THE UNIVERSITY OF MANITOBA

CHARACTERIZATION OF A SOMATOSTATIN BINDING PROTEIN

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

MASTER OF SCIENCE

DEPARTMENT OF PHYSIOLOGY

WINNIPEG, MANITOBA

May, 1977

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ACKNOWLEDGEMENTS

I wish to thank Dr. H.G. Friesen for his advice during the preparation of this thesis. I also thank Dr. N. Ogawa for the use of his data in this thesis. His work includes distribution and properties of somatostatin binding activity as well as studies on the biochemical composition of the binding activity (Ogawa et al 1977). During these studies I aided him with column chromatography.

I would also like to thank Nikki Ryan for her secretarial assistance in the preparation of this thesis.

ABSTRACT

A somatostatin binding protein, of molecular weight 77,000 daltons, was found in the cytosol fraction derived from several rat tissues. It has an isoelectric point of approximately 5.0. Studies of the binding protein provide no conclusive evidence as to whether it is a true hormone receptor or not, but the molecule appears to have similar characteristics in the variety of tissues studied. These similarities include pH and ionic optima, time course of binding, molecular weight, isoelectric point and electrophoretic mobility. The somatostatin binding protein has properties similar to the regulatory subunit of mammalian cytosol protein kinase.

INTRODUCTION

Brazeau et al (1973, 1974a) purified a tetradecapeptide from ovine hypothalami that inhibited growth hormone (GH) release in pituitary cultures, using the methods of Vale et al (1972a,b,c) and Vale and Grant (1974). The sequence of the peptide is:

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH

Schally et al (1975, 1976) isolated a growth hormone release inhibiting peptide, from porcine hypothalami, using the same bioassay as Brazeau et al (1973, 1974a). It has the same stucture and amino acid sequence as the tetradecapeptide isolated from ovine hypothalami.

These peptides are called somatostatin or growth hormone release inhibiting hormone (GH-RIH). They have many actions in vitro and in vivo and their mechanism of action has been studied. It is not yet known if somatostatin is a true hormone, since no significant levels have been found in the blood. Many of its observed actions may not even represent physiological roles of somatostatin.

I wish to review information on the biological effects of somatostatin and its mechanisms of action, and compare and contrast characteristics with those of other polypeptide and hypothalamic hormones. This may provide insight into the possible role of somatostatin and a basis for the

discussion of the significance of a somatostatin binding protein found in the cytosol derived from a variety of rat tissues. Recent reviews provide a general overview of somatostatin biochemistry and biological actions (Reichlin et al 1976, Hall and Gomez-Pan, 1976).

Structure, Forms and Analogues of Somatostatin.

Both ovine and porcine somatostatin are cyclic hormones, containing a disulphide bridge (Brazeau et al 1973, 1974a). They are identical to the synthetic hormone upon thin-layer chromatography (TLC) (Schally et al 1975, 1976).

Both synthetic and reduced or oxidized somatostatin exhibit identical biological activities (Vale et al 1973, Brazeau et al 1973, Rivier, 1974), but three linear somatostatin analogues with blocked cysteine residues: [Ala^{3}]-, [Ala^{3,14}]-, and [SMe-Cys^{3,14}]-somatostatin, cannot form intramolecular disulphide bonds. This suggests the presence of two cysteine residues in synthetic somatostatin analogues, that are free to form disulphide bonds is important in somatostatin bioactivity (Rivier et al 1975a, Serantakis et al 1973).

Deleting or modifying the N-terminal Ala-Gly affects bioactivity little (33-100% of native hormone)(Rivier et al 1975b, Brazeau et al 1974b, Vale et al 1973), so tyrosine may be substituted on the N-terminal to allow iodination of somatostatin without greatly affecting somatostatin bioactivity (Arimura et al 1973)

In man, the effect of a single intravenous, intramuscular or subcutaneous injection of somatostatin, lasts only 30 minutes (Besser et al 1974a). Somatostatin has a half-life of only five minutes in man (Hall et al 1973). To be of any therapeutic value, somatostatin analogues with a slower rate of degradation must be found. Modifications of the mode of injection of somatostatin and of its structure, lengthens the half-life of somatostatin in vivo, including:

i) acetylation of [Cys^3] which renders somatostatin less soluble and prolongs its action in rats (Brazeau et al 1974b) but not humans (Evered et al 1975).

ii) complexing somatostatin with protamine zinc increases its half-life in rats but not man or monkeys (Brazeau et al 1974c).

iii) mixing with arachis oil or gelatin increases somatostatin half-life in rats, but not man or monkeys (Besser et al 1974b).

iv) substituting [D-Trp^8] for [L-Trp^8] lengthens the effect of somatostatin on rat pituitaries 6 to 8 fold in vitro and in rats in vivo (Rivier et al 1975a). It is of interest that the major cleavage site of somatostatin in rat blood and brain homogenates is between [Trp^8] and [Lys^9] (Marks and Stern 1975), and substitution of [D-Trp^8] completely blocks cleavage at this site in the rat blood and brain homogenates (Marks et al 1976).

Immunoassay and Distribution of Somatostatin

Arimura et al (1975a) and Patel et al (1975a) developed immunoassays for somatostatin. Reduced and oxidized somatostatin are both immunologically identical to native somatostatin and synthetic forms of somatostatin show cross-reactivity to tissue extracts (Arimura et al 1975a,b, Patel et al 1975) and to tissues studied by immuno-histological methods (Alpert et al 1975, Pelletier et al 1974). Antisera to synthetic somatostatin can be used therefore for immunoassay. [Tyr^1]-substitution of somatostatin is necessary for iodination of somatostatin using lactoperoxidase.

Levels and contents of somatostatin in the cytosol extracts of various rat tissues are shown in the following table. Only tissues with high levels or contents are shown. Insignificant amounts of somatostatin are found in the heart, lung, thymus, spleen, kidney, adrenals and ovaries of rats.

ORGAN	CONCENTRATION OF SOMATOSTATIN (ng/mg protein)	TOTAL SOMATOSTATIN CONTENT (ng)	REFERENCE
pancreas	33.8	110.9	Arimura
stomach	11.7	199.0	et al
duodenum	1.6	12.8	(1975b)
jejunum (upper portion)	1.6	36.3	
hypothalamus	2.1	39.3	Brownstein
septum and preoptic area	0.6	24.7	et al
thalamus	0.2	17.5	(1975)
cerebral cortex	0.03	30.0	

A similar distribution for somatostatin was found by Patel et al (1975a). In the pancreas, somatostatin is located in the D-cells of the islets (Dubois 1975, Polak et al 1975, Goldsmith et al 1975).

Small quantities of somatostatin are found in many extrahypothalamic brain areas (Brownstein et al 1975, Patel et al 1975a, Setalo et al 1975, Alpert et al 1976) in the rat. The cerebrospinal fluid (CSF) in man contains low levels of somatostatin which are elevated in some patients with pinealoma and medulloblastoma (Patel et al 1975b).

Krulich et al (1968) showed high growth hormone release-inhibiting activity in the median eminence.
Using a specific radioimmunoassay for somatostatin, Brownstein et al (1975) showed that in the hypothalamus

the concentration of somatostatin was highest in the median eminence and arcuate nucleus, though all nuclei contain some activity.

Pelletier et al (1974), using immunohistochemistry with electron microscopy, showed that somatostatin is found in nerve endings (i.e. somatostatinergic neurons) in the external zone of the rat median eminence. Similarly, Alpert et al (1976) localized somatostatin in secretory granules in neurons of the rat hypothalamus.

Effect of somatostatin on pituitary hormone secretion

Somatostatin was purified on the basis of its ability to suppress GH secretion by rat pituitaries *in vitro* (Brazeau *et al* 1973, 1974a). Somatostatin suppresses both basal GH secretion and the GH response to all known stimuli of its secretion in various mammals, *in vivo* and *in vitro* including:

i) exercise (Hansen et al 1973)
ii) insulin-induced hypoglycaemia (Hall et al 1973)
iii) sleep (Parker et al 1974)
iv) arginine infusion (Siler et al 1973)
v) L-dopa (Siler et al 1973)
vi) sodium pentobarbital (Brazeau et al 1974c)
vii) isoprenaline and chloropromazine (Kato et al 1974)
viii) intrahypothalamic epinephrine injections (Sachs et al 1971)
ix) protein-caloric malnutrition (Pimstone et al 1975)
x) electrical stimulation of the ventromedial nucleus (Martin et al 1974)
xi) injection of crude GH releasing hormone (Szabo and Frohman 1975)

The rate of decline of GH blood levels during somatostatin infusion corresponds to a half-life of 24-34 minutes, which is approximately the half-life of GH. This suggests that somatostatin produces immediate inhibition of GH release (Pimstone et al 1975, Yen et al 1975).

Somatostatin decreases blood levels of a variety of pituitary hormones in rats and humans. These results are summarized in the following table.

HORMONE	EFFECT
GH	-Decreases GH levels and secretion *in vivo* and *in vitro* in various mammals to all known stimuli of GH release. -In acromegalics, GH-RIH decreases GH release in proportion to infusion rate or dose injected (Besser et al 1974b, Hall et al 1973, Yen et al 1974, Brazeau et al 1974c, Christensen et al 1974). -Maximal suppression of GH occurs with an infusion rate of 1.3 ug/min (Besser et al 1974b)
PRL	-In rat pituitary cell cultures, somatostatin administration decreases basal, oestrogen- and TRH-induced PRL secretion (Vale et al 1974) -Normal rats and human subjects show no decrease in basal, insulin-induced (Hall et al 1973) or TRH-induced (Carr et al 1975, Drouin et al 1976) PRL blood levels due to somatostatin injection or infusion. -Some acromegalics show decreased PRL levels after somatostatin infusion (Yen et al 1974) -Copinschi et al (1974) decrease PRL levels in normal subjects with somatostatin infusion, so data in humans is contradictory

HORMONE	EFFECT
LH-FSH	-Somatostatin infusion does not alter basal and gonadotrophin releasing hormone-induced LH and FSH levels in normal human subjects (Hall et al 1973, Siler et al 1973)
ACTH	-In normal humans, somatostatin does not alter basal and insulin-induced ACTH blood levels (Hall et al 1973). -Somatostatin decreased ACTH blood levels in four cases of Nelson's syndrome, Cushing' syndrome (Tyrrell et al 1975) and Addison's disease (Fehm et al 1976)
TSH	-In normal humans, somatostatin has no effect on basal TSH blood levels (Carr et al 1975) -In rat pituitary cell cultures, somatostatin has no effect on basal TSH secretion (Drouin et al 1976) -In human hypothyroids, somatostatin decreases TSH blood levels (Gomez-Pan et al 1976a) -In rat pituitary cell cultures, somatostatin decreases TRH-induced TSH release by 75% and this effect is not due to competitive inhibition at the TRH receptor. -Note that TRH stimulates (Labrie et al 1975) while somatostatin inhibits (Labrie et al 1975, Borgeat et al 1974) cyclic AMP accumulation in anterior pituitary tissue.

Effects on the endocrine pancreas

The effects of somatostatin on insulin and glucagon levels and secretion and its consequent effects of glucose metabolism, are summarized below.

In vitro somatostatin:

i) (1 ug/ml) decreases basal insulin and glucagon secretion (Fujimoto et al 1974) and glucose-, tolbutamide-, theophylline-, cytochalasin b- and Ca^{+2}-stimulated insulin secretion in monolayer cell cultures of isolated rat pancreatic islets, containing alpha and beta cells (Fujimoto 1975)

ii) decreases insulin and glucagon secretion on direct perfusion of isolated pancreas from various species (Alberti et al 1973, Iversen 1974, Curry et al 1974a, Efendic et al 1974, Weir 1974, Fujimoto et al 1974, Gerich 1974a, Johnson 1975)

In vivo somatostatin:

i) decreases basal blood levels of glucagon and insulin in man (Mortimer et al 1974, Alberti et al 1973, Christensen et al 1974, Yen et al 1974), baboon (Koerker et al 1974), dog (Sakurai et al 1974) and rats (Gerich et al 1975)

ii) decreases glucose-stimulated insulin secretion (Alberti et al 1973), arginine-stimulated glucagon secretion (Mortimer et al 1974) and the insulin and glucagon response to a meal and administration of tolbutamide (Gomez-Pan et al 1976b)

iii) decreases glucose tolerance in man (Mortimer et al 1974)

iv) reduces or abolishes the hyperglycaemia induced by pancreatectomy and alloxan, when infused in rats (Dobbs et al 1975)

v) in humans, with diabetes mellitus, with and without hypophysectomy, somatostatin infusion decreases blood glucose with a parallel fall in blood glucagon (Gerich et al 1974a).

Actions on the gastrointestinal tract and exocrine pancreas

The effects of somatostatin on various gastrointestinal hormones is summarized below.

Somatostatin:

i) decreases basal and meal-stimulated gastrin levels in man. Patients with elevated gastrin levels due to pernicious anaemia show partial gastrin suppression with somatostatin infusion (Bloom et al 1974)

ii) inhibits HCl-stimulated secretin release and pancreatic secretion of water, bicarbonate and protein in the dog (Boden et al 1975)

iii) decreases cholecystokinin (CCK) release due to intestinal perfusion with amino acids, sodium oleate, HCl or a peptone meal in dogs with pancreatic fistulas (Konturek et al 1976)

iv) decreases secretin-induced pancreatic bicarbonate secretion and CCK-induced pancreatic enzyme secretion in man (Creutzfeldt et al 1975)

v) decreases pepsin and gastric acid secretion stimulated by a test meal, pentagastrin, urecholine or histamine in dogs and man (Gomez-Pan et al 1975)

The actions of somatostatin on the digestive tract may be mediated by the decreased GH levels it produces in long term studies. This is because GH is known to have secretory and trophic influences on the digestive system (Enoch and Johnson 1975)

Effects on the kidney

Infusion of somatostatin (10 ug/min) to normal males with furosemide-induced renin secretion caused a 30% decrease in plasma renin activity (Gomez-Pan et al 1976c). Plasma aldosterone was unaffected. The infusion rate necessary for this effect however, is nearly ten times that needed to give maximal GH level suppression in humans (Besser et al 1974b).

Actions on hormone secretion by tumours

Somatostatin suppresses hormone secretion by a number of tumours, including:

i) GH (Besser et al 1974b, Hall et al 1973, Yen et al 1974) and ACTH (Tyrrell et al 1975) in pituitary tumours.

ii) insulin (Gomez-Pan et al 1976b; Scuro et al 1976) and glucagon (Mortimer et al 1974) in pancreatic islet tumours.

iii) gastrin, in a pancreatic tumour of Zollinger-Ellison syndrome (Bloom et al 1974)

iv) vasoactive intestinal peptide (VIP) in a patient with Verner-Morrison's syndrome (Bloom et al 1975)

Neurological effects of somatostatin

Somatostatin is found in synaptosomes of neurons of the hypothalamus and extrahypothalamic loci (Hokfelt et al 1974, Pelletier 1974). This strongly suggests somatostatin is a neurotransmitter. Further evidence, is a wide variety of actions of somatostatin in the nervous system which are listed.

In the central nervous system somatostatin:

i) gives similar responses whether in the cyclic or linear form

ii) potentiates L-dopa activity (Wilber et al 1976) like MSH-1 (Plotnikoff et al 1971), TRH and LHRH (Wilber et al 1976) but to a lesser degree.

iii) reduces the fatal dose of pentobarbital (LD_{50}) (Brown and Vale 1975), an effect opposite to TRH (Prange et al 1974, Breese et al 1975)

iv) decreases spike frequency in many neurons of the cerebral cortex, cerebellar cortex and hypothalamus like LHRH and TRH (Wilber et al 1976) when applied by micro-iontophoresis.

v) enhances Ca^{+2} uptake in synaptosome preparations (Tsang et al 1975)

vi) causes behavioral, motor and electrophysiological changes when administered cortically or to the hippocampus, including: patterns of stereotyped behavior, alteration of sleep-waking cycle, and co-ordination difficulties associated with drowsiness (Rezek et al 1976a) while hippo-campal administration also causes frequent dissociation of the EEG from behaviour (Rezek et al 1976b)

Electrical stimulation studies of several brain areas indicates these areas receive axon collaterals from tuberoinfundibular neurons. These areas include various hypothalamic and limbic loci (Renaud and Martin 1975, Harris and Singhera 1974, Renaud 1976, Renaud and Martin 1974). Tuberoinfundibular neurons have long been associated with neural control of anterior pituitary function (Szentagsthal et al 1968), so if somatostatin is a regulator of GH secretion it may act as a neurotransmitter via these neurons.

Collaterals from tuberoinfundibular neurons may explain the distribution of somatostatin throughout the brain, if such neurons were somatostatinergic. TRH is also widely distributed in the brain and ablation of the hypothalamic TRH regulatory area causes reduced TRH content in the cerebral cortex (Jackson and Reichlin 1975), indicating that axon collaterals from the TRH regulatory area extend to the cerebral cortex.

