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PHYSIOLOGY AND PHARMACOLOGY OF LHRH AND SOMATOSTATIN

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INTRODUCTION

In this review I will try to give a brief account of the current status of two of the hypothalamic peptides which were characterized on the basis of their actions on the anterior pituitary gland. The literature on this subject is vast and this review will not list all of the publications. In a number of cases we will refer to previous recent reviews. Both of these peptides have now been shown to have a much wider spectrum of action than originally envisioned. Somatostatin acts as an inhibitory peptide in many bodily systems, whereas luteinizing hormone releasing hormone (LHRH) appears to have widespread actions throughout the reproductive tract. For other references, the reader is referred to several excellent recent reviews (1–3).

LHRH

LH-releasing activity was first found in hypothalamic extracts utilizing the ovarian ascorbic acid depletion assay of Parlow to measure LH released following injection of acid extracts of stalk-median eminence (4). At about the same time similarly prepared extracts were shown to evoke ovulation following intrapituitary injection (5). The activity was rapidly purified, shown to be a peptide on the basis of enzymatic degradation studies, and ultimately its structure was determined in Schally's laboratory by Matsuo and confirmed by synthesis (6). The elucidation of structure and synthesis of LHRH paved the way for its synthesis in large quantities which made possible clinical studies, many more physiological studies, the development

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of antisera which could be used to study the physiological significance of the peptide, and the development of radioimmunoassay and immunocytochemical techniques to localize the peptide more precisely.

Localization of LHRH

It is now clear on the basis of bioassay, radioimmunoassay, and immunocytochemical localization that there are LHRH cell bodies in the septal-preoptic and anterior hypothalamic areas and that the axons of these neurons project particularly to the external layer of the anterior median eminence by passing caudally along a predominantly mediobasal course. The localization in the median eminence is presumably to permit release of LHRH into the hypophyseal portal capillaries. The axons also project to the organum vasculosum lamina terminalist (OVLT) and to more caudal structures (7). LHRH axons have been found even in brain stem regions thought to be involved in mating behavior (8). Recent evidence suggests that the distribution of LHRH and LHRH-like peptides is more widespread than originally thought and occurs throughout the reproductive system. LHRH has been found in the olfactory system by both immunoassay and immunocytochemistry (9). It has been found in testicular lymph by bioassay (10) and by immunoctyochemistry in the interstitial cells of the testis (11). An LHRH-like peptide, named gonadocrinin, has been detected in ovarian extracts by bioassay and apparently is even produced by granulosa cells in culture (12). LHRH-like materials have been reported in placenta (13) and also in milk (14). This leads to the suggestion that LHRH and similar peptides may be involved in reproductive processes at all levels.

Actions of LHRH on the Pituitary

LHRH acts on the pituitary to promote rapid release of LH and, to a lesser extent, follicle-stimulating hormone (FSH) from the gland. Pulse injection may lead to release of only LH; however, more prolonged exposure of the gland to the peptide leads to significant FSH release (15). The FSH-releasing potency is often of the order of 20% of the LH releasing potency; however, the FSH-releasing potency varies depending on the hormonal state of the animal and the frequency of LHRH injections. In immature rat and human the peptide tends to produce greater FSH release than in adults (16). Frequent pulses of the peptide provoke primarily LH release. However, with more infrequent pulses, a predominance of FSH release appears to occur, perhaps related in part to the longer half-time of disappearance of FSH from the circulation (17). Responsiveness to LHRH is quite high in the castrate (18). Following administration of estrogen to the castrate female, responsiveness declines rapidly in rat, man, and other species, and

this is followed by augmented responsiveness to the peptide (19, 20). In other words there is a biphasic response, first inhibition and subsequent augmentation in response, produced by estrogen. Progesterone appears to synergize with estrogen in the latter half of the cycle in rat and man to hold gonadotropin secretion in check (21, 22). Injection of progesterone can initially augment and then suppress responsiveness to the decapeptide in both rats and humans in the presence of estrogen (22, 23). Testosterone has an inhibitory action suppressing responsiveness (20).

During the menstrual cycle and rat estrous cycle, responsiveness to LHRH increases, presumably because of release of estrogen from the developing follicles. Responsiveness becomes maximal just prior to the preovulatory discharge of LH (20, 22, 24). This further enhancement in responsiveness is due not only to the action of estrogen but also to the so-called "self-priming action" of LHRH which augments response of the pituitary to subsequent LHRH (25, 26). The estrogen secreted by the follicles brings about enhanced LHRH release by an action on the preoptic region and this then produces the self-priming action. Responsiveness to LHRH may increase many fold above minimal levels at the time of the preovulatory release in man and rat. In the progestational phase of the cycle, responsiveness is suppressed, presumably because of inhibitory actions of both estrogen and progesterone. Responsiveness with regard to FSH release follows a similar pattern (20, 24).

