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A peptide as a possible transmitter in sympathetic ganglia of the frog

[luteinizing hormone-releasing hormone (luliberin)/slow potentials]

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ABSTRACT **A search was made in sympathetic ganglia of the bullfrog for a noncholinergic, nonaminergic transmitter that is released by a distinct group of preganglionic axons. These initiate a late slow excitatory postsynaptic potential which lasts for many minutes. The most promising candidate for the role of transmitter is a peptide that resembles luteinizing hormone-releasing hormone (LHRH; luliberin). The reasons are: (*i*) LHRH (1 μM) and some of its analogs cause a slow depolarization of ganglion cells. (*ii*) Radioimmunoassays established that 100–800 pg of a LHRH-like substance is contained in the lumbar chain of sympathetic ganglia. (*iii*) The LHRH-like material is specifically distributed in those spinal nerves that contain axons that initiate the slow noncholinergic synaptic responses. (*iv*) Five days after ipsilateral preganglionic axons are cut, 95% of the LHRH-like substance disappears from ganglia, while the LHRH immunoreactivity triples in the spinal nerves proximal to the cut region. (*v*) About 0.6% of the LHRH-like material within ganglia can be collected from the perfusate after 30 min of incubation in isotonic KCl; this release is Ca dependent. (*vi*) The candidate for transmitter has several chemical characteristics of a peptide and has a M_r near 1000.**

Since the late 1930s acetylcholine (AcCho) has been generally accepted as the excitatory transmitter contained in preganglionic fibers of sympathetic ganglia (1). In subsequent years cholinergic fibers were found to initiate, directly or indirectly, three types of synaptic responses: (*i*) the nicotinic fast excitatory postsynaptic potentials (epsps), which last for about 30–50 msec and generally give rise to conducted impulses (2); (*ii*) the muscarinic slow epsps, lasting 30–60 sec; and (*iii*) the slow inhibitory postsynaptic potentials of 1–2 sec duration which are also blocked by muscarinic blockers (3). In 1968 a fourth synaptic potential was discovered (4) and named the "late slow epsp," which lasts for 5–10 min and is not blocked by cholinergic blockers (4). Here we report experiments that suggest that the transmitter for the late slow epsp may be a small peptide.

MATERIALS AND METHODS

Physiological Experiments. The last four or five paravertebral ganglia in the sympathetic chains of bullfrogs were isolated and perfused at room temperature with 115 mM NaCl/2 mM KCl/3.6 mM $CaCl_2$/1 mM Hepes, pH 7.2. The mixing time for drugs added to the bath is a few seconds, while access to cells in the interior of ganglia takes longer. Preganglionic fibers were stimulated with suction electrodes. Similar electrodes also served for extracellular recording from postganglionic branches. The structural features of the nerve cells, viewed with Nomarski optics, resemble those in parasympathetic ganglia (5).

Radioimmunoassays (RIAs). Luteinizing hormone-releasing hormone (LHRH; luliberin) (Bachem, Torrance, CA) was radioiodinated by the chloramine T method as described (6). The anti-LHRH serum, pool R-42, was a gift of T. M. Nett. Frog sympathetic ganglia or other tissues were homogenized in 100 times the volume of 2 M acetic acid. After centrifugation at 8700 × *g* for 4 min, the supernatant was lyophilized and resuspended in 10 mM phosphate-buffered saline (pH 7.0) containing 0.1% gelatin (gel/NaCl/P_i). For RIAs (6, 7), 500 μl of sample or standard (LHRH in gel/NaCl/P_i) was mixed with 200 μl of anti-LHRH serum diluted 1:24,000 in 50 mM EDTA/NaCl/P_i containing 0.25% heat-inactivated normal rabbit serum. After 6 hr at 4°C, 100 μl of ^{125}I-labeled LHRH (^{125}I-LHRH) (approximately 10,000 cpm on the day after iodination) in gel/NaCl/P_i was added to the mixture. The iodinated and unlabeled peptides compete for binding sites on the antibody. Twenty-four hours later the free iodinated peptide was separated by thoroughly mixing into the solution 1 ml of an ice-cold dextran-coated charcoal suspension. The charcoal suspension was made up by adding 500 mg of activated charcoal (Norit A, Pfanstiehl Lab., Waukegan, IL) to 100 ml of dextran solution [25 mg of dextran (Dextran T70, Pharmacia) in 100 ml of NaCl/P_i). After 30 min at 4°C the free peptide, adsorbed to the charcoal, was separated by centrifugation at 2500 rpm at 4°C for 10 min. The radioactivity in the supernatant and charcoal pellet was measured separately by a Beckman gamma counter. We estimate that dextran-coated charcoal adsorbed over 95% of free ^{125}I-LHRH. Each estimate of LHRH was derived from RIAs on five to seven dilutions of tissue extracts.

