimulated GH, morphine sulfate (3 mg/kg i.v.) or normal saline was administered at 1105 to animals pretreated with either SK&F 64139 or vehicle at 0930. Samples were removed from 1000–1300, as described above.

GH and PRL radioimmunoassays

GH and PRL were measured in duplicate by double-antibody radioimmunoassays with materials supplied by the National Pituitary Agency, as described previously (29). Values were determined using the weighted Rodbrad method (30) and expressed in terms of the National Institute of Arthritis, Metabolic, and Digestive Diseases reference preparation, rGH-RP-1 and rPRL-RP-1. The minimum detectable amount of GH was 2.5 ng/ml, and the intraassay and interassay coefficients of variation were 3.8 and 9.6%, respectively (means of 10 determinations). The minimum detectable amount of PRL was 1.0 ng/ml, and the intraassay and interassay coefficients of variation were 3.4 and 10.9%, respectively (means of six determinations).

Catecholamine assay

Concentrations of EPI, NE, and DA in the hypothalamus were measured by a sensitive radioenzymatic assay. The catecholamines were extracted according to the method of Sole and Hussain (31) and separated by thin-layer chromatography according to Peuler and Johnson (32). The minimum detectable level of each catecholamine was 10 pg. All samples were measured in the same assay to avoid interassay variation.

Analysis of data

The secretory profiles of GH were compared in drug-treated and control animals to determine (a) the effect of SK&F 64139, SK&F 29661, and LY 78335 on pulsatile GH and PRL release and (b) the effects of clonidine and morphine on GH secretion in vehicle- and/or SK&F 64139-pre-

treated animals. The effects of saline vehicle, SK&F 64139, SK&F 29661, and LY 78335 on GH and PRL levels over time were analyzed by two factor, repeated measures analyses of variance (drugs \times time). In addition, single-factor analyses of variance, followed by Newman-Keuls tests, were used to compare (a) plasma GH and PRL levels in SK&F 64139-, SK&F 29661-, and LY 78335-treated rats and (b) plasma GH levels from 1100 to 1300 in vehicle- or SK&F 64139-pretreated animals that received drugs at 1105. Single-factor analyses of variance and Newman-Keuls tests (33) also were used to compare tissue levels of catecholamines 1.5, 2.5, and 3.5 h after receiving SK&F 64139, SK&F 29661, or vehicle. $P < 0.05$ was considered significant.

RESULTS

Effects of SK&F 64139, SK&F 29661, and LY 78335 on episodic GH and PRL secretion. The pattern of