There is clear evidence that there is increased LHRH release from the hypothalamus at the time of the preovulatory LH surge in rat (20) and suggestive evidence for this phenomenon in primates (27). However, Knobil's group has been able to reinitiate cycles by pulsatile administration of LHRH in Rhesus monkeys with arcuate nuclear lesions which suppress gonadotropin release. He proposes that the preovulatory surge in Rhesus monkey is due to invariant pulsatile release of LHRH from the region of the arcuate nucleus which leads to development of follicles and estrogen secretion (28). The estrogen then acts to augment responsiveness of the pituitary to this constant pulsatile release of LHRH, resulting in the preovulatory discharge. Spies' group, on the other hand, has been unable to induce cyclic release of gonadotropins with pulsatile LHRH administration in Rhesus monkeys with stalk sections and a permanent barrier placed between the cut ends of the stalk to block regeneration by portal vessels (29). In these stalk-sectioned monkeys the pituitary is presumably completely denervated, whereas it is possible that the arcuate nuclear lesions would leave intact the preopticotuberal LHRH pathway which could be stimulated by estrogen from the developing follicles and stimulate a preovulatory release of LHRH. Further work is necessary to resolve this issue.

Mechanism of Action of LHRH at the Pituitary

The initial event involved in LH-releasing action is combination with specific receptors on the surface of gonadotrophs. This has now been clearly demonstrated utilizing slowly degradable agonist analogs of LHRH (30). The numbers of receptors appear to fluctuate under various conditions; noteworthy is a decline in receptors at the time of the preovulatory surge of LH. Whether this decline is due to an actual decrease in receptor numbers or to occupancy by LHRH released in increased amounts at this time of the cycle remains to be determined.

According to one theory, the receptor hormone interaction activates adenylate cyclase, leading to generation of cAMP. This cAMP is visualized to act on a protein kinase which phosphorylates the cell membrane, leading to uptake of calcium. This then activates exocytosis of LH secretory granules (31). Unfortunately, the early enthusiasm for this concept is waning. It has not been possible to demonstrate increases in cAMP following exposure to LHRH in glands taken from female rats, although delayed increases in cAMP occur in males (32). Even in populations of purified gonadotrophs from females there was no increase in cAMP in spite of very lusty increases in LH release. On the hand, increases in cGMP in cells and in media have been demonstrated in certain conditions in response to LHRH (33). These observations suggested that cGMP might be more important than cAMP in the release mechanism. The delayed increases in cAMP seen in males might be involved in synthesis of LH. Recently, however, the essentiality of an increase in cGMP has been questioned, since it has been possible to obtain LH release in the face of treatments which hold cGMP levels constant (34). In the meantime, it is certain that increased availability of calcium is required for the releasing process, since removal of calcium from the medium or addition of calcium chelating agents blocks release (35).

It is now apparent that continued high dose administration of LHRH, although initially stimulatory, is inhibitory to gonadotropin release (17). The mechanism of this paradoxical inhibitory effect is not known but may involve down regulation of receptors. Apparently the gland can only accept pulsatile release of LHRH or it becomes desensitized in some manner to the peptide. This phenomenon is important to keep in mind when attempting to induce ovulation with LHRH.

Putative Synaptic Transmitters Involved in Control of LHRH Release

The possible roles of various transmitters in control of LHRH release are best considered under two modes of LHRH release. The first mode is the increased pulsatile release which occurs in the castrate. It occurs with a

certain period, characteristic of the species, as a single pulse of LHRH. The effect of the LHRH then dissipates and the LH released disappears from the circulation according to its half-life, to be followed by another pulse at the appropriate interval. The second mode is the preovulatory type of LHRH release which is brought about under normal conditions, mostly by estrogen, but in some species is also promoted by progesterone. Exogenous administration of both steroids will evoke the preovulatory type of release in the estrogen-primed animal. The evidence from the many studies carried out so far indicates that norepinephrine is important in the generation of the preovulatory LHRH release, probably by input via the ventral noradrenergic tract to the preoptic region. This statement is supported by the ability of lesions in the ventral noradrenergic tract to block preovulatory LH release, by the ability of norepinephrine injected into the ventricle to promote LH release in the estrogen-primed rat, and by the fact that lesions, or knife cuts, which interrupt the influence of the preoptic area over the basal tuberal region can block this mode of LH release. Inhibitors of norepinephrine synthesis also block this type of release and they are effective in blocking the release from preoptic electrochemical but not basal tuberal stimulation, suggesting that the noradrenergic synapse is located somewhere caudal to the point of stimulation in the preoptic region (36, 37).

There is a possibility that the endogenous opioid peptides may be involved in this release by a reduction in endogenous inhibitory tone at the time of the preovulatory release. This is supported by the facts that exogenous morphine (38) or opioid peptides (39) inhibit LH secretion and that naloxone injections to block opioid receptors augment the height of the surge (40). Serotonin may play a supportive role in the surge, since blockade of serotonin synthesis can suppress the surge (41).