RESULTS

The ganglion (principal) cells of paravertebral sympathetic ganglia fall into two groupings. The larger size group, B cells (average diameter ≈50 μm), send out axons that conduct at velocities of about 2 m/sec. C neurons are smaller (average diameter ≈30 μm) and their axons conduct at speeds around 0.2 m/sec (8). Preganglionic stimulation causes four different kinds of synaptic potentials. In B and C neurons one records one or more fast epsps that last for tens of msec. Normally, at least one of these potentials initiates an impulse (Fig. 1). The transmitter for the fast epsp is AcCho; transmission is blocked by nicotinic blockers (for example, 1 μM dihydro β-erythroidine). AcCho also initiates a much slower epsp, which lasts for about 1 min and is usually seen in B cells. The slow epsp is blocked by muscarinic blockers (e.g., 0.1 μM atropine). A third type of synaptic potential is an inhibitory postsynaptic potential (ipsp) of 1–2 sec, also blocked by muscarinic blockers. However, whether an adrenergic interneuron is involved remains to be determined (9, 10).

Our studies deal with the fourth response, named the late slow epsp (4). It lasts for about 5 min, which is approximately

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Abbreviations: LHRH, luteinizing hormone-releasing hormone (luliberin); ^{125}I-LHRH, ^{125}I-labeled LHRH; NaCl/P_i, 10 mM phosphate-buffered saline (pH 7.0); epsp, excitatory postsynaptic potential; ipsp, inhibitory postsynaptic potential; AcCho, acetylcholine; RIA, radioimmunoassay.

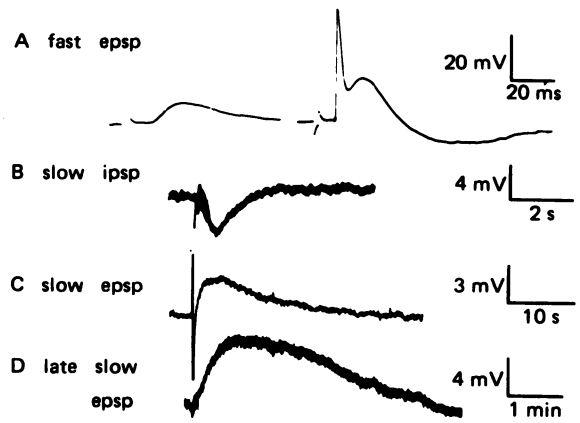

FIG. 1. Four types of synaptic signals in the 10th sympathetic ganglion recorded with intracellular electrodes. (*A*) Single nerve stimulus initiates a subthreshold epsp (left); with stronger stimulation (right), a second larger epsp produces an impulse. (*B*) Slow ipsp on stimulation of central portions of 7th and 8th spinal nerves (13 stimuli at 20/sec). The fast epsp was blocked by 1 μM dihydro β-erythroidine. (*C*) Four stimuli at 50/sec to sympathetic chain above the 7th ganglion result in a slow epsp lasting about 30 sec. The initial rapid deflections are four large conducted impulses. (*D*) Late slow epsp (300 sec duration) on stimulation of the 7th and 8th spinal nerves (50 stimuli at 10/sec). Note different time scales. No drugs used in *A*, *C*, and *D*. Upstroke of impulse in *A* touched up.