Mechanism of action of polypeptide hormones

It is widely believed that polypeptide hormones exert their biological effect by binding to membrane receptors, because when antisera against hormone receptors are administered to experimental animals the action of the hormone is inhibited (Shiu and Friesen 1976; Patrick and Lindstrom 1973). There are membrane receptors for many peptide and polypeptide hormones (Cuatrecasas 1974).

Cuatrecasas (1975) developed a number of criteria for the acceptance of a binding protein as a hormone receptor. These include:

i) strict structural and steric specificity

ii) saturability, indicating a limited number of binding sites

iii) tissue specificity, in accord with target glands for the hormone

iv) high affinity, permitting sensitivity to physiological concentrations of the hormone

v) reversibility, which is consistent with the disappearance of the hormone's effect after removal of the hormone from the system

Many hormones exert their effect by activating membrane bound adenyl cyclase. Cuatrecasas (1974) notes that fat cells contain membrane receptors for seven different hormones, all of which alter membrane-bound adenyl cyclase activity. If each of these membrane receptors, with molecular weights of 200,000-300,000 daltons are to interact physically with adenyl cyclase, it would be physically impossible for all these different receptors to be bound to adenyl cyclase at any one time.

To allow many hormones to interact with one membrane enzyme, Cuatrecasas (1974) developed a theory of hormone action dependent on the current concept of the membrane as a fluid matrix in which embedded proteins are free to move about and interact in a two-dimensional field (Singer and Nicholson 1972). In this case, formation of the hormone-receptor complex gives the receptor an affinity for a membrane enzyme such as adenyl cyclase. Binding of adenyl cyclase to hormone-receptor complexes activates or inhibits the enzyme. Activation of adenyl cyclase produces cyclic AMP which then mediates the action of the hormone in the cell.

An example of cyclic AMP mediation of hormone effect that has been elucidated is the action of epinephrine and glucagon in increasing phosphorylase activity in muscle (Sutherland and Rall 1960). Cyclic AMP then activates a phosphorylase kinase which activates a phosphorylase. The phosphorylase catalyzes breakdown of glycogen.

Mechanism of action of hypothalamic hormones

Somatostatin is thought to be a hypothalamic hormone that suppresses GH secretion after being transported to the pituitary in a manner similar to LH-RH and TRH from the hypothalamus (Redding and Schally 1971; Reichlin 1975). The mechanism of action of LH-RH and TRH may therefore be more relevant in understanding the mechanism of action of somatostatin.

Both TRH (Grant et al 1972; Wilber and Seibel 1973) and LH-RH (Borgeat et al 1972) act on pituitary cells by binding to specific membrane receptors, and appear to activate the adenyl cyclase enzyme system in the rat (Kaneko et al 1973; Borgeat et al 1972). There is no evidence however that the cells with increased cyclic AMP levels are actually LH- and TSH-producing cells.

TRH stimulates the secretion of prolactin and increases prolactin synthesis while decreasing GH synthesis, in isolated GH-cells from rat pituitary (Dannies and Tashjian 1973). TRH appears to increase PRL synthesis by increasing the accumulation of cytoplasmic mRNA for PRL (Dannies and Tashjian 1976b). There is only one class of high affinity TRH receptors on GH-cells (Hinkle and Tashjian 1975). Prolonged exposure of GH-cells to TRH causes decreased numbers of these receptors This process is dependent on protein synthesis since cycloheximide inhibits the response (Hinkle and Tashjian 1975).

In GH-cells, TRH increases intracellular cAMP accumulation (Dannies et al 1976). Intracellular cyclic AMP levels do not effect GH production because exogenously applied cAMP, its analogues and phosphodiesterase inhibitors had no effect on GH production (Dannies et al 1976). Prolactin synthesis and release are affected to different degrees by different analogues of TRH (Dannies and Tashjian 1976), indicating that the mechanism of action of TRH in these two effects is different. The effect of TRH on PRL release as with TSH release, appears to be mediated by cyclic AMP (Dannies and Tashjian 1976). The mechanism of action of TRH in affecting GH and PRL synthesis is as yet unknown.

LH-RH binds to a single, high affinity membrane receptor in a variety of rat tissues (Marshall et al 1975). In the pituitary there is also a low affinity, high capacity site (Marshall et al 1975; Spona 1973). The low affinity site is thought to mediate FSH release and the high affinity site, LH release. This is because loss of the low affinity site after long-term ovariectomy, parallels a decreased ability of somatostatin to elicit an FSH response to LHRH stimulation.

Borgeat et al (1972) showed that LH-RH stimulates cyclic AMP accumulation in rat anterior pituitary gland in vitro. Removal of Ca^{+2} from the media causes a 75% inhibition of this effect (Borgeat et al 1975). This is

consistent with other evidence that Ca^{+2} is necessary for hormonal activation of the adenyl cyclase system (Bar and Hoechter 1969; Sayers et al 1972; Leftkowitz et al 1970).

Both immunoreactive LH-RH and TRH have been localized in synaptosomes of mammalian hypothalami (Barnea et al 1975) indicating they may be neurotransmitters. LH-RH (Endroczi and Hilliard 1965; Piacsek and Meites 1966) and TRH (Jackson and Reichlin 1974a,b) also have widespread distribution in the extrahypothalamic rat brain. Burt and Snyder (1975) also showed receptors for TRH in rat brain membranes. This evidence indicates that TRH and LH-RH may be neurotransmitters.

The source of TRH throughout the rat brain is at least partially the hypothalamus since ablation of TSH-regulatory areas of the hypothalamus decreases TRH levels throughout the brain (Jackson and Reichlin 1975) suggesting innervation of many brain areas with neurons or collaterals from the hypothalamus.

Somatostatin as a hormone

Somatostatin is assumed to be a hormone, even though it has yet to be detected in significant amounts in sera (Arimura et al 1975a). Transport of somatostatin, like TRH, to the anterior pituitary gland via a hypophyseal venous portal system (Redding and Schally 1971) has not yet been shown.

There is some circumstantial evidence for somatostatin as a hypothalamic hormone. Hypophysectomy decreases somatostatin stores in the hypothalamus, indicating a possible feedback by GH on hypothalamic somatostatin levels and release (Baker and Yen 1976).

Terry et al (1976) and Arimura et al (1976) showed somatostatin antisera, injected into rats, inhibited stress-induced abolition of GH pulsatile release. The actual source of GH pulses is unknown but the ability of somatostatin antisera to inhibit the above response suggests somatostatin is physiologically important in suppressing GH levels, even if only in these limited circumstances.

Evidence for somatostatin being a neurotransmitter is strong. Somatostatin is widely distributed in the rat brain (Brownstein et al 1975) and is localized in synaptosomes in several rat brain areas (Hokfelt et al 1974, Pelletier 1974). Further evidence is the variety of actions of somatostatin in the nervous system, as previously discussed.

Because somatostatin may be a neurotransmitter does not mean it cannot be a hormone, because similar evidence of neurotransmitter function has been shown for TRH and LH-RH (Barnea et al 1975; Burt and Snyder 1975). Somatostatin has a variety of actions outside the brain and pituitary. While these actions may not be important in vivo, they correlate well with the location of somatostatin in D-cells of the

pancreatic islets and gastrointestinal tract (Arimura et al 1975b; Patel et al 1975a). This correlation indicates somatostatin may be a local messenger, analogous to prostaglandins, especially since somatostatin has not yet been found in the blood (Arimura et al 1975a).

It has been proposed that somatostatin-containing cells in the gut and pancreatic islets are embryologically derived from the neural crest as is the hypothalamus (Welbourn et al 1974). Substance P has a similar distribution to somatostatin and is localized in the brain and in specific endocrine cells of the gut of proposed neural crest origin (Pearse and Polak 1975). This may explain somatostatin's widespread distribution.

Mechanism of action of somatostatin

Like TRH and LH-RH, the action of somatostatin has been associated with adenyl cyclase, but somatostatin's mechanism of action is not so straightforward. Whatever may be somatostatin's mechanism of action, its effects are not blocked by agents that block protein and RNA synthesis (Vale et al 1973; Vale et al 1974) indicating an action independent of protein synthesis.

Cyclic AMP and somatostatin's action

Somatostatin inhibits basal and prostaglandin ($PGE_{2\alpha}$)-induced cyclic AMP accumulation in rat pituitaries *in vitro*, suggesting that it acts at a step preceding cAMP formation. Somatostatin's suppression of glucagon-induced

cyclic AMP accumulation in isolated rat pancreas (Gerich et al 1974b) leads to similar conclusions. This causes decreased insulin secretion because cyclic AMP stimulates insulin release (Montague et al 1971; Malaisse et al 1967). Robberecht et al (1975) showed that somatostatin inhibits secretin-induced cyclic AMP accumulation in the exocrine pancreas and therefore pancreatic hydrolase release. Secretin also stimulates cyclic AMP accumulation in the exocrine pancreas (Robberecht et al 1974). This evidence favours somatostatin acting at a step preceding cyclic AMP synthesis (i.e. at a membrane receptor).

Somatostatin inhibits cyclic AMP accumulation due to theophylline stimulation, in the rat pituitary and pancreas, to a much greater extent than basal levels (Borgeat et al 1974; Garcia et al 1976). Perracchi et al (1976) and Garcia et al (1976) showed that somatostatin inhibits GH and insulin release induced by exogenously administered dibutyryl cyclic AMP in humans and rats. This indicates an inhibition of cyclic AMP accumulation in various cells at a step distal to cyclic AMP synthesis. Theophylline is known to directly affect flux across some membranes (Johnson and Inesi 1969) so this effect may be due to the effect of theophylline on membranes and not due to phosphodiesterase activity.

Kenako et al (1974) found that somatostatin decreases cyclic AMP and increases cyclic GMP concentration in rat pituitaries. Because cyclic GMP enhances cyclic AMP

hydrolysis by phosphodiesterase (Hardman et al 1971; Klotz et al 1972), somatostatin may decrease cellular cyclic AMP by increasing cyclic GMP. Whatever the mechanism by which somatostatin decreases cyclic AMP accumulation, increased cyclic AMP levels may explain the decreased GH and TSH release seen on somatostatin administration, because cyclic AMP stimulates GH and TSH release (Belanger et al 1974). Inhibition of cyclic AMP accumulation may also explain the inhibition of gastrin secretion caused by somatostatin (Gomez-Pan et al 1975) because gastrin secretion is associated with an increase in intracellular cyclic AMP (Gabrys et al 1973).

It is important to note that in these studies, cyclic AMP levels were measured in whole pituitaries and whole pancreas or pancreatic islets. There is no direct proof that somatostatin decreases cyclic AMP accumulation specifically in GH-secreting cells of the pituitary or the alpha or beta cells of pancreatic islets.

Robberecht et al (1975) found that somatostatin inhibits secretin-induced cyclic AMP accumulation in the exocrine pancreas, causing inhibition of secretin-induced pancreatic hydrolase secretion. However, at high concentrations somatostatin actually stimulates cyclic AMP accumulation in the exocrine pancreas *in vitro* (Robberecht et al 1975) and pancreatic hydrolase secretion *in vivo* . The interaction of somatostatin and secretin on pancreatic secretion shows signs of competitive inhibition

by kinetic analysis (Konturek et al 1976).

The existence of competitive inhibition does not exclude the possibility that somatostatin may act by another mechanism to decrease cyclic AMP, especially since high levels of somatostatin are necessary for such an effect. Competitive inhibition between secretin and somatostatin would not be suprising however, because secretin and somatostatin have a four amino acid sequence in common (a.a's 5-8 in secretin and a.a.'s 10-13 in somatostatin).

Cellular calcium and somatostatin action

While inhibition of adenyl cyclase may be a sufficient mechanism to explain the action of somatostatin in some tissues, including the pituitary, it is not sufficient to explain somatostatin's effects on pancreatic insulin secretion.

Increased intracellular cyclic AMP increases insulin secretion (Malaisse et al 1967; Montague and Cook 1971) and somatostatin decreases both islet cyclic AMP levels and insulin secretion due to a variety of stimuli (Efendic et al 1975; Gerich et al 1974b; Borgeat 1974). Calcium ions are however, essential for insulin secretion (Curry et al 1968b) as for other secretory processes (Douglas and Poisner 1963).

Inhibition of insulin secretion by somatostatin is reversed by elevating Ca^{+2} concentration in the incubation media (Curry and Bennett 1974b). The inhibition of insulin release by somatostatin is also dependent on inhibition

of Ca^{+2} flux into the secretory cell (Taminato et al 1973). Fujimoto and Ensinck (1976) added ionophore A23187 to rat pancreatic islet cell cultures. This ionophore bypasses normal cell pores and allows Ca^{+2} flux through cell membranes (Reed and Hardy 1972). When added to islet cells in culture, the release of insulin and glucagon increased, in proportion to external Ca^{+2} concentration. Increased insulin and glucagon secretion occured despite the presence of somatostatin in the media. The abolition of somatostatin inhibition was probably due to Ca^{+2} influx because lack of extracellular Ca^{+2} on addition of A23187 meant there was no effect on insulin secretion (Wolheim et al 1975). This evidence suggests somatostatin suppresses insulin release, by inhibiting a plasma membrane Ca^{+2}-carrier system, so reducing Ca^{+2} influx and inhibiting insulin release.

The cyclic AMP and Ca^{+2} flux theories of controlling insulin release need not b mutually exclusive and in fact may be necessary to explain somatostatin's actions on islet cells. Garcia et al (1976) showed that somatostatin inhibits both insulin secretion and biosynthesis, and that high Ca^{+2} levels reverse this inhibition. However some investigators have shown that some extracellular Ca^{+2} concentrations cause considerable changes in insulin release (Curry et al 1968b) while not modifying insulin biosynthesis (Steiner et al 1972).

This indicates that Ca^{+2} fluxes cannot account for all the actions of somatostatin in the pancreas. Even

without this evidence it is recognized that increased cyclic AMP levels increase insulin release (Malaisse et al 1967; Montague and Cook 1971).

In isolated rabbit ileum, increased cyclic AMP, and theophylline acting via cyclic AMP, cause increased ion fluxes (Powell et al 1974). It is possible that cyclic AMP increases Ca^{+2} flux in beta-cells which then causes insulin release, explaining both theories of somatostatin action as one. Ca^{+2} is also known to be necessary for hormonal activation of adenyl cyclase (Bar and Hoechter 1969; Sayers et al 1972; Leftkowitz et al 1970).

Somatostatin and α-adrenergic receptors

Somatostatin is contained in pancreatic D-cells (Dubois; Polak et al 1975; Goldsmith et al 1975) and also in certain central and peripheral neurons (Pelletier et al 1974). Somatostatin may therefore act as a neuro-transmitter and may be important in pancreatic islet function. Since catecholamines inhibit insulin secretion through α-adrenergic receptors (Woods and Porte 1974), Smith et al (1976) using phentolamine (an α-blocker) infusion in rats, showed that phentolamine blocked somatostatin's suppression of insulin release. This could be due to pharmacological effects of phentolamine (Nickerson and Hollanberg 1967) or to unopposed endogenous β-stimulation(Yen et al 1974). The latter is unlikely since somatostatin inhibits insulin release due to isoproterenol (a β-agonist) (Gerich et al 1975a).

Therefore, there may be a direct interaction between somatostatin and α-adrenergic receptor mechanisms on pancreatic β-cells.

Alpha-adrenergic receptor activation causes inhibition of insulin release elicited by most secretagogues except arginine and secretin (Malaisse 1972) but somatostatin inhibits insulin secretion due to secretin and arginine as well (Chideckel et al 1975). Somatostatin's effects cannot therefore be due totally to activation of α-adrenergic receptors.

Epinephrine also causes decreased islet cyclic AMP levels but the inhibitory effect of α-stimulation on insulin secretion appears to be independent of its effect on cyclic AMP levels, and its inhibitory effect may rather be due to its inhibition of cellular Ca^{+2} uptake (Malaisse 1972, Porte and Robertson 1973).

The importance of α-adrenergic receptors in somatostatin's action is unknown but it does have some role in regulation of intracellular cyclic AMP levels and Ca^{+2} uptake by islet cells, and as previously mentioned, somatostatin also potentiates the actions of L-dopa in the central nervous system. This indicates an association with α-adrenergic receptors (Wilber et al 1976).

OBJECTIVES

A cytosol binding protein for somatostatin has been detected in various rat, human and bovine tissues. The objectives of this study are to characterize this binding factor in extracts from various rat tissues and consider its possible role as a mediator of the action of somatostatin. Various steroid hormones have cytosol receptors or binding proteins important in their action. These form reversible hormone-receptor complexes (O'Malley 1971; Feldman et al 1971; Feldman 1975). No polypeptide hormone has yet been shown to exert its effect via a cytosol receptor. Characterization of this somatostatin binding protein will help to determine whether this somatostatin binding protein is a hormone receptor.