Norepinephrine also seems to be important in the pulsatile release, because blockade of norepinephrine synthesis or administration of alpha receptor blockers can lower LH in the castrate and block the pulsatile release (36, 42). It is possible that this type of release is also partly under dopaminergic control because intraventricular injection of dopamine can increase LH release in the castrate (43). Furthermore, incubation of median eminence pieces from intact rats with dopamine can augment LHRH release and the response is blocked by pimozide, a dopamine receptor blocker (44). The results with dopamine receptor blockade in vivo have been equivocal. Sometimes a slight inhibition of castrate type release has been seen; at other times no inhibition and at other times even augmentation has occurred (45, 42). Furthermore, it is clear that high doses of dopamine or dopamine agonists given peripherally can inhibit LH release in the castrate, at least in part via inhibition of LHRH release (46, 47). Thus, dopamine apparently can inhibit or stimulate LH release depending on the circum-

stances. In the castrate high doses at least can inhibit. In the in vitro system it has been shown that the release of LHRH by dopamine and norepinephrine is greatly augmented by treatment of ovariectomized females with estrogen and progesterone (48).

A possible role for acetylcholine in generation of the pulsatile release is also evidenced by the ability of acetylcholine to increase LH release in castrates and of the muscarinic blocker, atropine, to suppress LH release in the castrate (49, 50). Acetylcholine appears to release LH via a dopaminergic step, since the response to acetylcholine is blocked by the dopamine receptor blocker, pimozide (50).

Recent studies in which conscious animals were cannulated and blood samples taken over a period of time, so that the pattern of pulsatile LH release could be constructed, indicate that there is a depletion of LHRH and dopamine from median eminence on the rising limb of the LH pulse, whereas norepinephrine in the suprachiasmatic region rises just prior to the pulse (51). This would suggest a stimulatory role for dopamine in generation of the pulses and that norepinephrine is probably also involved. Lesion studies by Soper & Weick (51) also fit with this concept in that pulsatile LHRH release was not blocked by anterior cuts or complete hypothalamic deafferentation nor was it blocked by lesions of the arcuate nucleus. However, when the lesions were combined blockade ensued. The arcuate region would be the source of the dopaminergic stimulatory drive, and the preoptic region the source of the noradrenergic drive. Apparently, pulsatile release can take place more or less in the absence of either of these drives, but if both are interrupted it ceases. One might postulate that the noradrenergic influence is exerted via fibers which reach the arcuate region, stimulate cholinergic neurons there, which in turn trigger release of dopamine, which then triggers LHRH release. Dendrites from the dopaminergic cells would synapse with the cholinergic neurons to suppress their activity. This would account for the interpulse interval and explains the dual actions of dopamine to both stimulate and inhibit LH release. The failure to see inhibitory actions of dopamine with the median eminence incubation system is presumably related to the fact that these inhibitory actions occur deeper within the hypothalamus in the region of the arcuate nucleus. It is also possible that the excitatory actions of dopamine involve different dopamine receptors than the inhibitory ones (53).

There may even be a role for the endogenous opioid peptides in the inhibition of pulsatile release, because naloxone administration to block opioid receptors is followed by an increase in pulsatile release (54). Other candidates to play a role in pulsatile release would be serotonin, which inhibits LH release in the castrate (55), and histamine (56), which stimulates it. However, the dose of histamine required to stimulate LH release

may be outside the physiological range. Furthermore, blockade of serotonin receptors does not alter pulsatile release (36). GABA clearly acts to stimulate LH release; however, since results with the GABA blocker, bicuculline, were equivocal, the physiological significance of GABA in LH release also remains to be established (57).

A veritable host of peptides have now been described in the hypothalamus and many of them have important actions on LH release. Most of the peptides have an inhibitory action. Cholecystokinin is the most potent inhibitor, acting in nanogram doses following intraventricular injection, to suppress LH release in the castrate (58). Larger doses of neurotensin and gastrin have a similar inhibitory action (59). Vasoactive intestinal peptide (VIP) and substance P can stimulate LH release. VIP is by far the most potent and is active in nanogram doses following intraventricular injection. It has been shown to act directly at the hypothalamic level, since it will release LHRH from a hypothalamic synaptosomal preparation (60).

Inhibin, the gonadal peptide, which appears to inhibit FSH release selectively by a pituitary site of action, can also act at the hypothalamus to suppress FSH release selectively (61). FSH release could be suppressed following intraventricular injection of purified inhibin preparations in the face of normal responsiveness to LHRH in terms of both FSH and LH release.