10,000 times as slow as the fast epsp, and is not affected by nicotinic or muscarinic cholinergic blockers. The late slow epsp can be recorded from B and C cells.

The pattern of innervation of the 9th and 10th ganglia is summarized in Fig. 2. The B cells receive their cholinergic input from the chain above the 7th ganglion and their noncholinergic input through the 7th and 8th spinal nerves (with a smaller component from the 9th nerve). C cells receive both their cholinergic and noncholinergic input through the 7th and 8th spinal nerves (8, 11).

Pharmacology of Late Slow epsp. To find the transmitter that mediates the late slow epsps we started with pharmacological tests while recording extracellularly the various synaptic

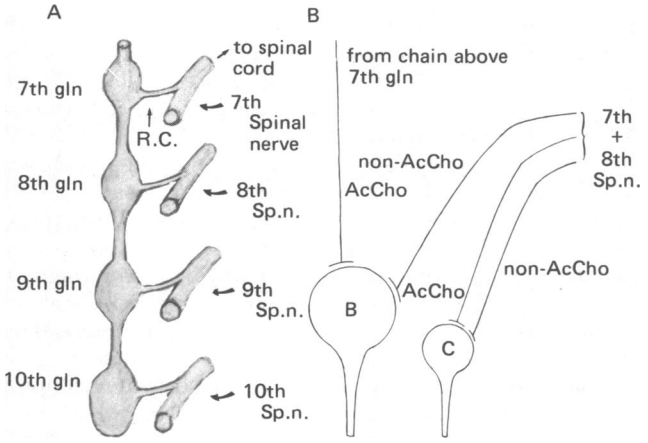

FIG. 2. (*A*) Sketch of the last four paravertebral sympathetic ganglia. Preganglionic innervation leaves the spinal nerves and enters ganglionic chain through the rami communicantes (R.C.). (*B*) Scheme of innervation of B and C neurons in 9th or 10th ganglion. A third cell type, the adrenergic interneurons, is omitted. Cholinergic axons for B neurons reach these ganglia through the sympathetic chain above the 7th ganglion, while preganglionic fibers for C neurons come through the 7th and 8th spinal nerves. Noncholinergic axons for all neurons in the 9th and 10th ganglia also enter through the 7th and 8th nerves.

potentials from a postganglionic branch where it emerges from the 9th or 10th ganglion. By perfusing substances through the bathing solution we first confirmed that the late slow epsp was not significantly altered by nicotinic and muscarinic blockers (4). Further, large carbachol concentrations (0.1 mM), which eliminate fast epsps and ipsps (presumably by desensitizing the AcCho receptors), failed to change the late slow epsp. Therefore, we generally added 1 μM dihydro β-erythroidine and 0.1 μM atropine to the bath to selectively eliminate the cholinergic contributions, leaving exclusively the late slow epsp.

We tested for the action of a series of transmitter candidates, such as γ-aminobutyric acid (up to 10 mM), D,L-octopamine (0.02 mM), UDP (1 mM), ATP (1 mM), serotonin (0.01 mM), and serotonin blockers (0.05 mM methysergide maleate or lisuride), as well as the α and β adrenergic blockers (0.05 mM phentolamine or propranolol). All of these produced no obvious effect on the generation of the late slow epsp.

Because sympathetic ganglia in the chicken had been reported to contain a peptide, substance P (12), we tested several peptides for their action on ganglion cells. Substance P, neurotensin, bombesin, somatostatin, thyrotropin-releasing hormone, and angiotensin I and II up to 0.1 mM had no obvious effect. However, one peptide, LHRH, produced a slow depolarization in ganglion cells.

Effect of LHRH and Its Analogs. In Fig. 3 a late slow epsp was first set up by a train of stimuli (5/sec for 5 sec) to the 7th and 8th spinal nerves. At the end of the late slow epsp, 20 μM LHRH was added to the bath, causing a slow depolarization during which the same nerve stimulation produced a smaller late slow epsp. Such actions of LHRH were reversible, and after 10 min of washing the nerve-evoked late slow epsp returned to its original size.