Characterization of the binding activity involves determining properties such as pH and ionic optima and the time course and specificity of the binding. Specificity is particularly important for the binding protein to be physiologically significant in mediating hormone action. Also to be determined are the biochemical composition of the somatostatin binding protein and its molecular weight, isoelectric point and electrophoretic mobility. By comparing the biochemical properties of the binding factor in various rat tissues, it will be determined if it is the same molecule in each tissues. This is an important criteria for the binding protein to be considered physiologically significant. Biochemical and binding studies

also provide a basis for comparison of the somatostatin binding protein to other hormone receptors and also to many cytosol enzymes through which somatostatin may be mediating its action. This would allow a better understanding of the physiological significance of the somatostatin binding protein.

MATERIALS AND METHODS

Hormone and drug preparations

Somatostatin (AY-24, 910), [Tyr^{1}]-somatostatin and luteinizing hormone-releasing hormone (LH-RH), products of Ayerst Research Laboratories, Montreal, Canada, were generously provided by Dr. M. Gotz. [Ala^{3,14}]-somatostatin, [D-Trp^{8}]-somatostatin and α-endorphine were gifts from Dr. R. Guillemin (Salk Institute, Lajolla, CA). Thyrotropin-releasing hormone (TRH) was a gift from Takeda Chemicals Industries, Ltd., Osaka, Japan. Methionine-enkephalin (Met-enkephalin) was obtained from Peninsula Lab. Inc.,L.A. CA. and naloxone was from Endo Drugs Ltd., Montreal, Canada. Human growth hormone (hGH, NIH HS2019G), dog growth hormone (dGH, NIH D-1001A), bovine growth hormone (bGH, NIH B-1003A), porcine growth hormone (pGH, NIH P-526B), ovine growth hormone (oGH, NIH O-743B), rat growth hormone (rGH, NIH RP-1), rat prolactin (rPRL, NIH RP-1), ovine prolactin (oPRL, NIH P-S-10), bovine thyrotropin (bTSH, NIH-TSH-B-3), human follicle-stimulating hormone (hFSH, NIH LER 1366), human luteinizing hormone (hLH, NIH LER960), ovine LH (oLH, NIH LH-SI8), and human placental lactogen (hPL) were obtained from the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, MD. Ovine placental lactogen (oPL0 was purified in this laboratory (Chan et al 1976). Synthetic lysine-8-vasopressin and synthetic oxytocin were gifts from Sandoz Ltd., Basel, Switzerland and

porcine glucagon (p-glucagon) was a gift from Eli Lilly and Company, Indianapolis Ind. Synthetic ACTH (Cortrosyn) and porcine insulin (p-insulin) were purchased from Organon Canada Ltd., Toronto, Canada and Connaught Lab. Ltd., Toronto, Ont., respectively. Dithiothreitol (DTT), trypsin (bovine pancreas), soybean trypsin inhibitor, ribonuclease (RNAase; bovine pancreas), deoxyribonuclease I (DNAase; bovine pancreas), phospholipase C and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. St. Louis, Mo. Mercaptoethanol, acrylamide and bis-acrylamide were purchased from Eastman-Kodak Co. N.Y., and guanidine-HCl from Schwarz-Mann, N.Y., U.S.A. Urea, NaCNS and H_2O_2 (30%, v/v, solution) were from Fisher Scientific Co.,N.J. Lactoperoxidase was from Calbiochem, La Jolla, Ca. $Na^{125}I$ and $Na^{131}I$ (carrier-free) were bought from New England Nuclear, Boston, Mass. Sephadex G-100 and Dextran Blue 2000 was obtained from Pharmacia, Uppsala, Sweden.

Preparation of cytosol fractions

Sprague-Dawley female rats (200-225 g, Bio Labs, N.J.) were killed by decapitation. Various tissues and organs were removed immediately and frozen on dry ice. They were thawed and homogenized on the same day.

Tissues were homogenized in 10 volumes (w/v) of tris-EDTA buffer (50 mM tris-HCl buffer pH 8.0, 25mM EDTA) at $4^{o}C$ with a Brinkman Polytron PT-10 at full speed for

2 minutes. Homogenates were centrifuged at 200,000g for one hour on a Beckman L5-65 ultracentrifuge. The supernatants were frozen and stored at -20°C.

Protein determination

All protein determinations were obtained using the absorbance of solutions at 280 nm. Bovine serum albumin was used as standards.

Gel filtration and molecular weight determination

For study of the properties of the binding protein of various tissues, crude cytosol preparations (10 ml) were applied to a Sephadex G-100 column (4cm x 60cm) and 5 ml aliquots collected and assayed for somatostatin binding activity. Peak tubes were pooled and concentrated by ultrafiltration on a UM-10 membrane. These purer fractions were used for isoelectrofocusing and electrophoretic studies as well as other studies.

To determine the molecular weight of the cytosol somatostatin binding protein in various tissues, crude cytosol preparations (1 ml) were applied to a Sephadex G-100 column (2 x 90 cm) and fractions of 2-2.5 ml were collected. Molecular weight was determined against the protein markers: cytochrome C (horse heart), myoglobin (sperm whale), human serum albumin, [^{125}I]-iodo-human growth hormone and ovalbumin. Void volume was determined with blue dextran 2000.

DEAE-cellulose ion exchange chromatography

Crude female Sprague-Dawley rat liver cytosol (1-5 mg/ml)

in (.02M tris-HCl, 10 mM EDTA pH 8.0) buffer was applied to a DEAE-cellulose column (5cm x 30cm) equilibrated with the same buffer. After sample application, the column was washed with the same buffer containing 100 mM NaCl until equilibrated. The column was then washed with several bed volumes of the buffer containing 200 mM NaCl. This final column effluent contained most of the somatostatin binding activity and was concentrated by ultrafiltration on a UM-10 membrane. This concentrate was used for electrophoresis and isoelectrofocusing studies.

Assay system of somatostatin binding activity

Iodination. [Tyr^1]-somatostatin must be used for iodination since somatostatin lacks amino acids that can be iodinated. The biological activity of [Tyr^1]-somatostatin compared to native hormone is reported as 100% (Ferland et al 1976) and 25% by Vale et al (I975). A modified lactoperoxidase method of Thorell and Johansson (1971) was used for iodination. Unlike the above method, the pH of the buffer (ammonium acetate .002M) was 4.2 instead of 7.4 and 5 µg of [Tyr^1]-somatostatin, 5 ug of lactoperoxidase and 10 µg of 30% H_2O_2 (1:50,000 dilution) with a reaction period one minute was used. The reaction was terminated by diluting the reactants with excess cold buffer (ammonium acetate .002 M, pH 4.6) and the somatostatin was eluted with a continuous gradient from .002 M to .25 M. The specific activity of the labelled hormone was 80 to 120 uCi/ ug, as determined by the amount (µCi) of the radioactivity which is TCA precipitable divided by

the number of μg of hormone added.

Assay of somatostatin binding activity. Cytosol protein was incubated with 15,000-20,000 cpm of [^{125}I]-iodo-[Tyr^{1}]-somatostatin with or without 0.1 ml of 10 μg/ ml somatostatin in tris-HCl-EDTA buffer (.05 M tris-HCl, pH 8.0, 25 mM EDTA and 0.5% w/v BSA) for a total incubation volume of .5 ml.

Samples were incubated for 24 h at 4°C. The reaction was terminated by adding 1 ml of dextran-coated charcoal solution (DCC, Arimura et al, 1973) in the assay buffer. Tubes were vortexed and left for 30 min before centrifugation at 2000g at 4°C for 30 minutes. The supernatant was aspirated and the pellet counted. These counts represent unbound somatostatin. Specific binding is the difference in counts between the pellets in the tubes with excess cold hormone and those with no cold hormone added, expressed as a percentage of total counts added.

Dissociation studies

Rat liver cytosol was incubated with [^{125}I]-iodo-[Tyr^{1}]-somatostatin at 4°C and then DCC was added and centrifuged. The supernatant (bound form) was added to various reagents and the dissociation of [^{125}I]-iodo-[Tyr^{1}]-somatostatin against time was examined over 24 hours.

To these supernatants were added a variety of reagents: 0.1 M acetic acid, o.1 NaOH, 2 mM dithiothreitol, 5 mM mercaptoethanol, 5 M $CaCl_2$, 8 M urea, 7 M guanidine-HCl,

or 5 M NaCNS for various periods of time and then DCC was added again and the cpm in the second pellet counted in an automatic gamma counter.

The pH and ionic optima

To determine the pH and ionic optima, assay systems were used with the assay buffer at different pH's and with different ionic constituents. All contained .5% BSA.

Specificity

Specificity of the binding protein for somatostatin was carried out by incubation of binding protein with [^{125}I]-iodo-[Tyr^{1}]-somatostatin and various concentrations of other peptide and polypeptide hormones.

Isoelectrofocusing

Analytical thin layer polyacrylamide gel isoelectric-focusing was performed with an LKB 2117 multiphor apparatus. Ampholytes were of a pH range 3.5-9.5. The gel was 6% (w/v) acrylamide, with cross-linking of 2.5%.

Crude cytosol protein from a variety of rat tissues was applied to separate channels of an isoelectrofocusing gel and focused. The gel channels were then cut into 5 mm slices and each piece was eluted in 1 ml of assay buffer overnight at 4^{o}C with constant agitation. The eluant was assayed for binding activity.

Binding protein, purified by DEAE-cellulose chromatography in the case of rat liver cytosol and by Sephadex G-100 chromatography in the case of other tissues, was labelled by incubation with [^{125}I]-iodo-[Tyr^{1}]-somatostatin and

the free tracer was removed by the dextran charcoal method. This was then applied to the gel and focused. It is assumed that the small size of the somatostatin tracer will affect the isoelectric point of the binding protein little when the two are bound. Each channel of the gel was cut into 5 mm slices after focusing for 3h with a constant power of 15 watts and then counted on an automatic gamma counter. Similar studies were done with tissue cytosol mixtures in which one tissue was labelled by preincubation with [^{125}I]-iodo-[Tyr^{1}]-somatostatin and the other with [^{131}I]-iodo-[Tyr^{1}]-somatostatin. These tissue samples are mixed and applied as one sample to an isoelectrofocusing gel. By mixing two differentially labelled somatostatin binding proteins from different sources, and applying them as one sample, you can compare the isoelectric points of binding proteins from different sources on the same channel of the gel. This eliminates the small variations between different runs, and different channels of the same run, so it can be more precisely determined if the binding proteins from different tissues are identical. In all the above isoelectrofocusing experiments, buffers contained .5% bovine serum albumin to reduce non-specific binding of labelled somatostatin to the gel. [^{125}I] and [^{131}I] activities were measured simultaneously on an automatic gamma counter.

Tissue mixtures were differentially labelled with [^{125}I] and [^{131}I]-iodo-[Tyr^{1}]-somatostatin as previously described, except buffers contained no BSA. The gels were

then stained with Coomasie Blue and destained with water, ethanol and acetic acid in a 8:3:1 ratio. The two major protein bands located in the area of the gel corresponding to the isoelectric point of the somatostatin binding protein were separately dissected from the gel and counted.

Cytosol fractions without bovine serum albumin in the buffers and without iodinated hormone bound, were also run on isoelectricfocusing and stained. Blank channels of each run were sliced into 5 mm slices after focusing and eluted overnight in distilled water, with constant agitation. The pH of the ampholytes eluted from each gel segment was then determined on a glass electrode pH meter, yielding the pH gradient across the gel.

Analytical gel electrophoresis

Polyacrylamide gel electrophoresis was performed according to a modified method of Davis (1964). A 7.0% (w/v) acrylamide gel was used with a pH of 8.8-9.0. Samples were also bound to somatostatin tracer as with isoelectricfocusing, to detect peak somatostatin binding activity.Gels were sliced and counted on an automatic gamma counter.

As with isoelectricfocusing studies, tissue mixture studies were done. One tissue cytosol binding protein (purified by gel chromatography on Sephadex G-100) is labelled with [^{125}I]-iodo-[Tyr^{1}]-somatostatin and another tissue binding protein is labelled with [^{131}I]-iodo-[Tyr^{1}]-somatostatin by preincubation with the tracer.

Free tracer is removed by addition of a DCC suspension followed by centrifugation. These two differentially labelled binding proteins are then mixed and applied as one sample to a disc gel electrophoretic gel. A current of 2 mAmp / gel is applied for 2h. The gels are then cut into 3 mm slices and counted for [^{125}I] and [^{131}I] activity simultaneously. As previously explained for isoelectric-focusing, such tissue mixture studies eliminate variation between different electrophoretic runs and different gels of the same run. This allows a more accurate examination of the similarity of somatostatin binding proteins from various tissues. Buffers used contained 0.5% BSA.

Cytosol preparations with and without tracer bound were also focused, but without BSA in the buffers. These were stained with Amido Black (1% w/v) and destained in 7% acetic acid. Protein bands in the region of the peak of somatostatin binding activity were cut out and counted to determine which constituted the binding protein.

RESULTS

I thank Dr. Norio Ogawa for the use of his results in this thesis, including figures 1-7 and tables 1-6. During the course of his studies I aided him with column chromatography. The data for which I am primarily responsible is found in figures 8-16.

Distribution and Properties of Somatostatin Binding Activity

Distribution of somatostatin binding protein.

A somatostatin binding protein has been found in the cytosol of a number of tissues from the rat. The distribution of somatostatin binding sites is shown in Table 1. Incubation of the 100,000g particulate fraction and the cytosol (100,000g) of rat liver with [^{125}I]-iodo-[Tyr^{1}]-somatostatin followed by centrifugation at 200,000g for 2h, showed that the supernatant of the 100,000g pellet and the cytosol contained most of the somatostatin binding activity (see Table 2). This means somatostatin binding sites probably are situated in the cytosol. Similar results were obtained with the submandibular gland. Tissue distribution of the somatostatin binding activity is shown in Table 3.

EDTA and Ca^{+2} effects on binding

Tris-HCl (5o mM) buffers with 25 mM EDTA, no EDTA, or with no EDTA but 1 mM $CaCl_2$, were used as homogenization

and incubation media in somatostatin binding studies. Figure 1 shows that Ca^{+2} reduces somatostatin binding markedly. EDTA (25 mM) which would remove trace Ca^{+2} levels from the cytosol greatly enhances binding over media with no EDTA or Ca^{+2}.

pH optimum

Figure 2 shows the effect of pH on somatostatin binding to the cytosol binding protein. The pH optimum appears to be from pH 8.0 to 8.5 in all tissues studied.

Time course and temperature effects

Specific binding in all rat tissues studied was maximal at 12 to 24 h of incubation at $4^{o}C$ (figure 3). Incubation at $24^{o}C$ or on ice resulted in lower maximal specific binding than at $4^{o}C$. An 18h incubation period was used in routine assays.

Effect of protein concentration

As shown in figure 4, increasing cytosol protein concentrations in incubations with [^{125}I]-iodo-[Tyr^{1}]-somatostatin yielded a direct relationship between specific binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin and protein concentrations of 3-50 ug per tube at $4^{o}C$.

Effects of various reagents on binding

The effect of exposure of cytosol to extreme temperature and pH, and to 85% ethanol is shown in Table 4.

Dissociation studies

Excess unlabelled somatostatin fails to displace [^{125}I]-iodo-[Tyr^{1}]-somatostatin over 18h at 4^{o}C with some displacement over 24h at 4^{o}C. Dithiothreitol and mercaptoethanol enhance dissociation greatly, whereas urea and guanidine-HCl have moderate effects as shown in Table 5. The effects of other reagents on dissociation are also shown.

Specificity of the somatostatin binding activity

Specificity studies

pituitary cytosol. Significant inhibition of the binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin occurred with 3 ng/ml (300 pg/tube) of somatostatin. [Tyr^{1}]-somatostatin and [D-Trp^{8}]-somatostatin (both cyclic forms) cross-reacted 150% and 40% respectively, as compared to native hormone. [Ala^{3,14}]-somatostatin, however, was only 10% cross-reactive. Synthetic ACTH (5%) and porcine glucagon (7%) were also cross-reactive. The cross-reactivity of other hormones is listed in figure 5, but none are more than 1% cross-reactive. Specificity was similar using either crude or semipurified somatostatin binding protein.

The effect of somatostatin binding protein on the radioimmunassay for somatostatin

Somatostatin binding activity decreases the binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin to anti-somatostatin antiserum (figure 6). The effect of increasing

concentrations of somatostatin binding activity in a standard radioimmunoassay is shown in figure 7. Semi-purified somatostatin binding protein (1 ug/tube) significantly decreases binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin to anti-somatostatin antiserum.

Biochemical Properties of Somatostatin Binding Activity

Biochemical nature of somatostatin binding activity

Incubation of cytosol with trypsin (50 ug/ml) caused a 60-70% decrease in [^{125}I]-iodo-[Tyr^{1}]-somatostatin (Table 5), so that the binding activity is probably a protein. Ribonuclease, deoxyribonuclease, and phospholipase C had no effect on somatostatin binding activity, therefore nucleic acids and phospholipids are not essential for somatostatin binding.