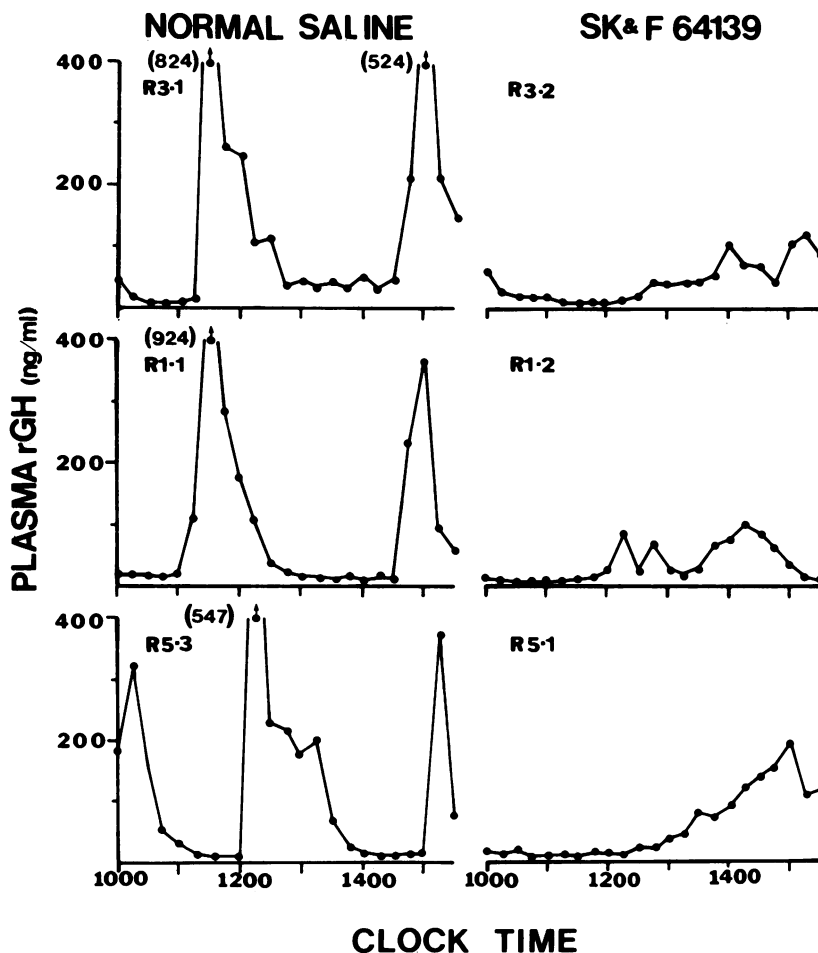


FIGURE 1 Effects of SK&F 64139, a central EPI synthesis inhibitor, on episodic GH secretion in individual male rats sampled for 5.5 h. Normal saline-treated (intraperitoneally) control animals showed typical episodic GH release with pulses occurring between 1100 and 1200 and between 1400 and 1500. SK&F 64139 (50 mg/kg i.p.) completely inhibited the first and partially suppressed the second pulse. Normal saline or SK&F 64139 were administered at 0930. "R" followed by a number in the upper left corner of each graph refers to individual rats.

GH secretion in individual animals confirmed previous observations (34) that male rats kept on a constant light-dark cycle have episodes of GH release that occur at approximately the same time (1100–1200 and 1300–1500) (Fig. 1, left). The centrally active SK&F 64139 suppressed ($P < 0.001$) pulsatile GH for 6 h (Fig. 1, right) in a dose-dependent manner (Fig. 2). Administration of the other centrally active EPI antagonist, LY 78335, also suppressed ($P < 0.001$) GH release, and this effect also was dose related (Fig. 3). However, the peripheral antagonist, SK&F 29661 (50 mg/kg i.p.) had no effect on GH when compared with the normal saline control group (Fig. 4).

SK&F 64139 caused a significant, dose-related increase in mean 3-h plasma levels of PRL (Table I). Administration of the peripheral EPI synthesis inhibitor, SK&F 29661, also elevated PRL levels (Table I). However, LY 78335 did not significantly affect plasma PRL when compared with the normal saline control group (Table I). None of the drugs caused a discernible change in the animals' behavior, and, specifically, there were no indications that the drug-treated animals were stressed.

Effects of SK&F 64139 and SK&F 29661 on hypothalamic EPI. As demonstrated previously (26, 35), the centrally active EPI synthesis inhibitor, SK&F 64139 (50 mg/kg i.p.), caused a significant reduction ($P < 0.05$) in the hypothalamic concentration of EPI in male rats from 1.5 to 3.5 h after administration, whereas SK&F 29661, the peripheral EPI synthesis inhibitor, had no effect when compared with time-

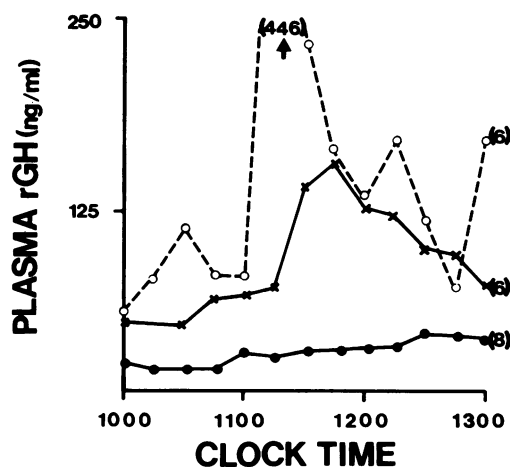


FIGURE 2 Dose-related suppression of episodic GH secretion by SK&F 64139 administered at 0930. SK&F 64139 inhibited ($P < 0.002$) GH release in doses of 25 (\times) and 50 (\bullet) mg/kg i.p. The 10 mg/kg dose (\circ) did not suppress GH significantly. Standard errors of the mean were omitted for clarity. In this and subsequent figures numbers in parentheses at 1300 refer to the number of animals in each treatment group.