The selective inhibition of FSH release following intraventricular injection of purified inhibin preparations is further evidence for a hypothalamic FSH-releasing factor (FSH-RF). It is likely that such a factor indeed exists. It is postulated that it is another peptide similar to LHRH which may have overlapping action to release LH, just as the decapeptide has overlapping FSH releasing activity. On the basis of stimulation (62, 63) and lesion experiments (64), the postulated FSH-RF would be found in cell bodies of neurons in the dorsal anterior hypothalamic area. These neurons appear to project preferentially to the caudal median eminence and OVLT, where amounts of FSH-RF are much higher than can be accounted for by the content of LHRH (65, 66). The question of a separate FSH-RF can only be resolved by the isolation and determination of structure of this postulated peptide.

Lastly, there may be important interactions amongst the various releasing hormones at the hypothalamic level. Somatostatin, which on intraventricular injection had a paradoxical action to elevate growth hormone (GH) release, presumably by suppression of its own endogenous release or by augmentation of release of GH-releasing factor (GRF), also inhibited FSH, LH, and thyrotropin (TSH) release, suggestive of an inhibition of release of LHRH and TSH-releasing hormone (TRH) (67).

The catecholaminergic influence on release of LHRH is probably mediated by prostaglandin E_2, as indicated by comprehensive in vivo and in vitro studies (68).

Extra Pituitary Actions of LHRH

The distribution of LHRH outside the regions known to be involved in control of gonadotropin release suggests other possible actions of the peptide. The first such action to be discovered was its ability to induce mating behavior in ovariectomized, estrogen-primed rats, as indicated by an increase in the lordosis response to mounting by males (69). This effect can be obtained not only following peripheral, but also central, administration of the peptide into preoptic region, basal tuberal region, and brain stem regions which contain LHRH (70). LHRH can alter firing rate of neurons (71), antisera to the peptide appeared to inhibit mating behavior when injected intraventricularly (72), and inhibitory analogs of the peptide can also inhibit mating (71). Thus, the facilitatory action of LHRH on mating behavior seems to be established in the rat. It also appears to be effective in the hamster (71). The evidence for the effect of LHRH on mating in males is less convincing, although suggestive. The evidence that LHRH can enhance mating in man is equivocal, although there are a number of anectdotal accounts of such actions. Further work is necessary to establish the role of LHRH in human sexual behavior (71).

It is now clear that receptors for LHRH are also localized to ovary (73), testis (74), and possibly to uterus (75). As indicated before, there is evidence for an LHRH-like peptide in the ovarian follicles (12) and testicular interstitial tissue (10). It has been demonstrated that high dosage LHRH therapy can have inhibitory effects at the gonadal level (76). These were first thought to be caused by inhibition of gonadotropin release; however, they are still apparent in the hypophysectomized animal and can even be seen in in vitro incubations (77). High doses of the peptide or its agonist analogs can act locally on the gonads to suppress steroidogenesis, oocyte maturation, and follicular maturation (77). However, short term exposure to high doses of LHRH or its analogs have a stimulatory action and have been shown to evoke follicular maturation, steroidogenesis, oocyte maturation, and ovulation in hypophysectomized immature rats and can even manifest some of these effects in vitro (78, 79). It has recently been shown in hypophysectomized animals that hypothalamic stimulation can evoke steroid output by the ovaries (80). It is possible then that the intragonadal LHRH-like peptides may play a stimulatory role in the normal course of events in follicular maturation, oocyte maturation, and ovulation. These actions could be mediated via ovarian nerves which might contain LHRH or, alternatively,

other putative transmitters which would stimulate release of the LHRH-like peptides from the granulosa cells in the ovary. Further work is necessary to determine which of these possibilities is correct. In the meantime it appears that there are clear neural influences on gonadal function. For example, there is a unilateral change in LHRH content in the hypothalamus following unilateral castration (81).

The recent suggestion of LHRH receptors in uterus (75) raises the possibility that the peptide may be involved in tubal transport and uterine motility as well. There is evidence now for LHRH-like peptides in the placenta (13) and they could play a role in stimulation of placental gonadotropin release. The finding of LHRH in milk of rats by immuno and bioassay (14) and the fact that gonadotropin levels fell in plasma of the infants following isolation from the mother suggest a role for LHRH delivered via the milk in maintenance of gonadotropin secretion in the litter. Obviously this work needs confirmation, but it suggests another important role of LHRH in reproductive function.

In summary, it is possible now that LHRH in the olfactory system may be involved in detection of sexual odors, that it may be involved in induction of mating behavior, that the peptide may then be involved in ovulation by release of gonadotropins and possibly also by release of LHRH-like peptides within the ovary. There are postulated roles for LHRH-like peptides in the uterus and placenta, and LHRH in milk may even be important in maintenance of gonadotropin secretion in the litter. In other words, LHRH would be the most powerful reproductive hormone in the body concerned with reproduction at all levels in the organism.