Molecular weight determination

Using a Sephadex G-100 column (2cm x 90cm), the molecular weight of somatostatin binding protein from a variety of female rat tissue cytosols was determined. The somatostatin binding protein in crude female rat liver, heart, stomach, submandibular gland, muscle and brain cytosols has a K_{av} of approximately .11 and a molecular weight of approximately 77,000 daltons (figgre 8) .

Isoelectricfocusing

Somatostatin binding protein from various tissues, bound to [^{125}I]-iodo-[Tyr^{1}]-somatostatin has an isoelectric point of approximately 4.8-5.0 (figure 9b). Somatostatin

binding protein from various tissues also has an isoelectric point of approximately 5.0 as determined by assay of the eluant of isoelectrofocusing gels after focusing (figure 9b). This illustrates that labelling the somatostatin binding protein is an accurate way to determine the presence of somatostatin binding activity The peak of somatostatin-labelled binding protein did not correspond to the peak of free [^{125}I]-iodo-[Tyr^{1}]-somatostatin (figure 10). Liver cytosol binding protein was also bound to [^{131}I]-iodo-[Tyr^{1}]-somatostatin-labelled somatostatin binding proteins from various tissues. Wjen these tissue mixtures were separated by isoelectrofocusing, identical distributions of [^{125}I] and [^{131}I] were detected, indicating somatostatin binding proteins in various tissues are similar to liver somatostatin binding protein and therefore to each other (figure 11).

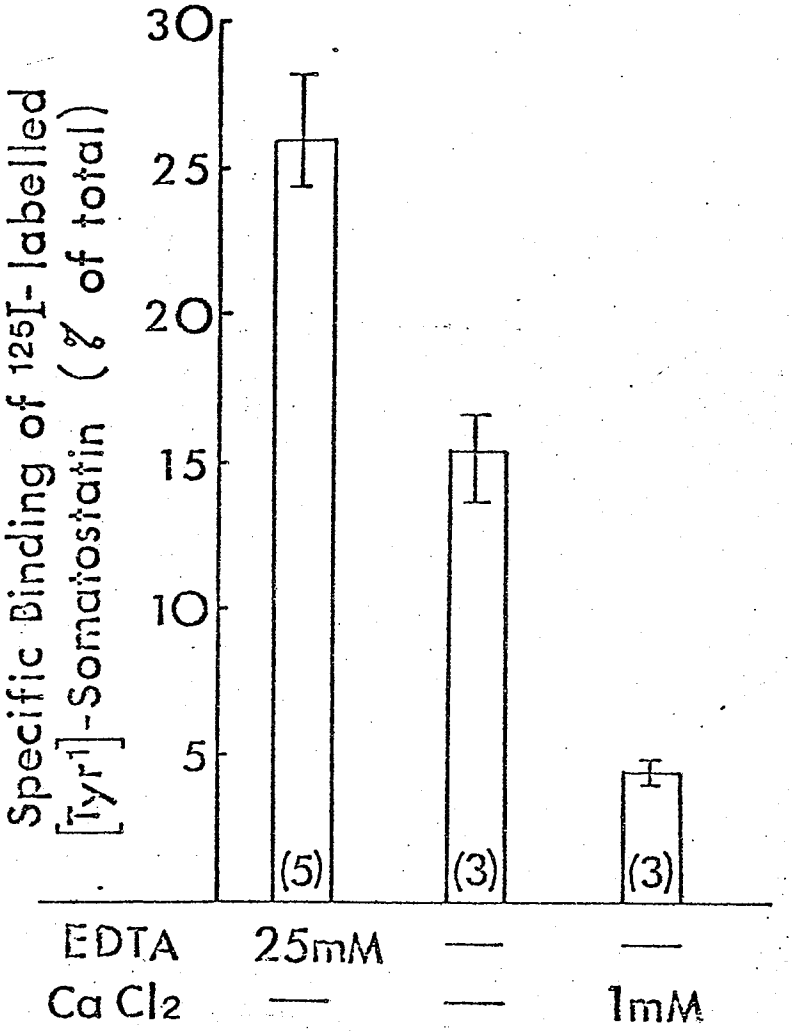
Protein stains of isoelectrofocusing gels of various tissues were also done, and there were two major protein bands corresponding to the area with somatostatin binding activity at pH 4.8-5.0 (figurew 12a,b). Protein stains were also done on tissue mixtures labelled with [^{131}I] and [^{125}I]-iodo-[Tyr^{1}]-somatostatin as above, except BSA was ommitted from all buffers. Two protein bands within the region of the peak of somatostatin binding activity were cut out and counted. In each case the bands

contained proportional amounts of [^{131}I] and [^{125}I] activity, indicating similarity of the binding proteins from various tissues. However, it was impossible to conclude which band represented the somatostatin binding activity, if either, since both segments contained large amounts or radioactivity.

Electrophoretic properties

Somatostatin binding protein from various tissues bound to [^{125}I]-iodo-[Tyr^{1}]-somatostatin showed an R_f of approximately 0.75 (figure 13). A second peak of [^{125}I] activity, at approximately R_f=.05 corresponds well to one of two peaks for free somatostatin tracer (figure 14). Tissue mixtures with one binding protein labelled with [^{125}I]-iodo-[Tyr^{1}]-somatostatin and liver somatostatin binding protein bound to [^{131}I]-iodo-[Tyr^{1}]-somatostatin gave similar patterns on gel electrophoresis when separated on one electrophoretic gel (figure 15). This indicates great similarity of somatostatin binding proteins from various tissues.

Protein stains of electrophoretic gels of various tissues show a protein peak at approximately R_f=0.75, corresponding to the peak of somatostatin binding activity (figure 16a,b). When [^{125}I]-iodo-[Tyr^{1}]-somatostatin-labelled binding protein was run on electrophoresis without BSA and stained for protein, the protein band at approximately R_f=0.75 was cut out and counted. It contained high radioactivity, indicating that it represents at least partially the somatostatin binding protein.



IGURE 1: Effect of EDTA and Ca^{2+} on the specific binding of ^{125}I-abelled [Tyr^1]-somatostatin. Three 50 mM-Tris/HCl buffers at pH 8 ontaining 25 mM-EDTA, no EDTA, or without EDTA but with 1 mM-$CaCl_2$ ere used for homogenization and incubation. 100 µg rat liver cytosol rotein per tube were incubated in ice for 5 h. Specific binding was etermined as described in Materials and Methods. Values shown are ean ± S.E.M., expressed as a percentage of the total [^{125}I]-iodo-[Tyr^1]-omatostatin per tube. The numbers in brackets represent the number of rials. (Ogawa et al 1977).

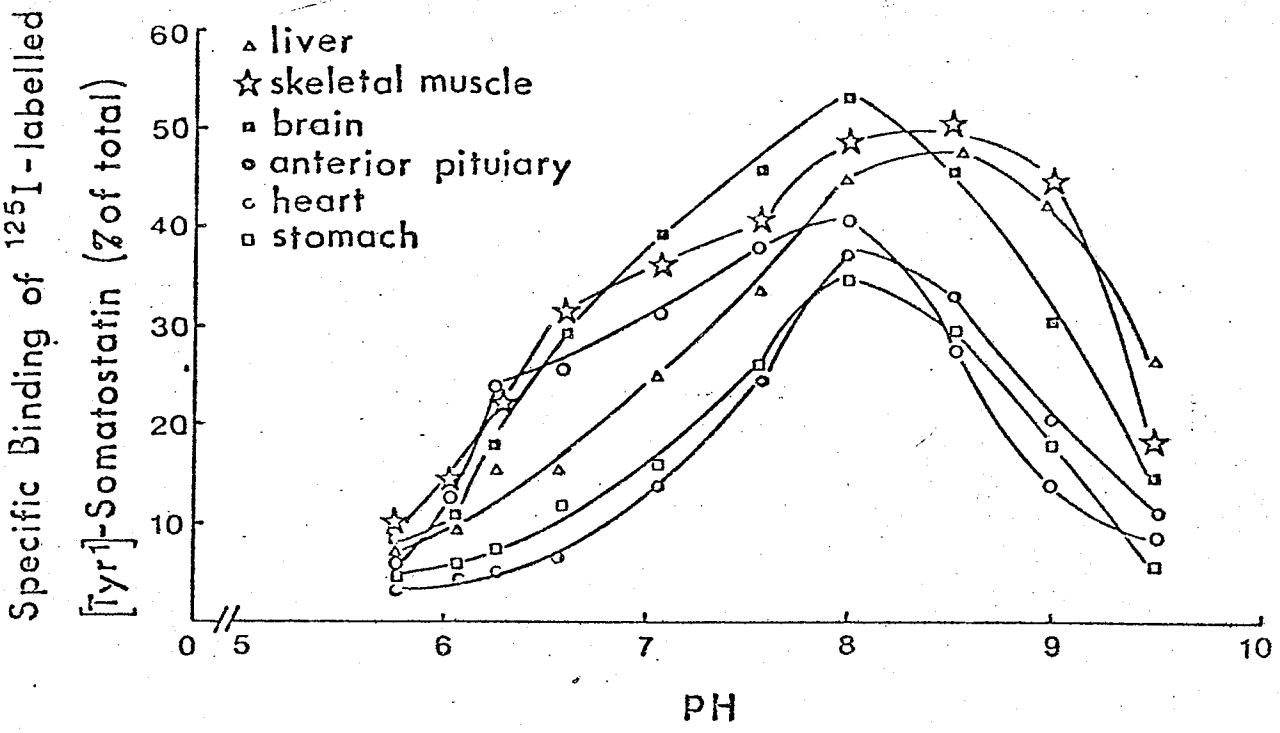


FIGURE 2: Effect of pH on the binding of [^{125}I]-iodo-[Tyr^1]-somatostatin. 0 µg of cytosol protein per tube were used for this experiment and determination of specific binding is described in Materials and Methods. The pH of the incubation buffer (0.2 M-Tris, 25, mM-EDTA) was adjusted with 6 M-HCl or 10 M-NaOH as required. All pH values are the final pH in the incubation media. Values shown are the means of triplicate determinations. ●, anterior pituitary; △, liver; □, stomach; ■, brain; O, heart; ☆, skeletal muscle. (Ogawa et al 1977).

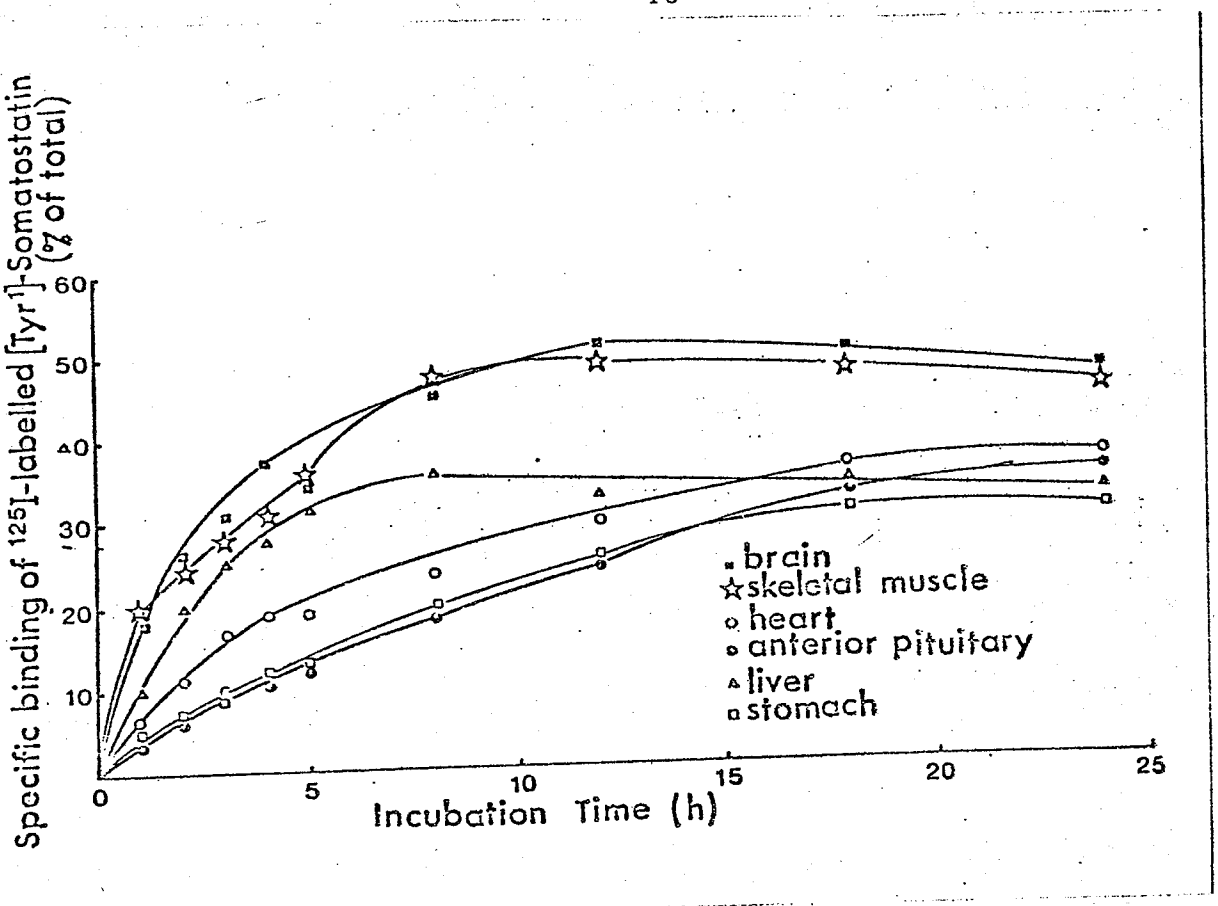
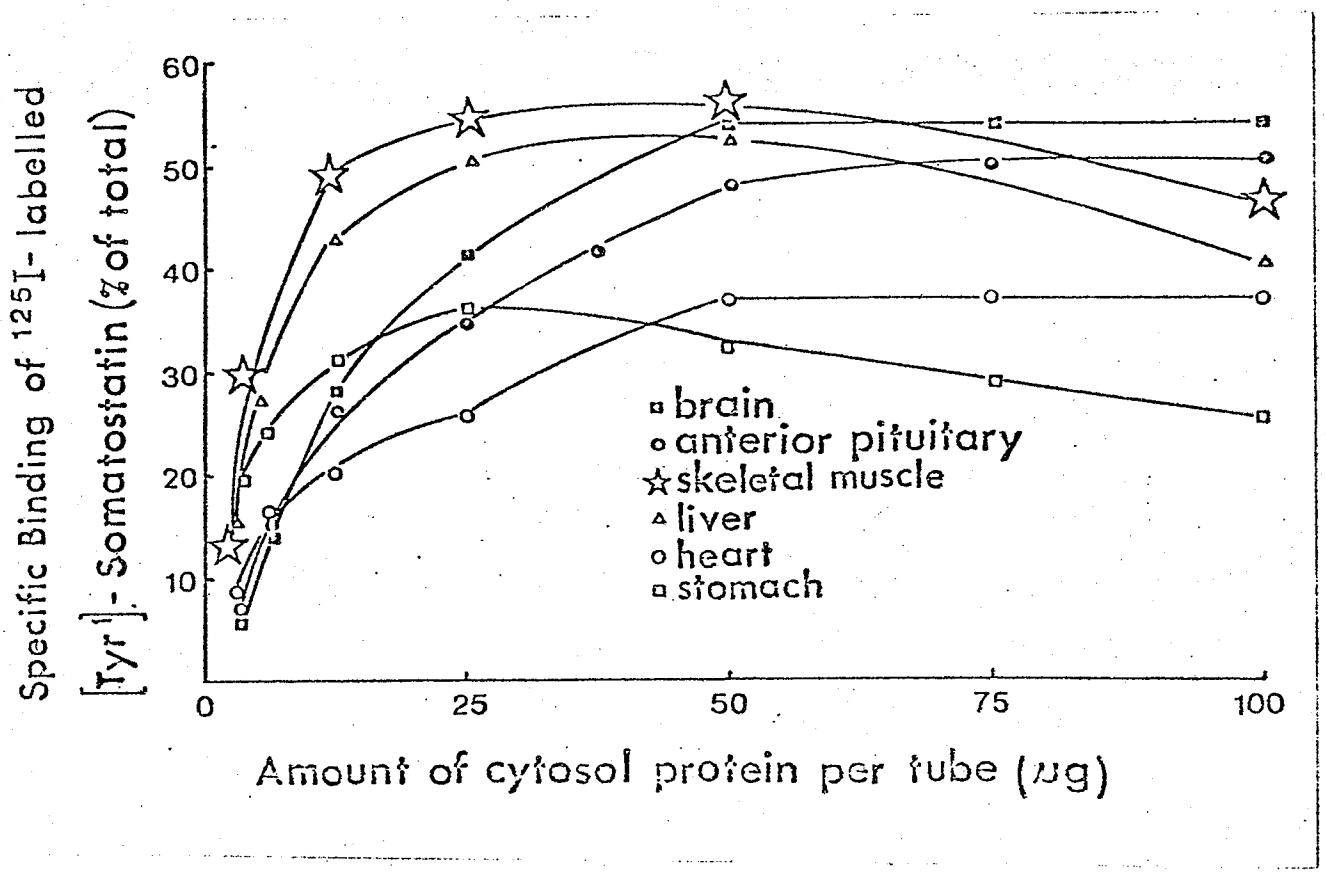
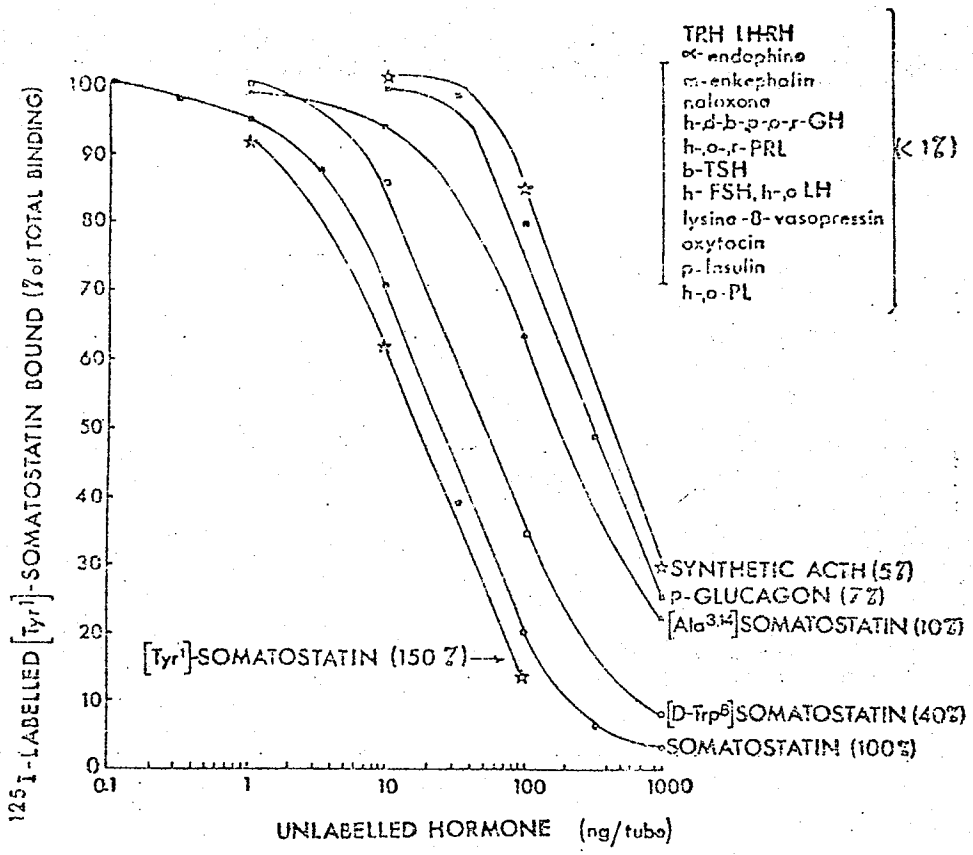