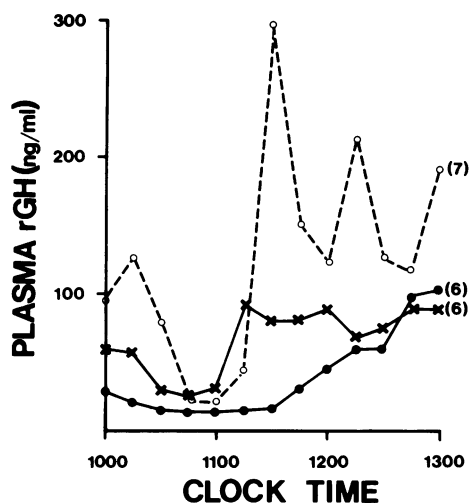


FIGURE 3 Dose-related inhibition of pulsatile GH release by the central EPI synthesis inhibitor, LY 78335, administered at 0930. Doses of 25 (\times) and 50 (\bullet) mg/kg i.p. inhibited ($P < 0.01$) GH. (\circ) Standard saline control. Standard errors were omitted for clarity.

matched controls (Fig. 5). Neither of the drugs affected tissue levels of DA or NE (Table II).

Effect of clonidine on GH in SK&F 64139-pretreated male rats. To test whether the inhibition of GH release by SK&F 64139 could be reversed by activation of central postsynaptic α -adrenergic receptors, clonidine (150 μ g/kg) or vehicle were administered at a time (1105) that coincided with the anticipated occurrence of a GH burst, as had been documented in the same animal on a previous control day. In SK&F 64139-pretreated rats, clonidine caused a significant elevation in mean GH levels above saline-injected controls ($P < 0.0001$, Fig. 6). Clonidine-stimulated mean GH levels were comparable to those seen during spontaneous pulses in untreated animals.

Effect of morphine on GH in SK&F 64139-pretreated male rats. To determine whether EPI was involved in morphine-induced GH release, morphine sulfate (3 mg/kg i.v. at 1105) was administered to vehicle- or SK&F 64139-pretreated male rats. In the vehicle-pretreated group, morphine had an immediate stimulatory effect on GH that resulted in mean plasma levels $>1,000$ ng/ml (Fig. 7), significantly higher than the ($P < 0.01$) group receiving saline vehicle (not shown). Previous studies have shown that morphine-stimulated GH release in the rat is inhibited by naloxone (17), indicating that this is a specific effect due to activation of opiate receptors. In the present study, the stimulatory effect of morphine on GH was completely blocked by pretreatment with SK&F 64139 (Fig. 7).

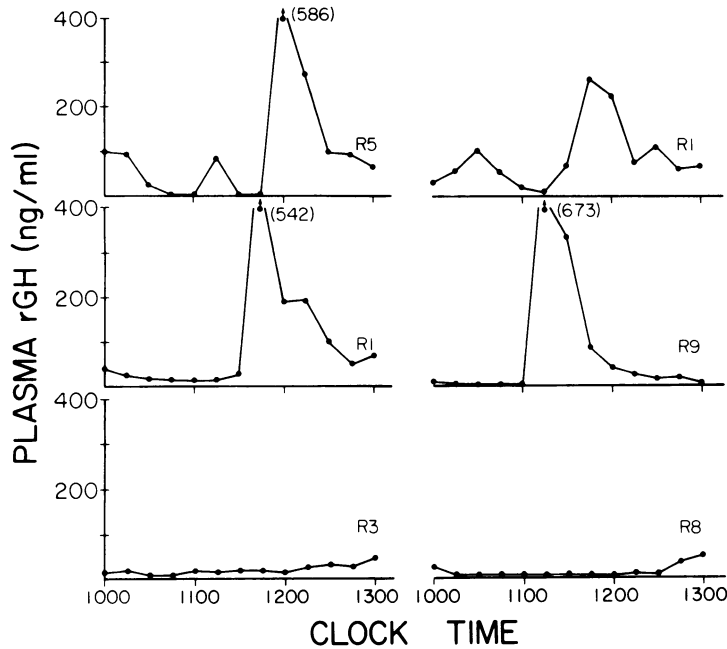


FIGURE 4 Effects of SK&F 29661, a peripheral (EPI) synthesis inhibitor, and SK&F 64139 on episodic GH secretion in individual male rats. Normal saline (intraperitoneally)- and SK&F 29661 (50 mg/kg i.p.)-treated animals showed pulsatile GH release (top and middle, respectively). SK&F 64139 (50 mg/kg i.p.) completely suppressed episodic GH secretion (bottom). The letter R followed by a number in the lower right corner of each graph refers to individual rats.