Clinical Use of LHRH and its Analogs

With the synthesis of LHRH, it has been possible to show that the complete decapeptide structure is nearly essential for its activity. Removal of a single amino acid from the C terminal end causes a 90% loss of activity. Many analogs have now been synthesized, some which inhibit the action of LHRH and others which have markedly enhanced potency above that of the natural product. The superagonist analogs have been used in trials to evoke ovulation. It was with their use that the paradoxical inhibitory actions of LHRH were discovered. It is now apparent that with suitable regimens of intermittent LHRH therapy given either by subcutaneous injection (82), intranasal spray (83), or preferably by pulsatile minipumps (84, 85), which mimick the pulsatile surges of LHRH, ovulation can be induced in humans with the peptide and its analogs. LHRH has also been used as a diagnostic tool to differentiate between different causes of hypogonadism and to determine pituitary gonadotropin reserve (86). It is clear that LHRH tests are

never diagnostic in themselves. This should be apparent from the realization that the steroid background produces profound alterations in the responsiveness to the peptide.

It is possible that Kalman's syndrome may represent a congenital absence of LHRH, since it is associated with anatomical alterations in the same regions of the brain which normally contain the peptide (87). This is yet to be proven.

In view of its inhibitory action when given in high doses over a protracted interval of time, LHRH has now been tried experimentally in patients with prostatic cancer and has been shown to reduce steroid levels and even to reduce nocturnal penile tumescence (88). It may be of use in the treatment of this disorder; however, inhibitory effects on sex behavior might mitigate against this usage.

SOMATOSTATIN

While screening fractions obtained in purification of growth hormone (GH)-releasing factor (GRF) by Sephadex G-25 gel filtration, fractions were found which inhibited GH release by pituitaries incubated in vitro. There was no effect of the inhibitory zone on the release of other pituitary hormones. The inhibitory factor, named GH-inhibiting factor (GIF), blocked
vitro but appeared to have no effect on synthesis of GH. It was purified by further chromatography on carboxymethyl cellulose and analytical Sephadex columns. The early findings were obtained by bioassay of GH by the tibia test but these results were later confirmed using radioimmunoassay for the hormone and it was localized to various sites within the hypothalamus [for review, see (89)]. Several years later, Brazeau et al (90), taking advantage of a highly sensitive assay for the inhibitor, monolayer cultured pituitary cells, isolated, characterized, and synthesized the tetradecapeptide which they renamed somatostatin. The elucidation of structure and synthesis of the molecule was a breakthrough which paved the way to many additional studies of the physiology of GIF in a variety of species including man and to the development of antisera which made possible its radioimmunoassay and immunocytochemical localization. Somatostatin is probably the best example of a hypothalamic factor characterized on the basis of its action on the pituitary gland, which is now known to have widespread distribution not only throughout the brain but also in other tissues and to have an equally widespread action not only on the central nervous system but on the gastrointestinal tract and other organs as well.

Distribution of Somatostatin in Various Organs

It is clear from bioassay, radioimmunoassay, and immunohistochemistry that somatostatin has a widespread distribution. The highest concentrations are in the hypothalamus, as initially realized (89). There are somatostatin-containing nerve cell bodies in the paraventricular hypothalamus whose axons project to the median eminence to terminate in juxtaposition to hypophyseal portal capillaries there. After transport to the pituitary via the portal vessels, GIF conveys the inhibitory hypothalamic control over GH secretion. Other fibers terminate in the OVLT, which has neurohemal contacts and is a storehouse for a large number of other biologically active peptides. The function of somatostatin released in the OVLT is unclear. There are also numerous terminals with somatostatin immunoreactivity in the suprachiasmatic nuclei, and in the arcuate and ventromedial nucleus. The presence of GIF in the ventromedial nucleus was missed in early studies because extracts of this region released GH, presumably because of the presence of sufficient GRF in this region to overcome the inhibitory action of GIF [for review see (91)].

Somatostatin is also widely represented in the limbic system—for example, in the amygdala, hippocampus, nucleus accumbens, and olfactory tubercle—which suggests a role in vegetative nervous system functions (91). Apparently, fibers from the somatostatinergic neurons in the amygdala project to the median eminence, since declines in somatostatin in the median eminence follow lesions in the amygdala (93). Immunoreactive fibers have been localized to the caudate nucleus, cerebral cortex, and even in the substantia gelatinosa of the spinal trigeminal nucleus, as well as in the dorsal horn of the spinal cord. The endings in the substantia gelatinosa are central processes of small diameter afferent neurons (91). Somatostatin has also been localized to the retina (94). At the electron microscopic level somatostatin immunoreactivity has been demonstrated in synaptosomes (91).

Neural processes containing somatostatin immunoreactivity are found in the muscular coats of the GI tract distal to the stomach, particularly surrounding ganglion cells of Auerbach's plexus. Fibers are also found in the submucous plexus and at the base of crypts and glands. Cells containing somatostatin are also found amongst argentophylic cells of the submucosa throughout the GI tract (90).