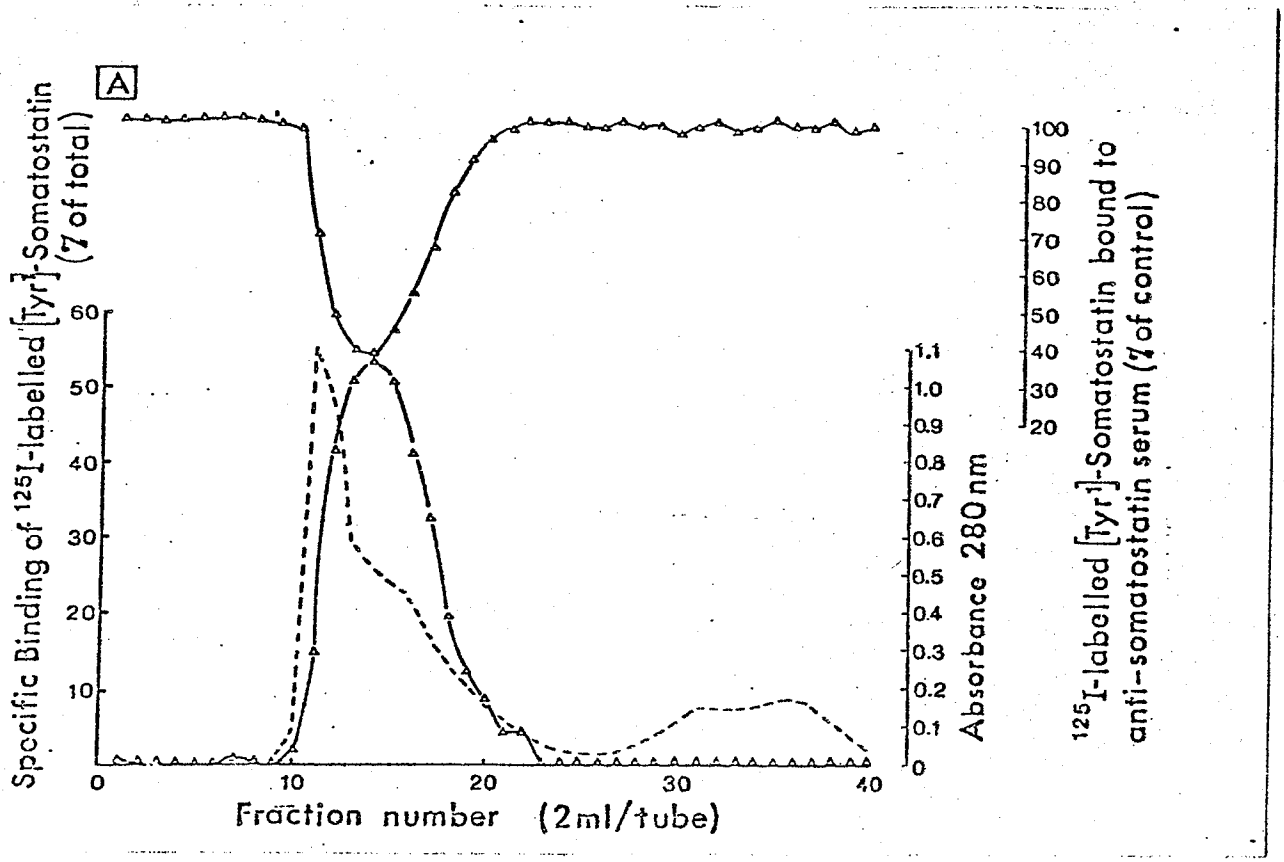
FIGURE 3: Effect of incubation time on the specific binding of ^{125}I-labelled [Tyr^{1}]-somatostatin to cytosol fractions of various rat tissues. 50 µg of cytosol protein per tube were used for this experiment and procedures for determining specific binding are in Materials and Methods. Values are expressed as percentage of total [^{125}I]-iodo-[Tyr^{1}]-somatostatin in each tube. Data shown are means of triplicate determinations. The symbols are identical to those for Figure 2. (Ogawa et al 1977).



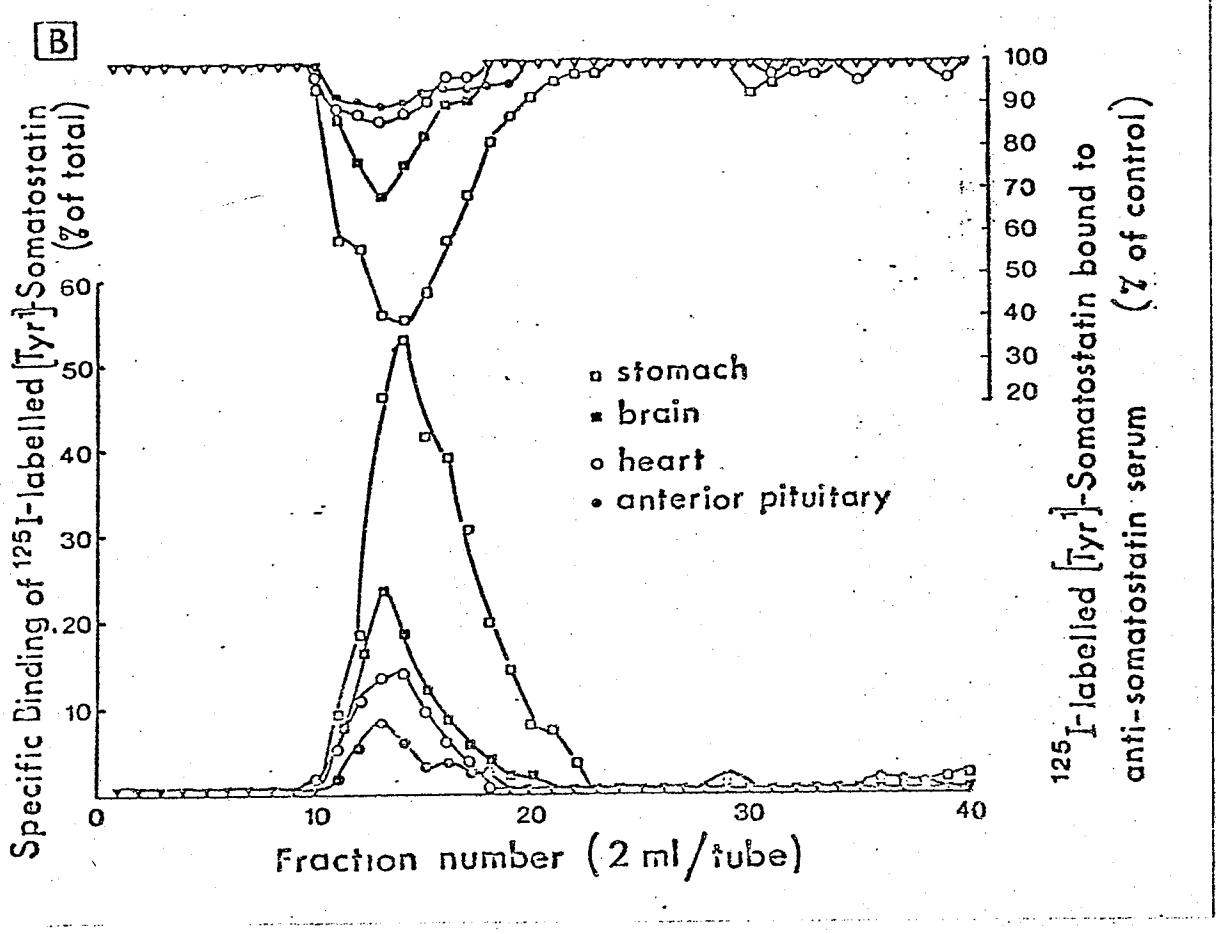
IGURE 4: Effect of increasing cytosol on the binding of [^{125}I]-iodo-Tyr^1]-somatostatin. Incubation and determination of specific binding of ^{125}I]-iodo-[Tyr^1]-somatostatin and protein determination are described n Materials and Methods. Symbols are the same as in Figure 2. (Ogawa et l 1977).

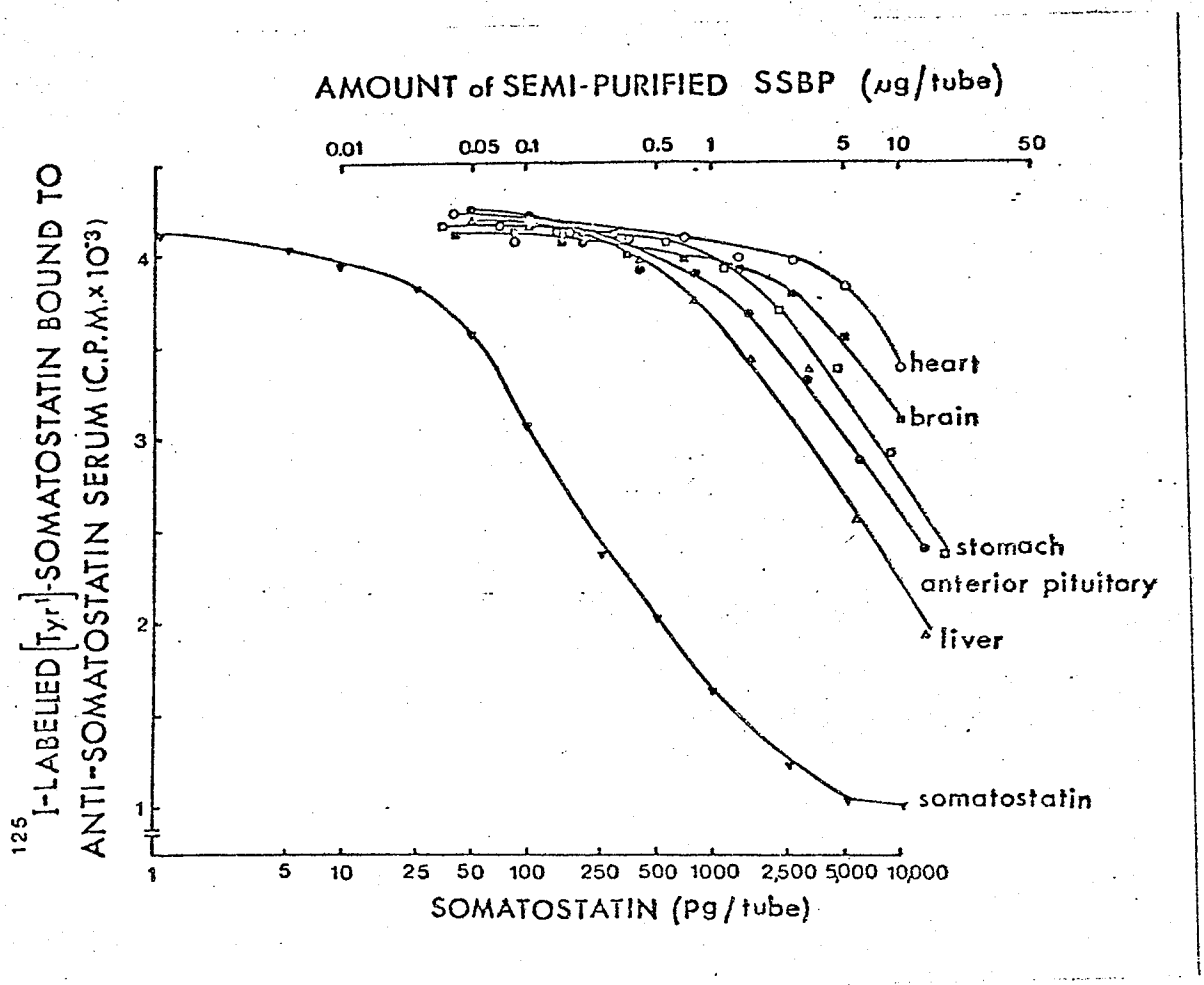


IGURE 5: The specificity of binding of somatostatin to SSBP. Aliquots f fractions after Sephadex G-100 gel filtration with maximal somatostatin inding activity (semi-purified SSBP, 10 µg protein per tube) were used nd incubated 48 h at 4°C. Non-specific binding of the tracer was estab- ished by adding excess somatostatin (5 µg/tube) to incubation mixtures rior to addition of semi-purified SSBP. This value was subtracted from ll samples. Competitive binding of ^{125}I-labelled [Tyr^{1}]-somatostatin expressed as a percentage of binding in the absence of unlabelled somato- tatin) was measured in the presence of increasing amounts of somatostatin ●), [Tyr^{1}]-somatostatin (★), [D-Trp^{8}]-somatostatin (□), [Ala^{3,14}]- omatostatin (▲), p-glucagon (■), or synthetic ACTH (☆). Other hormones isted were tested at a single dose (1 µg/tube) and are represented by he square bracket. The numbers in brackets represent % cross-reactivity f somatostatin. Abbreviations of hormones are in Materials and Methods. Ogawa et al 1977).



ure 6: Distribution of SSBP in fractions after gel filtration. A
adex G-100 column (1.2 x 50 cms) was used. [A] 5.9 mg cytosol protein
rat liver (△) was applied. The eluates were monitored for protein (--)
absorbance at 280 nm. [B] 1 mg of rat anterior pituitary (●), 7.6 mg of
nach (□), 6.3 mg of brain (■) and 5 mg of heart (o) cytosol protein were
lied. The specific binding of somatostatin and the effect of addition of
quots (50 µl) from each fraction on a radioimmunoassay (RIA) are indicated.
was performed by using 1:1,000 diluted rabbit anti-somatostatin serum
times greater than concentrations used in the standard RIA) with 24h
ubation at 4°C and bound and free hormones were separated by the double
ibody method. (Ogawa et al 1977).