DISCUSSION

The results of this study show that inhibition of CNS EPI synthesis by SK&F 64139 and LY 78335 completely suppressed physiologic, pulsatile, GH release

TABLE I
Effects of SK&F 64139, SK&F 29661, and LY 78335 on Mean 3-h PRL Levels

	Plasma PRL
	ng/ml
Normal saline (i.p.)	4.7±1.1 (10)
SK&F 29661 (50 mg/kg i.p.)	20.2±4.8 (10)*
SK&F 64139 (i.p.)	
10 mg/kg	14.4±4.1 (6)*
25 mg/kg	25.8±7.5 (6)*
50 mg/kg	39.7±8.0 (9)*
LY 78335 (i.p.),	
25 mg/kg	6.5±1.5 (6)
50 mg/kg	9.7±1.3 (6)

Values are given as mean±SE. Numbers in parentheses indicate the number of animals in each group.

* $P < 0.05$ compared with normal saline.

in male rats. In contrast, the peripheral EPI synthesis blocker, SK&F 29661, had no effect on GH secretion. This indicates that inhibition of brain rather than ad-

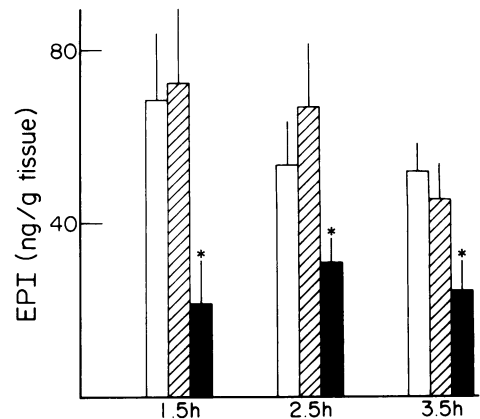


FIGURE 5 Effects of SK&F 29661 and SK&F 64139 on the concentration of EPI in the hypothalamus 1.5, 2.5, and 3.5 h after administration at 0930. SK&F 64139 (solid bar; 50 mg/kg i.p.) caused a significant reduction in EPI, but 29661 (striped bar; 50 mg/kg i.p.) had no significant effect. (open bar, normal saline intraperitoneally) Asterisk indicates $P < 0.05$. There were eight animals in each treatment group. Vertical lines represent SEM.

TABLE II
Effects of SK&F 29661 and SK&F 64139 on Hypothalamic Concentrations of DA and NE in Male Rats 1.5, 2.5, and 3.5 h after Injection

	Normal saline (i.p.)	SKF 29661 (50 mg/kg i.p.)	SKF 64139 (50 mg/kg i.p.)
	<i>ng/g tissue</i>		
DA			
1.5 h	223.5±17.8	213.4±9.9	242.8±6.5
2.5 h	221.8±11.4	219.6±10.3	250.1±11.8
3.5 h	206.1±16.6	190.9±13.3	231.2±15.0
NE			
1.5 h	1,498.5±60.8	1,384.0±63.0	1,313.0±36.0
2.5 h	1,503.0±118.0	1,433.0±111.0	1,327.0±7.5
3.5 h	1,275.2±61.3	1,300.0±62.0	1,300.0±84.0

Each group consisted of eight animals.

renal EPI synthesis is responsible. The inhibition of episodic GH secretion by SK&F 64139 was accompanied by a significant reduction in the hypothalamic concentration of EPI, whereas SK&F 29661 had no effect. Earlier studies have shown that LY 78335 inhibited hypothalamic EPI synthesis (28). None of these drugs altered hypothalamic NE nor DA.