The early findings that somatostatin can inhibit insulin and glucagon release (95) led to examination of the pancreas for its presence and it was found in the D-cells of the islets. These are located in the periphery of the islets adjacent to α-cells and at a further distance from β-cells (91). Pancreatic somatostatin has been isolated and has the same amino composition

and chromatographic characteristics as the tetradecapeptide (97). Somatostatin and β-endorphin have been colocalized to D-cells (98). The peptide has even been found in a parafollicular position in the thyroid gland (91).

Paravertebral sympathetic ganglion cells even contain somatostatin, and in this case the neurons were also adrenergic, as indicated by staining after treatment with antisera to dopamine beta oxidase (91). This widespread distribution of the peptide suggests its role in other systems and we will review this briefly.

Considerable work now suggests that somatostatin may be synthesized as other peptides as a precursor molecule which is then split and released. There is evidence for amino terminal elongation of the molecule to provide 25 and 28 amino acid forms (99–101). The 28 amino acid form is biologically active; however, its spectrum of activity seems to be somewhat different from that of the tetradecapeptide. It is possible that these other forms may be released as such and have physiological significance. The nucleotide sequence has been elucidated for a cloned structural gene coding for a precursor of somatostatin (102). An 18,000 dalton polypeptide, designated preprosatostatin, has been synthesized using mRNA isolated from angler fish islets and translated in the wheat germ cell free protein-synthesizing system (103).

Regulation of Release of Somatostatin from the Hypothalamus

The release of the peptide from basal hypothalamic fragments or median eminence fragments has been evaluated using in vitro static incubation systems. It is probable that the peptide is released by depolarization of cell membranes, since high potassium media increase its release (104). Release of somatostatin from neurohypophysis and median eminence tissue in vitro is also increased by electrical stimulation (104). Increased availability of calcium is required for release of the peptide, as is the case with other putative transmitters (104). Low doses of dopamine released somatostatin from the median eminence and the effects were blocked by the dopamine receptor blocker, pimozide, whereas higher doses of norepinephrine were stimulatory and these effects were blocked by the alpha receptor blocker, phentolamine (105). Sensitivity of the median eminence to the somatostatin-releasing actions of dopamine and norepinephrine was decreased by ovariectomy and increased by estrogen and progesterone replacement therapy (48). GABA inhibited somatostatin release from hypothalamic cells in culture and the action was blocked by the GABA blocker, bicuculline (106). Evidence has been presented suggesting that opioid peptides may inhibit release of somatostatin (107).

Release of somatostatin in vivo has been followed in hypophyseal portal blood by collection of blood from the cut stalk of male rats anesthetized with urethane. Somatostatin concentrations increased after intraventricular injection of dopamine, norepinephrine, or acetylcholine, but were unaffected by serotonin (108). Thus, both in vivo and in vitro studies support a catecholaminergic control of somatostatin release and the in vivo data suggest a stimulatory role for acetylcholine as well. Recently, increased somatostatin has been detected in hypophyseal portal blood of the rat following lateral ventricular injection of neurotensin (109). The effect was blocked by the H_1 receptor blocker, diphenhydramine.

In contrast to the situation with LHRH, prostaglandins appear to have no clear role in the release of somatostatin; however, they may be involved in the release of GRF, since prior injection of anti-somatostatin serum resulted in a remarkable enhancement of the GH release in response to intraventricular PGE_2. Thus, the increase in GH release induced by PGE_2 is not caused by inhibition of somatostatin release but rather is brought about by release of GRF (110).

GH suppresses its own release via a short loop feedback mechanism (111). That this may involve somatostatin is suggested by the decreases in somatostatin content observed in median eminence and other hypothalamic areas after hypophysectomy (112).

Somatostatin may also act via an ultrashort loop feedback to suppress its own release, since intraventricular injection of the peptide led to a paradoxical rise in plasma GH (67). It may also suppress release of LHRH and TRH, since plasma levels of FSH, LH, and TSH fell.

Action of Somatostatin on the Pituitary Gland

The early findings (89) have been confirmed by later work and indicate that somatostatin has a dramatic inhibitory effect on the release of GH by the somatotrophs. TSH release is also substantially inhibited (113). Under certain conditions release of prolactin can also be blocked, but there is little effect on release of ACTH, FSH, and LH; however, recently somatostatin has been reported to block ACTH release in vitro (114). It is possible that, given in sufficient dosage, the inhibitor could block release of all pituitary hormones.

Somatostatin is a powerful suppressor of GH release both in vitro and in vivo in animals and man. In man, it blocks the GH release in response to exercise, insulin-induced hypoglycemia, L-Dopa injection, and injection of arginine (2). In rat it blocks induction of GH release following pentobarbital anesthesia, electrical stimulation of the ventromedial and dorsolateral amgydala, and episodic GH surges (115). These effects of somatostatin on GH levels are shortlived because of the rapid disappearance of the hormone

from the circulation. In man somatostatin infusion suppresses the release of TSH in response to TRH (113).