B
Specific Binding of ^{125}I-labelled [Tyr]-Somatostatin (% of total)
^{125}I-labelled [Tyr]-Somatostatin bound to anti-somatostatin serum (% of control)
stomach
brain
heart
anterior pituitary
Fraction number (2 ml/tube)
0
10
20
30
40
10
20
30
40
50
60
20
30
40
50
60
70
80
90
100



URE 7: Effect of semi-purified SSBP on the binding of [^{125}I]-iodo-^{1}]-somatostatin to anti-somatostatin serum. Semi-purified SSBP (fraction 14 after Sephadex G-100 gel filtration; Fig. 6) obtained from rat anterior uitary (●), liver (△), stomach (□), brain (■), and heart (O) were used samples in a radioimmunoassay for somatostatin. RIA was performed as cribed in Materials and Methods. (Ogawa et al 1977).

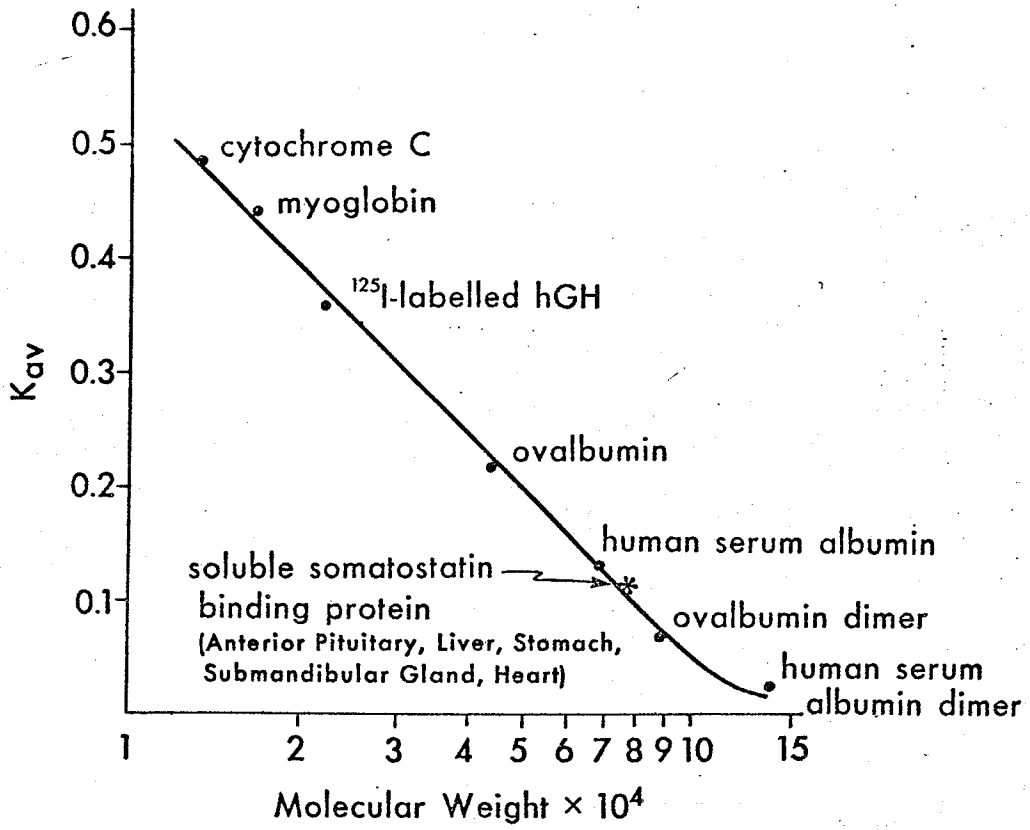


Figure 8. Molecular weight determination of the somatostatin binding protein. The molecular weight of the somatostatin binding protein from a variety of tissues was determined by gel chromatography on Sephadex G-100. The column was 2 cm by 90 cm. The K_{av} for the molecular weight markers are indicated on the graph. The somatostatin binding protein has a K_{av} of .11 which is equivalent to a molecular weight of 77,000. Methods used are explained in Materials and Methods.

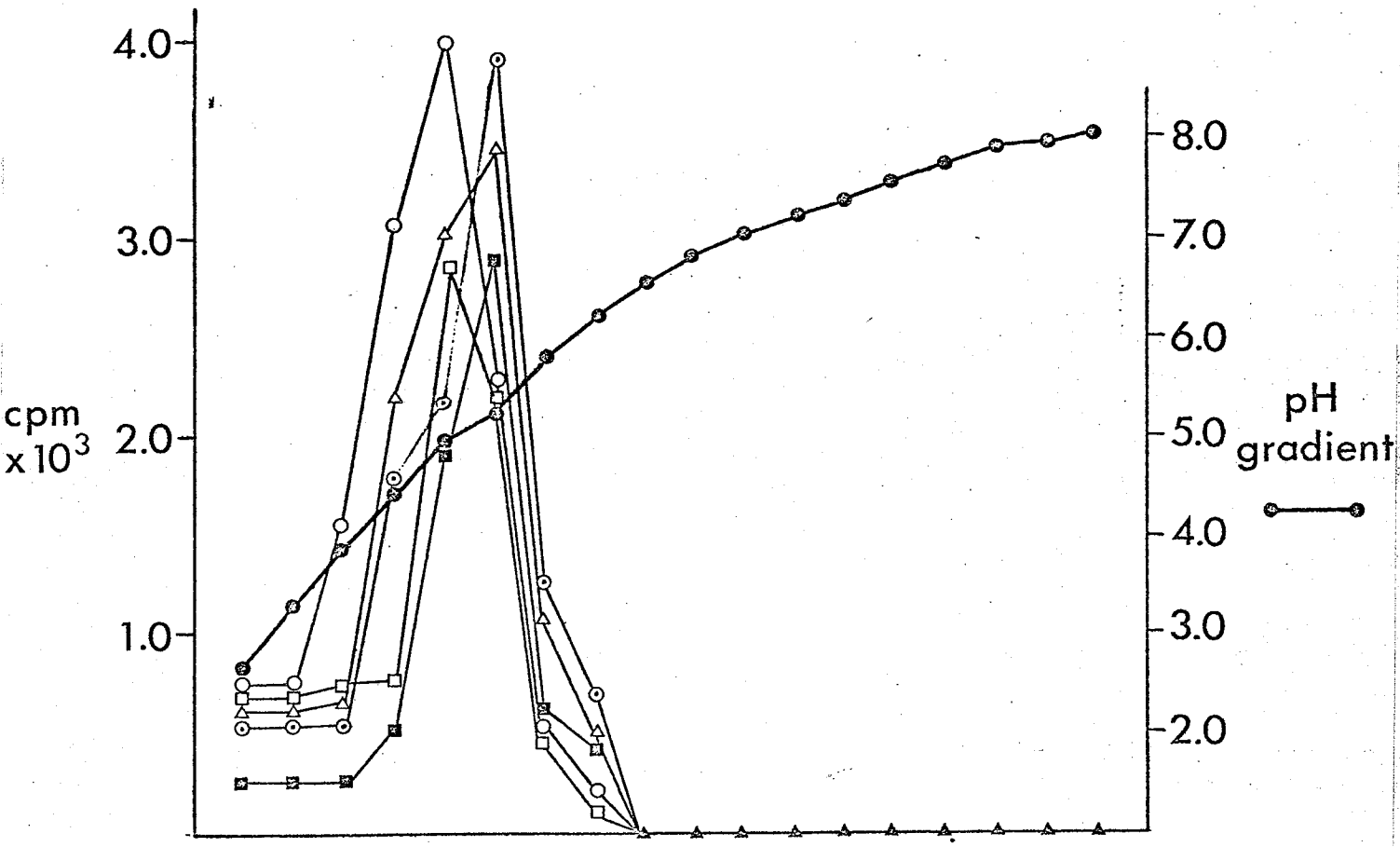


Figure 9a. Isoelectric point of the binding protein. The somatostatin binding protein from various female rat cytosol preparations were purified by gel chromatography on a Sephadex G-I00 column (4cm x I00cm). Semi-purified binding proteins were incubated separately with [^{I25}I]-iodo-[Tyr^{I}]-somatostatin for 24h at 4C and the unbound tracer removed by the addition of a dextran-coated charcoal suspension followed by centrifugation at 3000g for 30 min. Samples of the supernatant containing approximately I5,000-20,000 cpm were applied to a polyacrylamide isoelectric focusing gel and focused over 3h. The symbols representing various tissues are the same as in figure 2 except skeletal muscle is (⊙). (●) represents the pH gradient across the gel . The isoelectric point appears to be approximately pH 5.0-5.2.

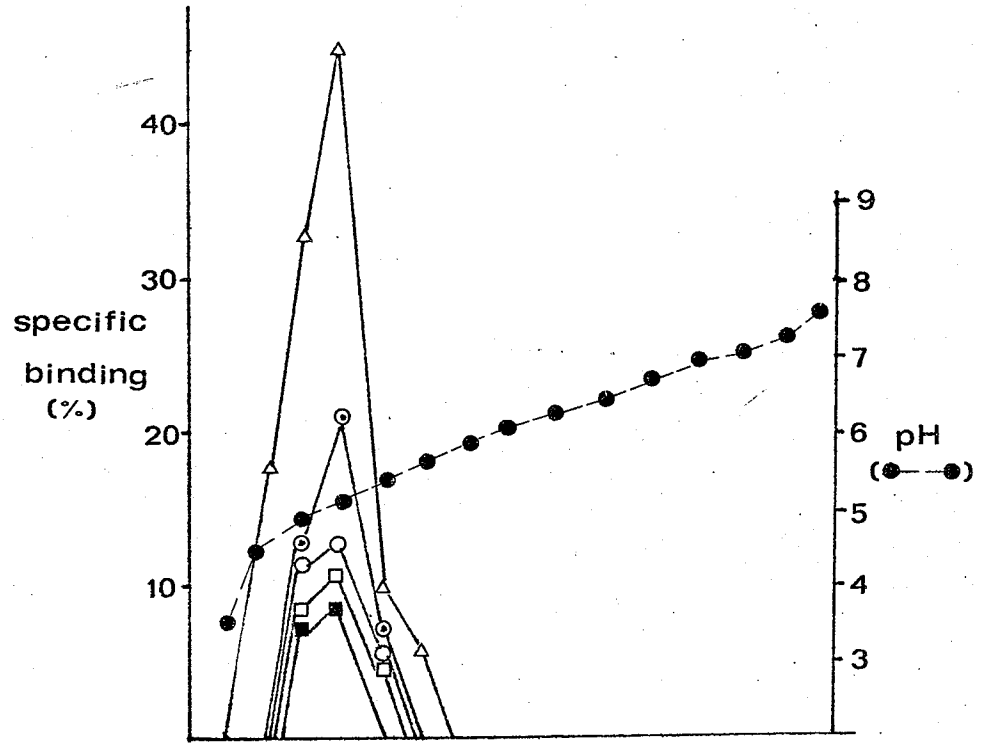


Figure 9b. Isoelectric point of binding protein. Crude cytosol protein (500 ug) from a variety of rat tissues was applied to an isoelectricfocusing gel and focused over 3h at a power of 15 watts. Each gel channel was then cut and each slice eluted overnight in assay buffer at 4°C with constant agitation. The eluant was then assayed for somatostatin binding activity as indicated in Materials and Methods. The symbols used are the same as figure 2 except skeletal muscle is (⊙).

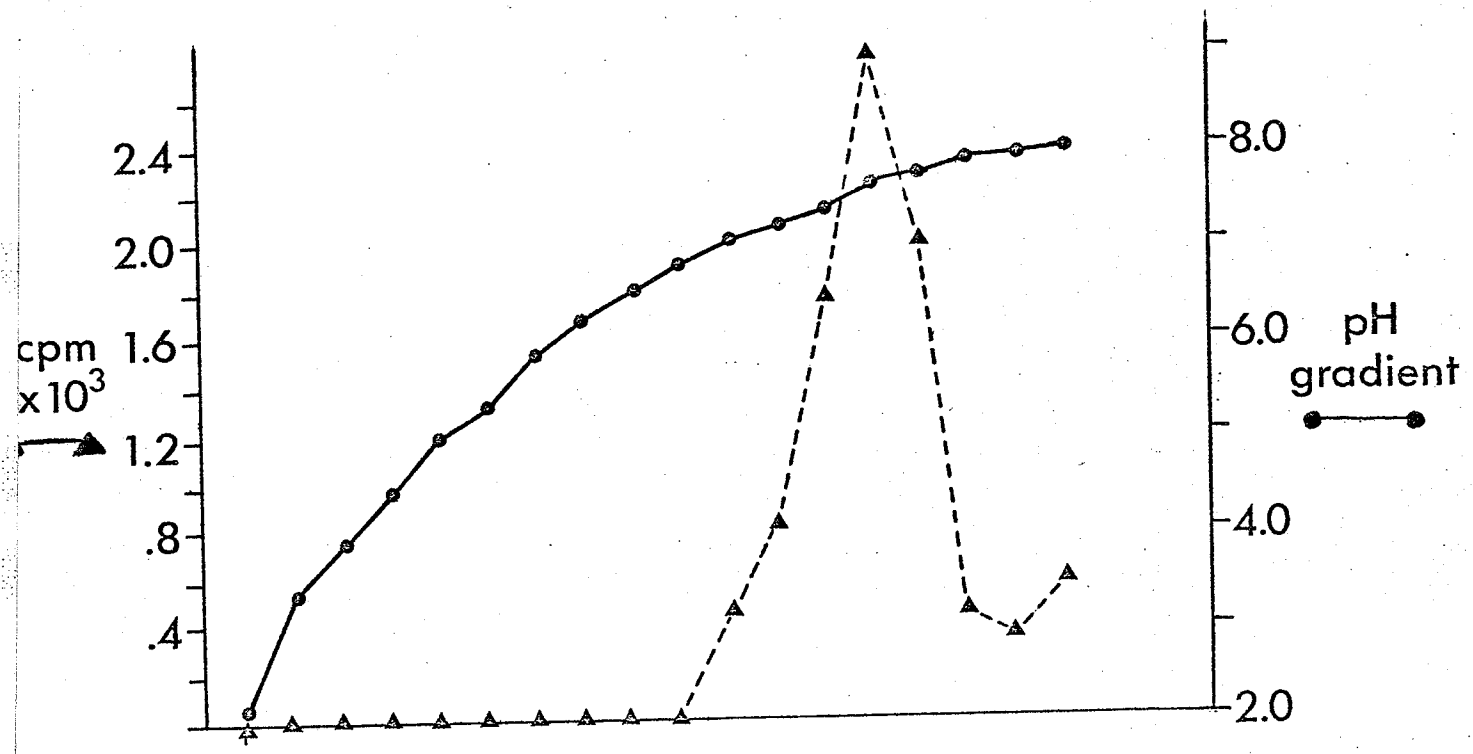


Figure I0. Isoelectric point of [^{I25}I]-iodo-[Tyr^{I}]-somatostatin. Approximately I5,000 cpm of [^{I25}I]-iodo-[Tyr^{I}]-somatostatin was applied to an isoelectric focusing gel and focused over 3h. The gel was then cut and counted, yielding the above result. (●) represents the pH gradient across the gel as measured by elution of blank channels of the gel in distilled water, overnight with agitation. The isoelctric point of somatostatin is approximately 7.7.