Previous attempts to investigate the function of EPI in GH regulation in man and experimental animals were limited by the poor penetrability of the blood-brain barrier by EPI and the unavailability of agents that directly affect brain EPI neurotransmission. In

man, peripheral administration of EPI in doses sufficient to produce significant hyperglycemia did not induce GH secretion (21–23) nor did it inhibit arginine-stimulated GH release (24). However, large doses of EPI initiated a marked and prompt increase in plasma GH levels in female rhesus monkeys (18). Moreover, intracerebroventricular or intravenous EPI administration to rats caused release of bioassayable GH (19,

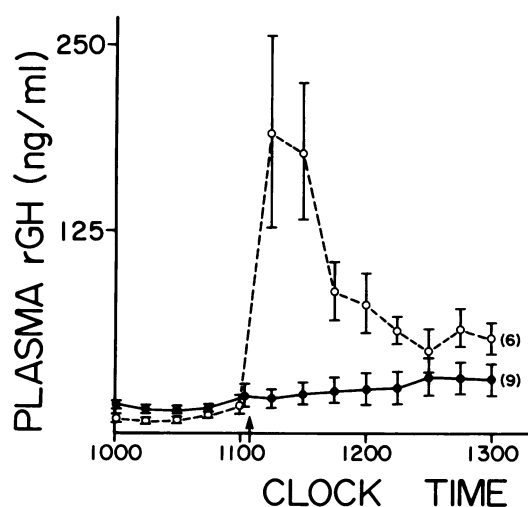


FIGURE 6 Effect of clonidine, a centrally active α -adrenergic receptor agonist, on GH secretion in SK&F 64139-pretreated (0930) male rats. Clonidine (O; 150 μ g/kg i.v.) administration at 1105 (indicated by arrow) caused a significant elevation in GH levels (●; saline intravenously at 1105). Vertical lines represent SEM.

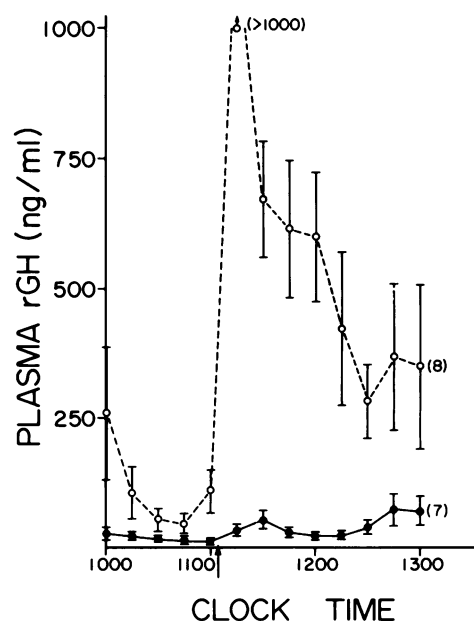


FIGURE 7 Effects of morphine sulfate on GH secretion in SK&F 64139(●, 50 mg/kg i.p.)- and normal saline (O, intraperitoneally)-pretreated (0930) male rats. Morphine sulfate (3 mg/kg i.v.) administration (1105, indicated by arrow) to saline-pretreated rats caused a significant elevation in GH levels. This effect was blocked completely by pretreatment with SK&F 64139. Vertical lines represent SEM.

20). Data from the present experiments, in which selective EPI antagonists were used, indicate that the central EPI system has a major function in the generation of episodic GH secretion in the rat.

Morphine and the endogenous opioid peptides, met-enkephalin and β -endorphin, have a potent stimulatory effect on GH secretion in numerous experimental animals, an effect prevented by the specific opiate receptor blocker, naloxone (1, 17, 36–38). Furthermore, the synthetic enkephalin analogue, FK-33-824, causes GH release in man (39). None of the opioids appear to act directly at the pituitary level, and it is likely that they influence the release of GH either by modulating the secretion of releasing-inhibiting factors and/or by affecting neurotransmitters (40, 41). Evidence supporting the latter hypothesis was provided in a recent study demonstrating that α -adrenergic receptor blockade with phenoxybenzamine or inhibition of NE and EPI synthesis with diethyldithiocarbamate, a dopamine- β -hydroxylase inhibitor, abolished the GH-releasing effect of morphine in the rat (17). These effects were attributed to noradrenergic mechanisms. Data from the present study suggest that morphine enhances GH secretion by activation of CNS EPI systems, because the selective EPI antagonist, SK&F 64139, completely blocked morphine-induced GH release. These results do not necessarily exclude a similar role for NE.