The actions of somatostatin to inhibit GH and TSH release appear to have physiological significance, since antisera to the hormone can elevate levels of GH and TSH in rats. From such studies with antisera it appears that somatostatin is involved in inhibition of GH release in response to stress in the rat and for the suppression of GH release during starvation in this species (115). Basal TSH levels are elevated following injection of antisomatostatin serum and there is enhancement of both cold-induced and TRH-induced TSH release in the rat, indicating a physiological role of somatostatin to suppress TSH release (116).

The mechanism of action of somatostatin to inhibit the somatotrophs has not been completely clarified. It is probable that the initial action of somatostatin is to combine with specific receptors on the cell surface (117, 118); however, there has been little work to define these receptors, probably because of the rapid degradation of the peptide. The hormone was found to inhibit in vitro secretion of GH induced by a number of secretagogues, such as cyclic AMP derivatives, prostaglandins, and theophylline, as well as hypothalamic extracts (119). Since the hormone inhibits the response to cyclic AMP and prostaglandins, this would suggest an inhibitory action beyond the actions of these two agents in stimulation of GH release. Further work also suggests that the hormone may block uptake of calcium or release of calcium from intracellular sites important in release of GH (120). On the other hand, it has been reported in other studies that somatostatin inhibits cyclic AMP accumulation in anterior pituitaries in vitro, which suggests an effect on the adenylate cyclase system (118). It is likely that there are actions of both sorts, an action to inhibit cyclic AMP formation and also an action to inhibit calcium mobilization.

Actions of Somatostatin on the Nervous System

The widespread distribution of the peptide within the CNS certainly speaks for CNS actions. It was first shown that somatostatin will antagonize strychnine convulsions and enhance the duration of barbiturate anesthesia (121). It also has actions in a number of other behavioral tests (122) and will induce barrel rotation, a peculiar longitudinal rotation usually to the left side, when injected into the ventricular system (123).

Intraventricular injection of the peptide inhibited both feeding and drinking behavior, suggesting possible inhibitory roles in the control of these vegetative functions which appear reasonable since the peptide is localized in the vicinity of ventromedial and lateral hypothalamic areas known to be involved in these behaviors (123).

In line with the postulated inhibitory effects, somatostatin has been shown to have inhibitory effects on firing of central neurons. However, increased firing has been found recently in a number of loci in the rat cortex (124). It is possible in these instances that the iontophoretically applied somatostatin may have inhibited inhibitory neurons, thereby releasing other neurons from inhibition. It inhibits cAMP accumulation in cultured mouse brain cells (125).

Localization of somatostatin to dorsal root ganglia and fibers projecting both peripherally and centrally from these ganglia suggests a role for somatostatin as an inhibitory transmitter in sensory pathways (91).

Its inhibition of release of acetylcholine in the myenteric plexus (126) has been confirmed, suggesting that this is one mechanism of its inhibitory action there. Extrapolating from these results, we see that it may also act centrally by inhibiting cholinergic pathways in the CNS. The peptide has recently been reported to inhibit release of labeled norepinephrine from slices of hypothalamus but not cortex (127).

Actions of Somatostatin on the Gastrointestinal Tract

It was early shown that somatostatin could inhibit salivary secretion and it has recently been demonstrated in the ducts of the submaxillary gland in the monkey, where it may play a physiologically significant inhibitory role (128).

Somatostatin given peripherally can suppress gastric acid, gastrin, and pepsin secretion and also gastric emptying (129, 130). It has been found in the pyloric antrum and could represent the long sought antral chalone which has been extracted earlier but never isolated (131).

The release of all known gastrointestinal hormones—gastrin, secretin, cholecystokinin, gastric inhibitory peptide, vasoactive intestinal peptide, and motilin, is inhibited (132). The peptide may have a paracrine action in the sites of secretion of these hormones to suppress their release.

In addition to suppressing gastric emptying and inhibiting gastrin secretion, the peptide depresses duodenal motility, pancreatic exocrine secretion (133), and gall bladder contractions, the latter probably via its action to inhibit cholecystokinin release (132). It even blocks ion transport in the colon (134). Somatostatin inhibits gluconeogenesis in cultured liver cells in response to VIP, GIP, and secretin (135).

In addition to the actions on the GI tract itself somatostatin appears to have actions in the portal circulation to dilate vessels there (136, 137).

Recently, it has been shown that somatostatin can inhibit the formation of stress ulcers in rats (138) and it has been used to treat oesophageal bleeding and ulcer in man (139, 140). Amazingly enough, years ago we showed that crude hypothalamic extracts would block ulcer formation in

rats but never published the results (I. E. Danhof, A. P. S. Dhariwal, and S. M. McCann, unpublished data, 1967). The evidence now would suggest that these results were due to the presence in the extracts of somatostatin, or possibly bombesin, which also inhibits gastric function.