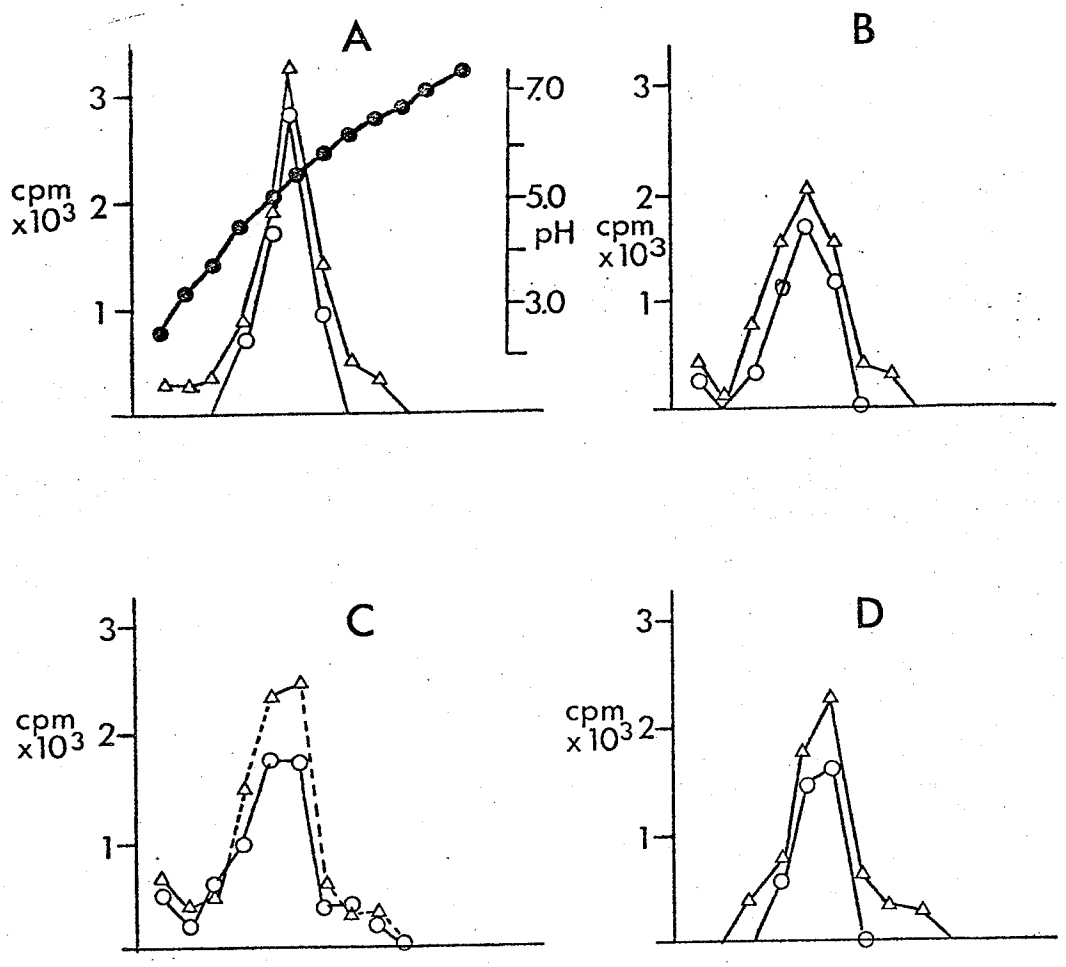


Figure II. Comparison of isoelectric point of somatostatin binding proteins from different tissues. Somatostatin binding proteins from various rat tissues, partially purified by gel chromatography, were preincubated with [^{I25}I]-iodo-[Tyr^{I}]-somatostatin and the unbound hormone removed by addition of a dextran-coated charcoal suspension. Such [^{I25}I]-labelled somatostatin binding proteins from various tissues were mixed with liver cytosol binding protein previously labelled with [^{I3I}I]-iodo-[Tyr^{I}]-somatostatin in a ratio of I:I From such mixtures I0,000-I5,000 cpm of [^{I25}I] and [^{I3I}I] activity were applied to an isoelectric focusing channel and the electric field applied. The [^{I3I}I] and [^{I25}I] activities were counted simultaneously on an automatic gamma counter. In all the above graphs (O) represents [^{I3I}I]-labelled binding protein from the liver. (Δ) represents (A) [^{I25}I]-labelled binding protein from the heart, (B) muscle, (C) stomach and (D) brain.

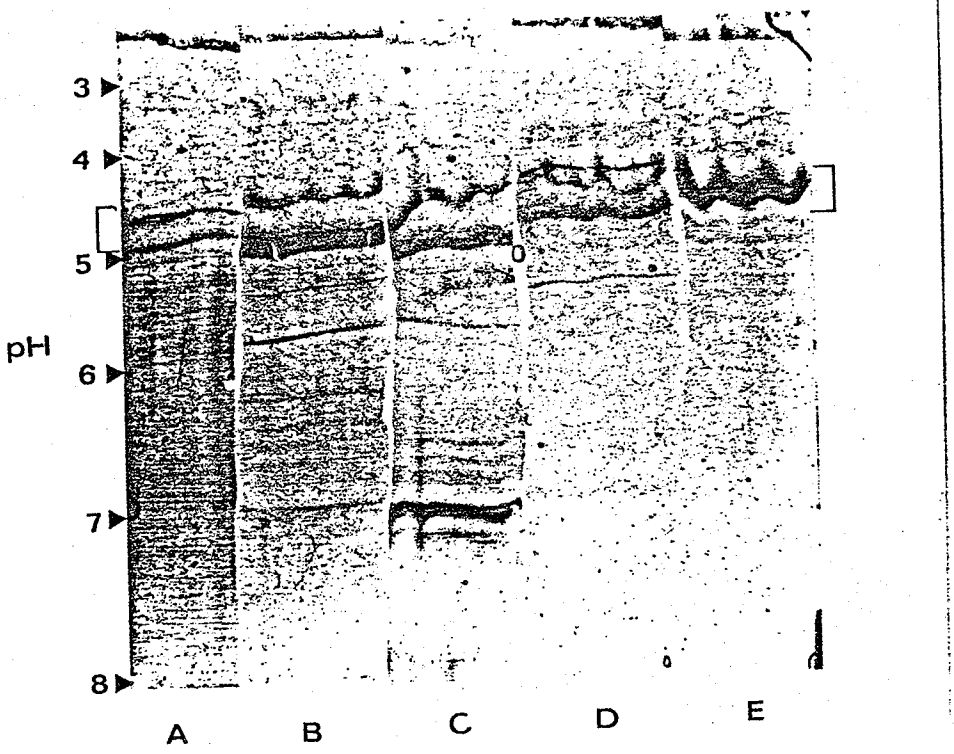


Figure 12a. Distribution of rat cytosol protein on isoelectricfocusing. Rat cytosol protein from various tissues containing somatostatin binding protein was partially purified on Sephadex G-100 and applied to an isoelectric-focusing gel. One hundred ug of protein from each tissue was run for 3h at a constant power of 15 watts. The gel was stained with Coomasie Blue as indicated in Materials and Methods. The brackets indicate the area of peak somatostatin binding activity. The tissues stained were (A) liver, (B) heart, (C) stomach, (D) muscle and (E) brain.

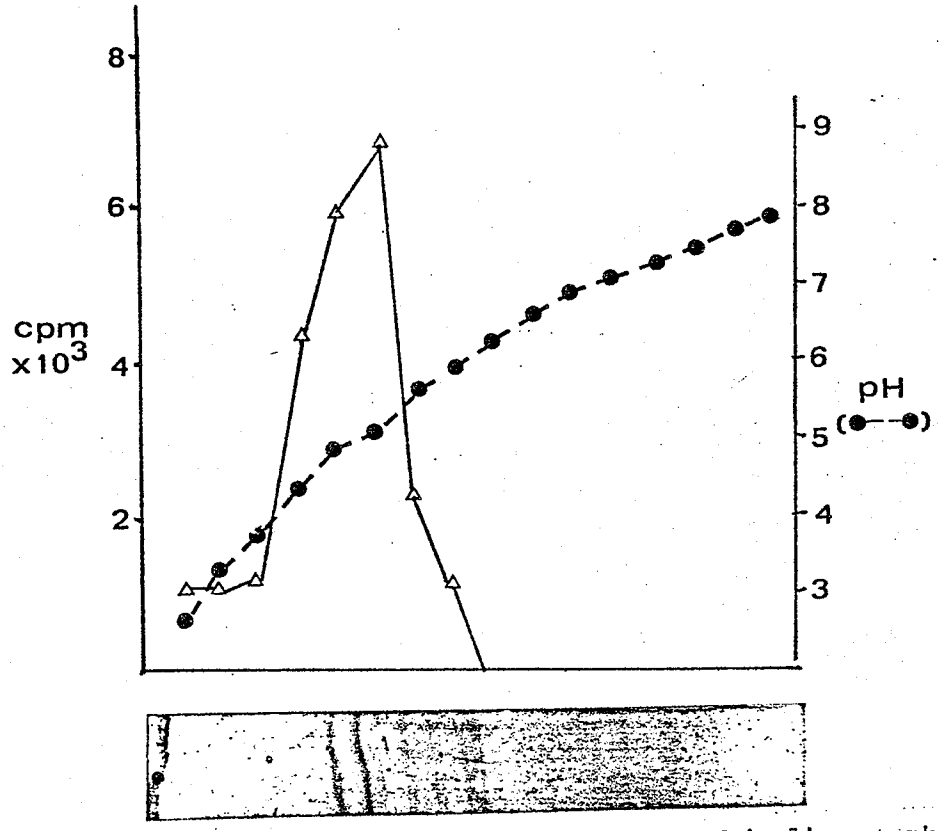


Figure 12b. Distribution of somatostatin binding activity on isoelectricfocusing gels. The data in the graph indicates the distribution of [^{125}I] activity obtained after focusing rat liver cytosol somatostatin binding protein, purified on carboxymethyl cellulose, and labelled with [^{125}I]-iodo-[Tyr^{1}]-somatostatin. This is the same data as figure 9a. The band below the graph indicates the protein distribution of 100 ug of rat liver cytosol protein that was focused on the same gel. This data is the same as that of figure 12a. The two major protein bands occur within the peak of somatostatin binding activity.

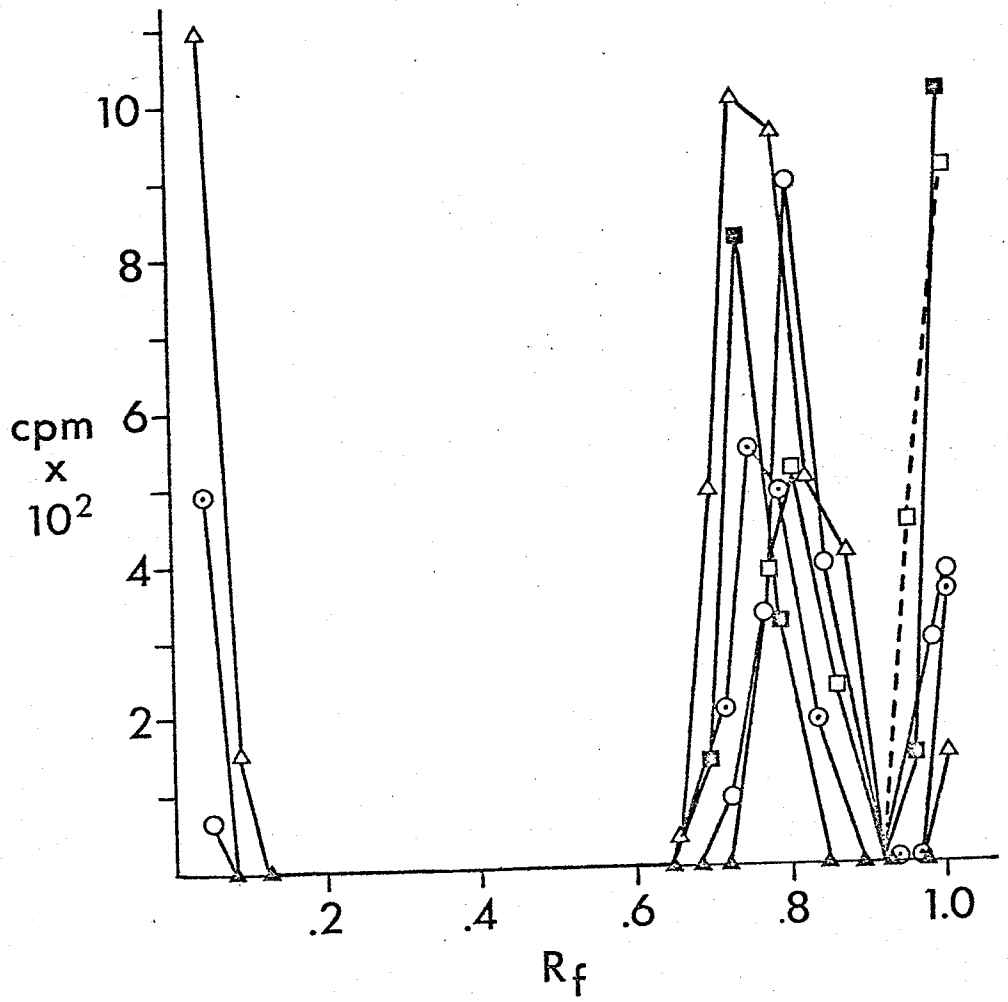


Figure I3. Electrophoresis of somatostatin binding proteins. [^{I25}I]-labelled somatostatin binding protein from various tissues were prepared and applied to alkali disc electrophoretic gels and a 2 mA/gel current was applied for 2h. The gels were then cut and the distribution of radioactivity was determined. Symbols sed are the same as figure 2 except skeletal muscle is (⊙). The R_f of somatostatin binding protein in the various tissues appears to be approximately .8.

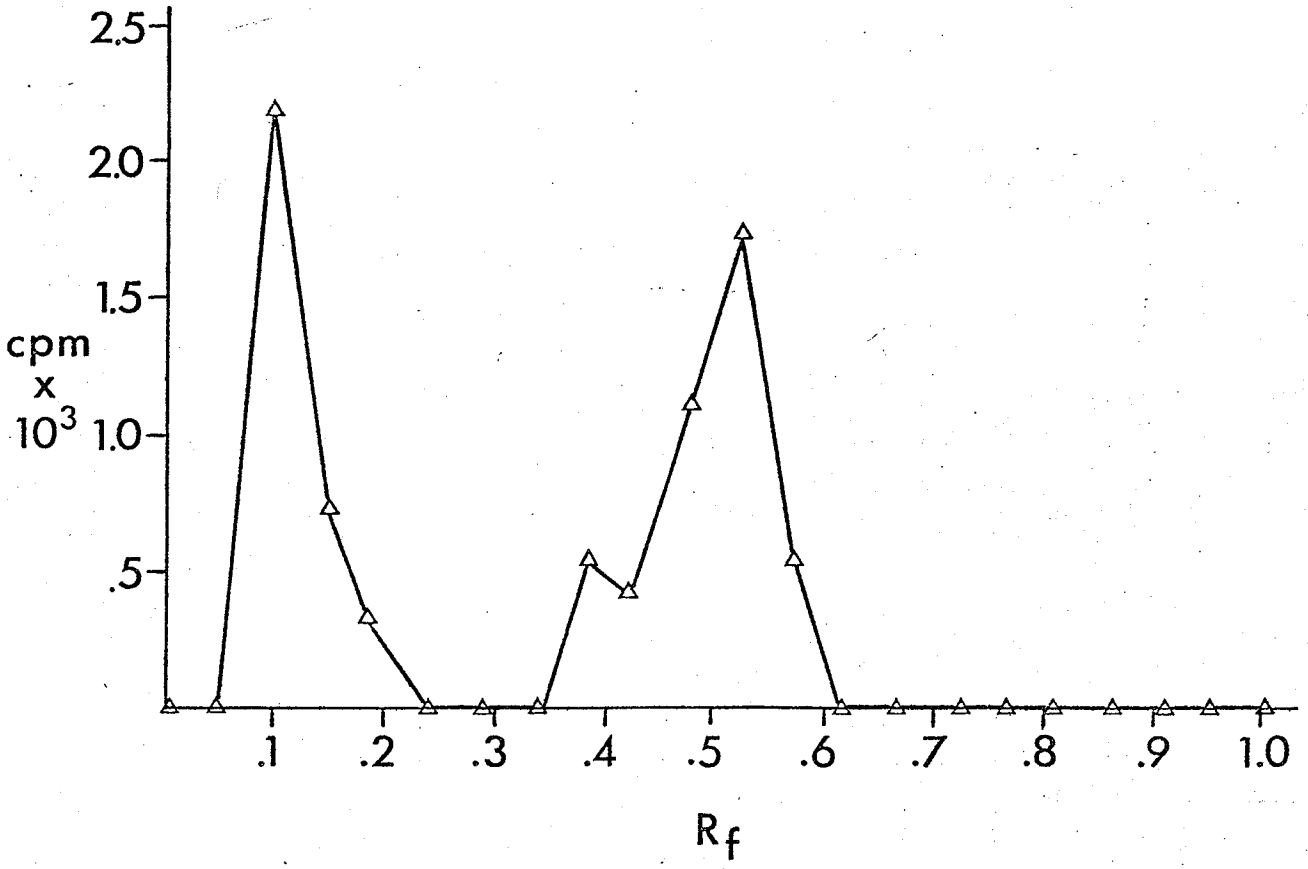


Figure I4. Electrophoretic properties of [^{I25}I]-iodo-[Tyr^{I}[-somatostatin. Approximately I5,000 cpm of [^{I25}I]-iodo-[Tyr^{I}]-somatostatin was applied to disc electrophoretic gels (pH 8.8-9.0) and a 2mA/gel current applied for 2h. The gel was then cut and counted, yielding the above pattern. The reason for there being two peaks is unknown. The R_f of somatostatin is approximately .I-.I5 with a second peak at approximately .5-.55.