In mammals, several analogs of LHRH are 10–100 times more effective than LHRH in releasing gonadotropin from the pituitary (13). We therefore tested two such analogs ([D-Ala^6]-LHRH and ethylamide-[D-Trp^6,Pro^9]LHRH) and found both about 100 times more potent than LHRH. Thus, whereas 1 μM LHRH will detectably depolarize ganglion cells, only 0.01 μM of the LHRH analog is needed to do the same. In Fig. 4*A* the analog ethylamide-[D-Tryp^6,Pro^9]LHRH (1 μM) caused a large slow depolarization which reached a ceiling effect within 1 min, during which nerve stimulation caused no detectable late slow epsp. All these actions were reversible; the recovery of the membrane potential, however, was more rapid than that of the late slow epsp. Therefore, the diminution of the nerve-evoked response was not simply a consequence of depolarization, nor was it due to a general blocking action on nerve terminals since the fast epsps were still seen. To find out whether the LHRH action included a direct effect on the postsynaptic cell, we removed Ca^{2+} from the medium and added 4 mM Mg^{2+} so that nerve-evoked chemical transmission was blocked. Yet, the LHRH analog still caused a large slow depolarization (Fig. 4*B*). Therefore, the effect of the peptide is largely postsynaptic.

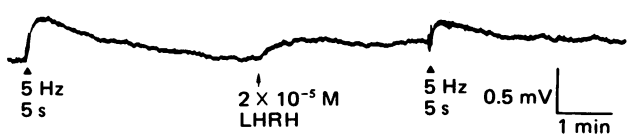

FIG. 3. Late slow epsp and the depolarizing effect of LHRH recorded with suction electrodes from the ramus communicans of the 10th ganglion. Preganglionic axons were stimulated (triangle) in the proximal portion of the 7th and 8th spinal nerves with 25 stimuli at 5/sec. After bath application of LHRH (arrow), the neurons depolarized and the synaptic response was reduced. The cholinergic synaptic potentials were blocked by 1 μM dihydro β-erythroidine and 0.1 μM atropine.

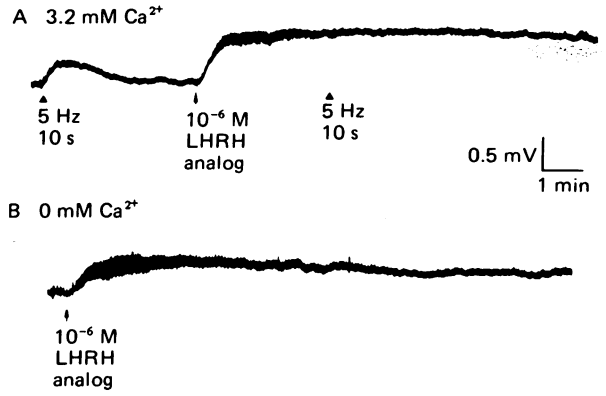

FIG. 4. Postsynaptic action of a LHRH analog. (*A*) In normal Ringer's solution nerve stimulation (triangle) initiates a late slow epsp. During depolarization by the bath-applied LHRH analog, ethylamide-[D-Tryp^6,Pro^9]LHRH (arrow), the same neural stimulation is not effective. (*B*) Synaptic transmission was blocked by removing Ca^{2+} and adding 4 mM Mg^{2+}. The analog (arrow) still produced a depolarization. Recording and stimulation as in Fig. 3.

The experiments thus far raise the possibility that the late slow epsp is mediated by a LHRH-like substance. In pursuing this hypothesis, we used RIA to detect the possible presence of LHRH-like substances in the ganglia.

LHRH-Like Substances in Sympathetic Ganglia. The standard curve of Fig. 5 *upper* shows that RIA detected a few pg of ^{125}I-LHRH. After extracting the ganglia with 2 M acetic acid or with a mixture of acetone and 1 M HCl (50:50, vol/vol), we found that the extract inhibited the binding of ^{125}I-LHRH to antibody (Fig. 5 *lower*).