Action of Somatostatin on the Pancreas

With the discovery of the inhibitory effect of synthetic somatostatin on insulin and glucagon release and its localization to the D-cells of the pancreas, it appeared that somatostatin might have a paracrine function to suppress glucagon and, to a lesser extent, insulin release (96, 132). The mechanism may be as in the pituitary via decreases in cyclic AMP and/or decreased availability of calcium. Antisomatostatin serum enhances both glucagon and insulin secretion by isolated islets, which suggests a physiologically important pararcine action of the peptide (142).

Unger and his coworkers have shown that somatostatin is released into the venous effluent from the isolated perfused pancreas in response to high concentrations of glucose, an amino acid mixture, or a number of gastrointestinal hormones including gastrin, cholecystokinin, secretin, and gastric inhibitory polypeptide (132). VIP (141), Substance P, and neurotensin also release somatostatin (143). Glucagon, itself, is a potent stimulant of somatostatin release from islets (144). Others have also reported that glucose can cause release of somatostatin (145). Increases in portal and arterial blood somatostatin occur after feeding of various meals (146, 147). Furthermore, inhibition of absorption of nutrients following a protein and fat meal (148) was induced by somatostatin injections. These findings led to the suggestion that somatostatin is released from the D-cell and that this release is increased during elevations in concentrations of nutrients and gastrointestinal hormones. Pancreatic somatostatin may regulate the absorption of nutrients from the gastrointestinal tract in coordination with insulin-mediated regulation of nutrient disposal (132).

Other Actions of Somatostatin

Somatostatin has widespread inhibitory actions in other parts of the body. For example, it appears to inhibit furosimide-induced secretion of renin (149). It has recently been found in the bladder of the toad (150), which is involved in excretory function, and even in the renal tubules in mammals (151). So it may have important inhibitory actions in the renal tubules (150).

Somatostatin also inhibits release of parathyroid hormone and calcitonin (152, 153), which would fit with its general inhibitory action on release of all hormones of gastrointestinal origin.

It has been shown to alter aggregation of platelets (149) and to block leukocytosis in response to endotoxin. It even inhibits granuloma formation (154). Release of histamine has been reported to be unaffected or stimulated by somatostatin (155). This is the only example of what would appear to be a stimulatory action of the peptide. Even actions on skeletal muscle have been found in which somatostatin alters release of amino acids from muscle (156); however, no action on cardiac muscle has yet been described.

Thus it is clear that somatostatin is a powerful inhibitor in a number of systems including central and peripheral neural, anterior pituitary, gastrointestinal tract, vascular smooth muscle function, and even, to a limited extent, skeletal muscle. It also appears to be active in the kidney. The mechanism of action may be similar at all sites but has not been completely elucidated. It may involve decreases in cyclic AMP and almost certainly actions beyond the cyclic AMP step, such as to block uptake or release of calcium associated with activation of cellular function. From all of these findings, it appears that the name "GH-inhibiting hormone" as well as the name "somatostatin" are clearly misnomers. A more appropriate name in English would be the inhibitor, or preferably the GrecoRoman contraction, panhibin. The term inhibin is in use already to signify a gonadal factor which has selective capability to suppress FSH secretion. It would seem possible that somatostatin could be inhibin, and yet FSH release is one of the few pituitary functions not markedly suppressed by somatostatin. We propose to rename somatostatin or growth hormone inhibiting hormone, panhibin.

Analogs of Somatostatin

Because of its widespread inhibitory actions, there is the potential that somatostatin could be used to suppress hyperfunction in various organ systems. There are two problems with its use: its very short duration of action and its wide spectrum of effects. We have already alluded to its use in correcting oesophageal bleeding and acute ulcers. It might be useful in treatment of diabetes mellitus, except for the fact that it suppresses not only glucagon but also insulin release. Its very short duration of action has prompted attempts to prepare long acting analogues of somatostatin. Attempts have also been made to prepare analogues that would selectively suppress one or another function. Indeed, protamine zinc somatostatin has been claimed to have a longer duration of action than the natural product; however, a dissenting view has already been voiced (157). Many analogues of somatostatin have been prepared and some have actually been reported to have a more selective action to suppress glucagon than insulin release (158). Thus, somatostatin or its analogs still provide potential tools for

treatment of a number of disease processes. One of the disease processes which could potentially be treated would be acromegaly. There is no evidence as yet that acromegaly is due to a deficiency of somatostatin release, since there appear to be normal somatostatin levels in CSF in the disease (159). However, if long acting somatostatin preparations were obtained that selectively suppress GH release, this could be a medical treatment for acromegaly.

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