Previous studies have shown that clonidine restores pulsatile GH release in rats after blockade of NE and EPI synthesis with FLA-63, a dopamine- β -hydroxylase inhibitor (11). In the present study, clonidine administration to SK&F 64139-pretreated animals elevated GH to levels that occur during episodic release. Clonidine also stimulates GH secretion in man and several species of experimental animals (7–11). The stimulatory action of clonidine is most likely due to activation of postsynaptic α -adrenergic receptors. Clonidine activates postsynaptic or presynaptic EPI receptors in discrete brain regions (42). It is not possible at present to determine whether the effect of clonidine is due to activation of receptors normally occupied by NE or EPI. However, the ability of clonidine to stimulate GH release in SK&F 64139-treated rats demonstrates that the EPI synthesis inhibitor does not compromise the ability of the pituitary gland to release GH.

In addition to its NMT-inhibiting actions, SK&F 64139 has been reported to have some adrenergic blocking effects mediated via α_2 - and α_1 -adrenoreceptors *in vitro* (43). Thus, caution should be exercised when interpreting the interaction between SK&F 64139, EPI, and GH, because NMT inhibition or α -receptor blockade could produce similar effects on GH release. It is unlikely that these α -blocking actions can account for the inhibitory effects of SK&F 64139 on

pulsatile GH for several reasons. First, SK&F 64139 has little postsynaptic adrenergic blocking action, but does have presynaptic antagonistic properties (43). However, this would enhance NE and EPI release (44), and thus would not be expected to block GH release. Second, central EPI synthesis inhibition by LY 78335, which does not possess α -blocking properties (28), also inhibited GH secretion. Finally, postsynaptic α -blockade by SK&F 64139 would be expected to inhibit clonidine-stimulated GH release (45), but it did not.

The tetrahydroisoquinoline NMT inhibitors, SK&F 64139 and SK&F 29661, both elevated PRL levels. The mechanism underlying this effect is unknown but does not appear to be related to EPI synthesis inhibition, because elevation of PRL was not obtained with the benzylamine NMT inhibitor, LY 78335. It is possible that the SK&F compounds exert a direct action on pituitary lactotrophs unrelated to their effects on EPI synthesis.

The dissociation between the effects of the SK&F agents and LY 78335 on PRL also argue against the possibility that the drug-induced suppression of GH release is due to stress. Stress suppresses pulsatile GH release and stimulates PRL release in rats (46). Both SK&F 64139 and SK&F 29661 elevated plasma PRL, but only SK&F 64139 suppressed GH secretion. LY 78335 also inhibited pulsatile GH release but had no effect on PRL. These dissociations favor the interpretation that the inhibitory effects on GH are due to inhibition of central adrenergic neurotransmission.

Because octopamine can serve as an alternate substrate for NMT (47), in addition to EPI it is conceivable that the result of that interaction, or others like it, could have a role in CNS regulation of GH secretion. However, the distribution and role of octopamine in the mammalian CNS are unclear, and there are no reports that other known metabolic products of alternative NMT substrates are active at α -receptor sites within the CNS.

In summary, results of the present experiments indicate (a) that central EPI systems exert a major stimulatory effect on episodic GH secretion, (b) that morphine-stimulated GH release is mediated by central EPI systems, and (c) that clonidine enhances GH release by activation of postsynaptic α -adrenergic receptors. The selective EPI antagonist, SK&F 64139, is currently being investigated in man as an antihypertensive agent (48). This drug may also prove useful in the diagnosis and treatment of disorders of GH secretion.

ACKNOWLEDGMENTS

We especially thank Charles Longserre and Cecil Lynch for their sincere dedication and expert technical assistance. Mrs. Ann Marie Bobrowski provided excellent secretarial assistance.

tance. Drs. Solomon Solomon, John Whitaker, and Seymour Reichlin provided expert critical reviews of this work. The generous gift of SK&F 64139 and SK&F 29661 by Dr. R. Pendleton and Smith Kline & French Laboratories and of LY 78335 by Dr. R. Fuller and Eli Lilly & Company Research Laboratories is appreciated greatly.

We are grateful to the Research Service of the Veterans Administration and the National Institutes of Health (grants HD-13703 and GM 2666-20) for financial support. The generous provision of radioimmunoassay materials by the National Pituitary Agency is acknowledged.

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