The antiserum used in these experiments is very specific for LHRH, as determined by Nett *et al.* (14), who showed that their RIAs agreed with their bioassays. Further, of the many analogs of LHRH that they tested, most did not inhibit the binding of ^{125}I-LHRH. We examined four additional analogs and found that [D-Ala^6]LHRH was ≈1/100th and [*p*-aminophenyl^1]-LHRH 1/1000th as effective as LHRH in inhibiting the binding of ^{125}I-LHRH to antibody (Fig. 5 *upper*). The analogs ethylamide-[D-Tryp^6,Pro^9]LHRH and LHRH-dinitroanilide were totally ineffective even when 10 ng was used for each assay, indicating a relative activity of less than 1 in 6000. Other peptide hormones, such as substance P, somatostatin, neurotensin, bombesin, angiotensin I and II, thyrotropin-releasing hormone, luteinizing hormone, and follicle-stimulating hormone, did not inhibit the binding of ^{125}I-LHRH to antibody. Their relative activity, if any, must be less than one part in 60,000.

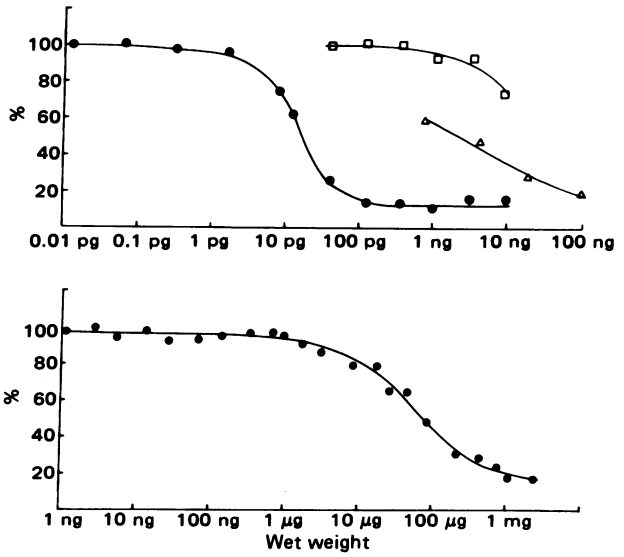

FIG. 5. RIA for LHRH. (*Upper*) Standard curve provides the amount of ^{125}I-LHRH displaced by varying amounts of unlabeled LHRH (●) from its binding sites on the antibody. Also included are inhibition curves by two analogs: □, [*p*-aminophenyl^1]LHRH; △, [D-Ala^6]LHRH. (*Lower*) Inhibition curve by an extract from sympathetic ganglia; 100% expresses amount of ^{125}I-LHRH (bound/total) when no unlabeled LHRH or no tissue extract was added. By comparing the midpoints in the *Upper* (●) and *Lower* curves, the immunoreactivity of the ganglionic extract is estimated as about 260 pg of LHRH-like material per mg of tissue.

Since the antiserum used in the RIA was highly specific for LHRH, we assumed that there was a LHRH-like substance in the sympathetic ganglia. To exclude tissue contaminants, such as the small blood vessels in the ganglia, we did RIAs on extracts from heart, liver, skeletal muscle, and whole brain (Table 1). There was no detectable LHRH-like substance in these tissues, except in the brain. To test the efficiency of the extraction procedure, we added 1 ng of [^{3}H]LHRH (38.3 Ci/mmol, 1 Ci = 3.70 × 10^{10} Bq, New England Nuclear) to one chain of sympathetic ganglia just before extraction. About 90% of the [^{3}H]LHRH was recovered in the extract. The inhibition curve produced by this extract containing [^{3}H]LHRH was then compared with that of the control chain from the same frog. The comparison, given in Fig. 6, demonstrates that the difference can be quantitatively accounted for by the introduction of [^{3}H]LHRH, indicating that inhibition curves are due to a LHRH-like substance. Each sympathetic chain contained 100–800 pg of the LHRH-like substance (20 frogs). Some chemical properties of this substance will be described below.

Distribution of LHRH-Like Substance in Sympathetic Chain. Stimulation of the central portions of the 7th and 8th spinal nerves (Fig. 2) initiates the late slow epsps in the 9th and 10th ganglia. The 9th nerve usually makes only a small contribution, while the large 10th spinal nerve has no preganglionic fibers. To see if the distribution of the LHRH-like substance is also restricted to the 7th and 8th spinal root outflow, we did RIAs on extracts of nerve segments between the spinal cord and rami communicantes (Fig. 2). Such central segments from the 7th and 8th spinal nerves contained 3–5 pg/mg of wet weight of the LHRH-like substance, while a corresponding part of the 9th spinal nerve contained either no LHRH-like material or, in some frogs, only 0.2 pg/mg of wet weight. No LHRH-like immunoreactivity was found in the 10th spinal nerve. This was not because some substance in the 10th nerve extract interfered with the assay, since mixing the extract from the 10th nerve with synthetic LHRH did not affect the ability of the synthetic LHRH to bind to antibody in the RIA. Therefore, the distribution of the LHRH-like substance in the spinal nerves agreed

Table 1. Distribution of LHRH-like substance in different tissues

Tissue*	Specific activity,† pg/mg wet weight
Brain	70
Heart	<1
Liver	<1
Skeletal muscle	<1
Sympathetic chain‡	600

* All five different tissues were from the same frog.
† This is expressed in equivalent amounts of synthetic LHRH in terms of their ability to inhibit the binding of ^{125}I-LHRH to antibody.
‡ About 1 mg wet weight.

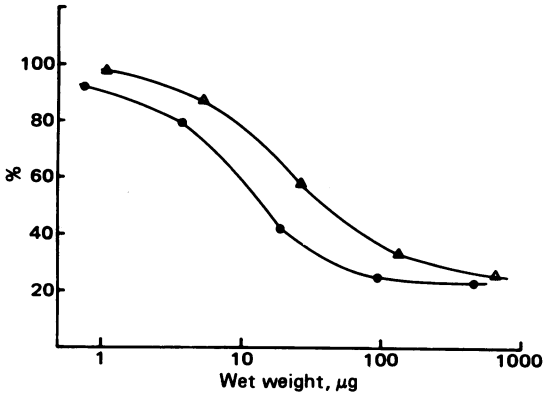

FIG. 6. Two inhibition curves of ^{125}I-LHRH binding to antibody, one from the extract of a sympathetic chain (△), the other from the extract of a corresponding chain from the other side of the same frog to which has been added 1 ng of synthetic LHRH (●). By comparing these curves with the standard curve (not shown), we estimate that each chain contains about 550 pg of LHRH-like substance.

with the distribution of preganglionic fibers for the late slow epsp.

To further test the correlation between axons giving rise to late slow epsps and structures containing the LHRH-like substance, we subdivided the 7th or 8th spinal nerves into three segments: one central segment between the spinal cord and the ramus communicans, one short segment containing the ramus, and one distal segment between the ramus and the peripheral targets (Fig. 2). The first two segments again contained 3–5 pg/mg of wet weight of LHRH-like substance, while the distal segment contained only 0.7 pg/mg of wet weight. LHRH-like substance, therefore, predominates in those segments of the spinal nerves that carry preganglionic axons to the ganglia.

Effect of Denervation. The preceding findings suggest that the LHRH-like substance should disappear after denervation of ganglia. Previous physiological and anatomical studies have shown (unpublished result) that after the preganglionic fibers were cut, synaptic transmission failed and nerve terminals of cut axons degenerated within 5 days, while the ganglion cells retained their membrane potentials and gave impulses. In about 10 days there were signs of sprouting of remaining preganglionic fibers on the ipsilateral or contralateral side. About 3 weeks later, some of the sectioned preganglionic fibers grew across the cut region and reinnervated the ganglion cells. Therefore, we looked at the distribution of LHRH-like substance 5 days after denervation, when the terminals had degenerated but sprouting or regrowth had not taken place.

In two frogs the 9th and 10th ganglia on one side were denervated by cutting their connections to the 7th and 8th nerves and by severing the chain above the 7th ganglion. After 5 days the amount of LHRH-like substance in the 9th and 10th ganglia had dropped to 7 pg/mg of wet weight, compared to 120 pg/mg of wet weight on the control side. The 6% of the LHRH-like substance remaining after denervation may be due to contralateral innervation, which frequently makes a small contribution to the input to these ganglia. We conclude that after appropriate denervation the LHRH-like substance disappears in the ganglia.

In another series of tests on five frogs, the 7th or 8th ramus was cut on one side of the animal. After 5 days the central segments of the operated nerves contained about 3 times more LHRH-like substance. In three denervated frogs there was about 15 pg/mg of wet weight in the central portions of the nerves with cut rami, as compared to 5 pg/mg of wet weight on the control side. In the other two frogs the ratio was similar, with 7 pg/mg of wet weight on the denervated side, and 2 pg/mg of wet weight on the control side. Such an accumulation of a LHRH-like substance may mean that axons proximal to the cut ramus proliferate or that they accumulate more of the LHRH-like substance. Analogous accumulations of substance P were seen in the cat after ligating dorsal roots close to the spinal cord (15).

Ca^{2+}-Dependent Release of LHRH-Like Substance. If the transmitter for the late slow epsp were a LHRH-like substance, it should be released either by stimulation of the preganglionic fibers or by raising the external potassium concentration. Further, since late slow epsps could be evoked only if solutions contained Ca^{2+}, one would expect the release of a LHRH-like substance to depend on external calcium. To test this prediction two separate experiments were done, each on six sympathetic chains from three bullfrogs. Six chains were pooled and treated with collagenase to speed diffusion through the tissue, washed thoroughly, then placed sequentially in 300 μl of (*i*) isotonic KCl containing 4 mM Mg^{2+} and no Ca^{2+}, (*ii*) normal frog Ringer's solution (containing 3.2 mM Ca^{2+} and no Mg^{2+}), and (*iii*) isotonic KCl containing 3.2 mM Ca^{2+} and no Mg^{2+}. Gelatin (0.1%) was added to all solutions, and also a mixture of 20 μM bacitracin and 10 μg of leupeptin, antipain, and elastatinal per ml to reduce proteolysis. Between each incubation the preparations were thoroughly washed. Physiological checks have shown that the neurons were depolarized in isotonic KCl and then fully recovered after washing. The ganglia were immersed at room temperature in each of the three solutions for about 30 min. The bathing medium was then collected, mixed with ice-cold 2 M acetic acid, lyophilized, and resuspended in gel/NaCl/P_i for RIA. Three hundred microliters of each of the three solutions without incubating with the sympathetic chains served as controls, showing that salts and the protease inhibitors in the solutions did not reduce the binding of ^{125}I-LHRH to antibody. Twenty picograms of a LHRH-like substance was collected in the isotonic KCl solution that contained Ca^{2+}. This was about 0.6% of the total content in the sympathetic chains. In contrast, no detectable LHRH-like substance was found after the chains were incubated in isotonic KCl without Ca^{2+} or in normal frog Ringer's solution. Ca^{2+}-dependent release of peptides has previously been shown (e.g., ref. 16).

Is the LHRH-Like Substance a Peptide? Fig. 7 shows that the LHRH-like immunoreactivity in the extract from sympathetic ganglia was not affected by heating in a boiling water bath for 10 min. Subjecting the extract to α-chymotrypsin, on the other hand, totally destroyed the ability of the extract to inhibit the binding of ^{125}I-LHRH to antibody. This was also true

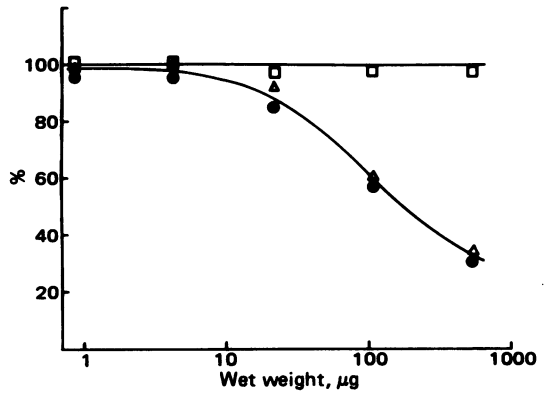

FIG. 7. RIA of ganglionic extracts. Boiling at 100°C for 10 min (●) did not alter the LHRH-like immunoreactivity (△). Digestion by α-chymotrypsin (□) at 25°C for 1 hr, however, destroyed the LHRH-like immunoreactivity.

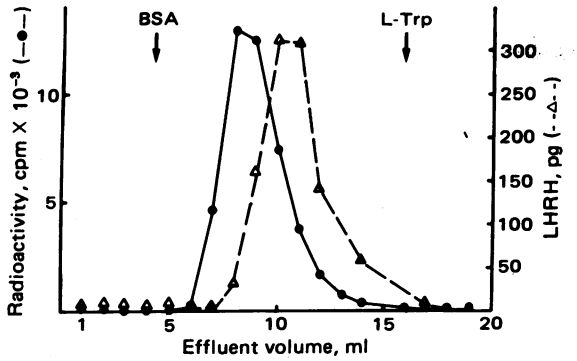

FIG. 8. Column chromatography of LHRH-like substance. After the ganglionic extract was passed through a Sephadex G-25 column (1.5 × 7 cm, equilibrated with 2 M acetic acid), the eluted fractions were examined by RIA. LHRH-like immunoreactivity is plotted as the equivalent amount of LHRH (△). For comparison, the profile of [^{3}H]LHRH (●) from a separate run is given on the same plot. Also indicated are the effluent volumes for bovine serum albumin (BSA) and L-tryptophan.

for synthetic LHRH. If the ganglionic extract was passed through a Sephadex G-25 (Pharmacia) column and the various fractions were assayed for LHRH-like immunoreactivity, the LHRH-like substance was eluted at roughly the same effluent volume as [^{3}H]LHRH (Fig. 8). We are not sure whether the small difference in effluent volume for [^{3}H]LHRH and the LHRH-like substance was real because they were chromatographed in separate runs. In any case, the LHRH-like substance probably has a M_r of around 1000, as does the decapeptide LHRH. The small M_r, together with the resistance to heat and sensitivity to a protease, indicates that the LHRH-like substance is a peptide.

DISCUSSION

Several lines of evidence support the hypothesis that the late slow epsp in sympathetic ganglia may be mediated by a LHRH-like peptide: (*i*) LHRH and its analogs cause a slow depolarization of the principal cells; (*ii*) a LHRH-like peptide is concentrated in those spinal nerve portions whose stimulation initiates late slow epsps; (*iii*) the LHRH-like peptide can be released from the ganglia with isotonic KCl, a release that requires Ca^{2+}; and (*iv*) after degeneration of the terminals of appropriate preganglionic fibers, almost all of the LHRH-like material disappears from ganglia.

The support for the transmitter hypothesis remains far from complete for several reasons. (*i*) We have only indirect evidence that the LHRH-like peptide is contained in the same axons that initiate the late slow epsp. Immunohistochemistry of the nerve terminals containing the peptide has not yet been done. (*ii*) Although the LHRH-like substance depolarizes the ganglion cells and diminishes the late slow epsp, we do not know whether the peptide and the nerve-released transmitter produce the same physiological effects.

We have used mammalian LHRH (17, 18) and antiserum highly specific for this peptide (14). Therefore, it is most likely that the LHRH-like peptide in frog sympathetic ganglia is structurally similar to mammalian LHRH. In this context, one would like to know whether the ganglionic LHRH-like substance is used as a releasing hormone in the central nervous system of the frog.

Slow noncholinergic synaptic potentials have also been detected in the guinea pig's inferior mesenteric ganglion and myenteric plexus (19, 20), where the transmitters are not known. However, in the myenteric plexus, substance P may be a candidate (20). In this and other autonomic nervous systems, peptides have been demonstrated (12, 21) and, therefore, they are possible transmitters for slow potentials. Other models for peptides as transmitters are being developed in invertebrates and in extensive studies of the vertebrate central nervous systems (22, 23). Autonomic ganglia may provide a good system for detailed studies for the role of peptides, because they have only a few cell types which are readily accessible.

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