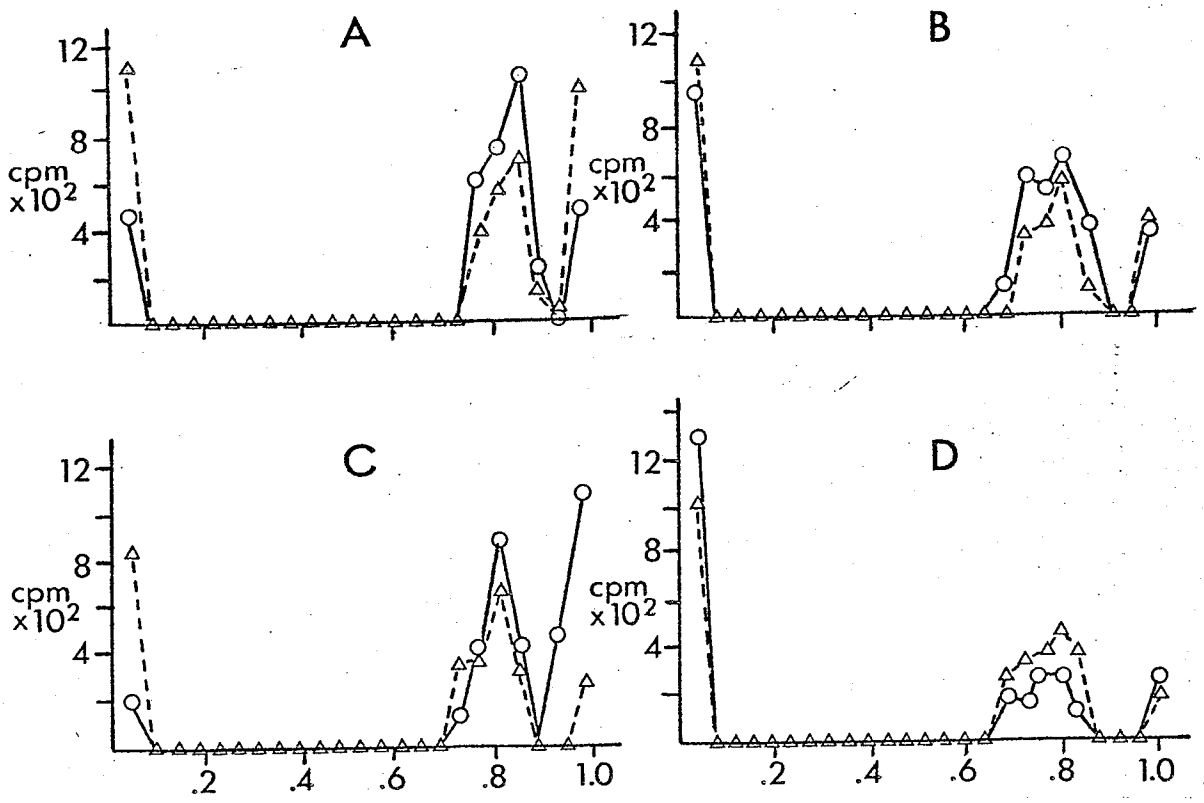


Figure I5. Comparison of electrophoretic patterns of somatostatin binding proteins from various tissues. Somatostatin binding proteins from various rat tissues, partially purified by gel chromatography, were pre-incubated with [^{I25}I]-iodo-[Tyr^{I}]-somatostatin and the unbound hormone removed by addition of a dextran-coated charcoal suspension. These [^{I25}I]-labelled somatostatin binding proteins from various tissues were mixed with liver cytosol binding protein, labelled with [^{I3I}I]-iodo-[Tyr^{I}]-somatostatin in a ratio of I:I From such mixtures approximately 5,000 cpm of [^{I25}I] and [^{I3I}I] activity were applied to a disc gel isoelectro focusing gel and a 2 mA/gel current was applied for 2h. The gels were then cut and [^{I25}I] and [^{I3I}I] activities were counted simultaneously on an automatic gamma counter. In all the above graphs (O) represents [^{I3I}I]-labelled liver cytosol somatostatin binding protein. (Δ) represents in (A) [^{I25}I]-labelled heart cytosol somatostatin binding protein, (B) muscle, (C) stomach and (D) brain.

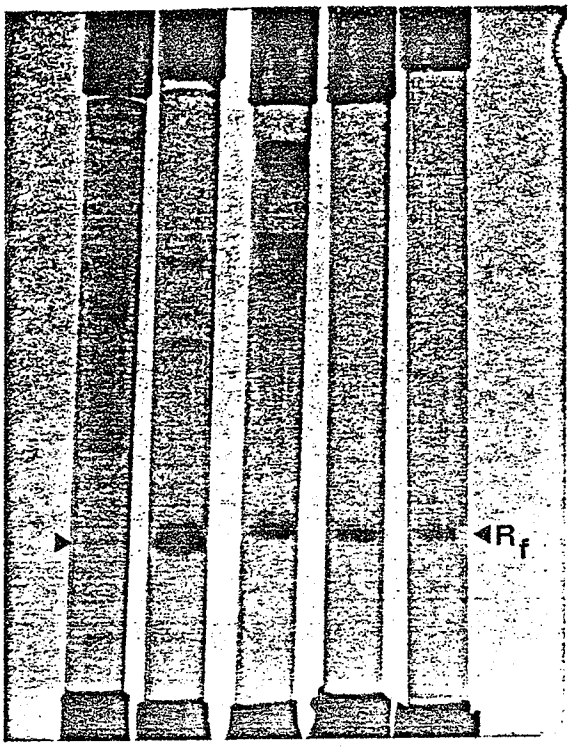


Figure 16a. Distribution of rat cytosol protein on disc gel electrophoresis. Rat cytosol protein from various tissues (100 ug) containing somatostatin binding protein was partially purified on Sephadex G-100 and applied to a disc gel electrophoretic gel. A 2 mAmp/ gel current was applied for two hours. The gels were stained for protein with Amido Black stain as explained in Materials and Methods. The tissues used were (A) liver, (B) heart, (C) stomach, (D) muscle and (E) brain. The arrow indicates the band containing the peak somatostatin binding activity.

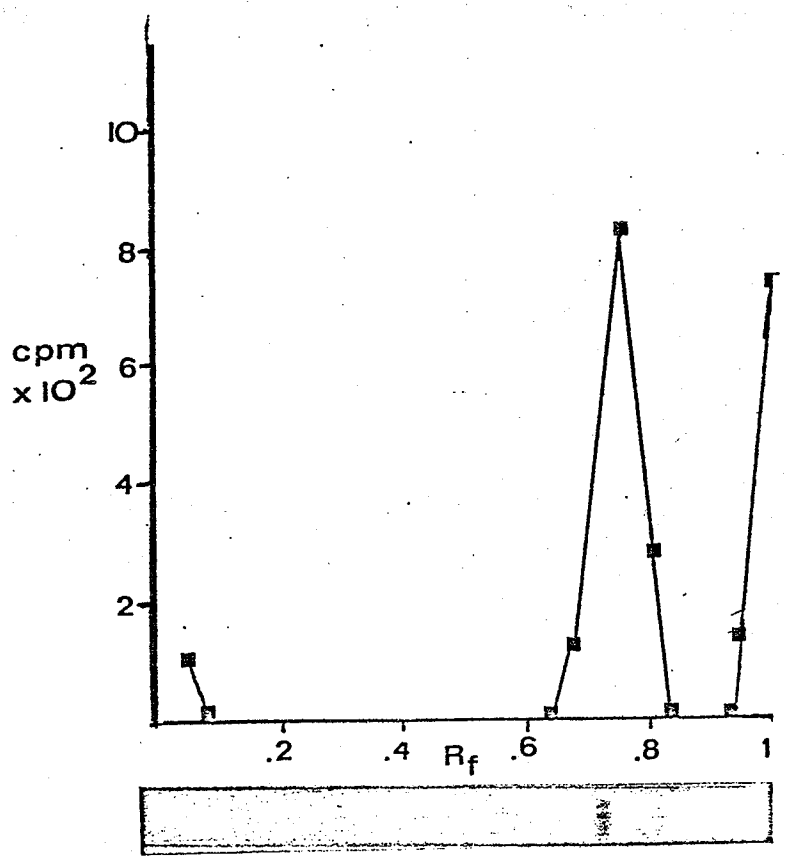


Figure 16b. Distribution of somatostatin binding activity on disc gel electrophoresis. This figure shows the distribution of [^{125}I] activity after electrophoresis of rat brain cytosol somatostatin binding protein, semipurified on Sephadex G-100, and labelled with [^{125}I]-iodo-[Tyr^{1}]-somatostatin. This is the same data as figure 13. The band below the graph is the protein distribution (Amido Black stain) after 100 ug of protein from rat brain cytosol was run on electrophoresis. This is the same data as figure 16a. Note that both maximal somatostatin binding activity and the major protein band occur at approximately R_f=.75.

TABLE I

Tissues	(No.)	% Specific binding		
		900 x g Pellet	15,000 x g Pellet	100,000 x g Pellet
.ver	(6)	25.7±1.0	25.8±0.6	28.8±0.4
ıbmandibular gland	(4)	18.1±0.5	14.2±0.8	12.9±1.4
:eletal muscle	(4)	4.0±0.9	4.0±1.0	7.2±0.4
)leen	(4)	5.0±0.5	4.7±0.2	5.5±0.6
:omach	(4)	4.3±0.7	4.4±0.8	3.7±0.7
:art	(4)	9.2±1.2	4.6±0.3	2.6±0.8

ABLE I: Distribution of specific binding of ^{125}I-labelled [Tyr^{1}]-)matostatin in subcellular fractions of rat tissues. Particulate :actions were obtained from several female rat tissues using methods :milar to those described in studies of prolactin receptors (Shiu et ., 1973; Posner et al., 1974). 0.1 ml of each fraction (containing))0 µg protein in 25 mM-Tris/HCl buffer pH 7.6) was incubated in glass ıbes for 2 h with [^{125}I]-iodo-[Tyr^{1}]-somatostatin (approximately 2.5 x $^{-4}$ c.p.m.) in a total volume of 0.5 ml. The buffer used for dilutions :cept for particulate fractions and additions was 50 mM-Tris/HCl buffer, 3 mM-$CaCl_2$, 0.5% BSA (W/V) pH 8.0. All reagents and reaction tubes :re kept in ice before and during the assay. At the end of the incubation :riod, cold (4°C) ethanol was added to a final concentration 85% (V/V), ıd reaction tubes centrifuged at 3,200 rev./min for 20 min at 4°C. The :dioactivity in the pellets was counted in a gamma counter. Specific .nding is the difference between radioactivity in the pellets with or :thout 10 µg/ml somatostatin (1 µg/tube), expressed as a percent of the)tal radioactivity added to the incubation mixture. Data are shown :an±S.E.M. (Ogawa et al 1977).

TABLE 2

METHOD		RESULT	
		Specific binding of GH-RIH Tracer (% of total cpm.'s)	
		Particulate Fraction	Cytosol
STEP I	Incubate {Particulate fraction or Cytosol} With somatostatin tracer With or without somatostatin	I7.5% (ETOH method)	22.2% (ETOH method)
STEP II	Centrifuge reaction mixture from {Particulate fraction or Cytosol} at 200,000xg for I h. and pour off supernatant and count pellet.	3.7%	0%
STEP III	Test binding of supernatant from {Particulate fraction or Cytosol}	I3.9% (ETOH) 20.9% (DCC)	22.9% (ETOH)]9.2% (DCC)

TABLE 2: Identification of subcellular binding sites for somatostatin. The particulate fraction (9I50 µg protein/ 500 µl) and cytosol fraction (I00 µg protein/500 µl) from female rat liver were incubated separately with [^{I25}I]-iodo-[Tyr^{I}]-somatostatin, with or without I0 µg/ ml somatostatin in a total volume of 7.5 ml for 4 h on ice. Half a ml of reaction mixture was used for determinaion of specific binding using ethanol to precipitate bound somatostatin (ETOH method) as described in Materials and Methods [Step I]. The remainder of the reaction mixture was centrifuged at 200,000xg for 60 min and the radioactivity in the pellet counted [Step II]. Half a ml of supernatant was treated with ethanol method or dextran-coated charcoal method (DCC) as described in Materials and Methods [Step III]. Specific binding is the difference in % of total counts bound with or without I0µg/ml of somatostatin. (Ogawa et al 1977).

TABLE 3.

imals	Tissues	(No.)	% specific binding 10 µg cytosol protein (Mean ± S.E.M.)
ıale rat	skeletal muscle	(4)	35.6 ± 1.4
	liver	(4)	33.3 ± 1.1
	small intestine	(4)	28.9 ± 0.7
	anterior pituitary	(4)	26.7 ± 1.2
	stomach	(4)	26.3 ± 0.3
	spleen	(4)	25.2 ± 0.6
	brain	(4)	24.8 ± 1.1
	submandibular gland	(4)	24.6 ± 2.4
	uterus	(4)	21.2 ± 1.7
	heart	(4)	19.4 ± 1.1
	kidney	(4)	19.2 ± 1.4
	lung	(4)	14.9 ± 1.3
	peripheral blood cells	(2)	2.6 ± 0.3
	plasma	(2)	1.2 ± 0.2
ıan	liver	(4)	22.4 ± 3.8
	anterior pituitary	(3)	15.7 ± 1.6
	plasma	(2)	1.2 ± 0.8
·ine	anterior pituitary	(4)	20.7 ± 1.0

ıLE 3: Distribution of specific binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin cytosol fractions. Incubation conditions and procedures for determining ecific binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin were carried out using hods similar to those in Materials and Methods except that 10 µg cytosol otein and 24 h incubation were employed, for all tissues. The number in ckets represents the number of animals studied. Because of the small e of rat anterior pituitary glands, 4 pituitaries were used as one sample. ues represent mean ± S.E.M. (Ogawa et al 1977).

TABLE 4.

Treatment			Specific Binding of ^{125}I-labelled [Tyr^1]-somatostatin (% of control)	
			Rat Liver	Bovine Anterior Pituitary
0.1 M Acetic Acid	30 min	24°C	23.1	30.8
0.1 M NaOH	30 min	24°C	16.2	23.3
85% ETOH	20 min	4°C	58.5	-
	30 min	56°C	0	9.0
	1 min	100°C	0	0
freezing and thawing (20 times)			95.0	94.6

E 4: Effect of various reagents on somatostatin binding. Female rat
: cytosol fraction and bovine anterior pituitaries were used for these
:iments. The effects of extremes of pH were examined by exposing cytosol
:ions to acetic acid or NaOH at a final concentration of 0.1 M for 30 min
)d before neutralization. The effect of ethanol was examined by adding
(4°C) absolute ethanol to a final concentration of 85% (V/V) and the
:ion tubes were centrifuged at 3,200 rev./min for 20 min at 4°C. The
:natant was decanted and the mouth of the tube blotted on absorbant
:. The pellet was dissolved in Tris-EDTA-BSA buffer, and assayed.
:rature effects were studied after cytosol fractions were exposed to
for 30 min, to 100°C for 1 min, or frozen and thawed 20 (thawing =
for 20 min). Cytosol fractions exposed to these various conditions
:ll as un-treated cytosol fractions (as a control) were adjusted to
protein per tube prior to assay. Data shwon are mean of triplicate
:minations. (Ogawa et al 1977).

TABLE 5.

Treatment		% Dissociation of total binding 1.5 h	12 h	24 h
l Acetic Acid	A	37.9	25.2	23.5
	B	56.0	34.6	23.8
l M NaOH	A	65.9	59.9	54.6
	B	67.9	61.9	46.4
nM Dithiothreitol (DTT)	A	59.7	65.6	77.9
	B	56.1	74.9	84.7
nM Mercaptoethanol	A	53.8	66.6	78.7
	B	61.7	71.5	80.9
M $CaCl_2$	A	29.5	20.5	16.1
	B	44.1	24.7	16.5
M $MgCl_2$	A	29.5	22.2	15.3
	B	43.1	24.3	18.3
M Urea	A	47.4	35.0	31.0
	B	47.1	32.1	22.5
M Guanidine-HCl	A	52.7	46.4	46.2
	B	55.6	40.7	31.2
M NaCNS	A	32.9	22.3	17.6
	B	35.6	22.8	15.9
ntrol (Tris-EDTA-BSA buffer)	A	34.9	20.9	14.7
	B	43.7	22.0	13.1

BLE 5: Dissociation of the binding between [^{125}I]-iodo-[Tyr^{1}]-somatostatin d SSBP using various reagents. Female rat liver cytosol and bovine terior pituitary cytosol were incubated with [^{125}I]-iodo-[Tyr^{1}]-somatatin for 1.5 h, 12 h, or 24 h at 4°C and then DCC was added followed centrifugation at 3,000 rev./min for 10 min. The supernatants (bound rm) were exposed to various reagents at final concentrations indicated r 30 min at 24°C, followed in each case by DCC prior to a second centrigation. The radioactivity in the second pellet (free tracer) was counted. cause adsorption of [^{125}I]-iodo-[Tyr^{1}]-somatostatin by DCC was reduced the presence of some reagents (80 to 91% of total radioactivity adsorbed stead of 98%) appropriate correction factors were applied. Data shown e mean of triplicates. A, female rat liver cytosol; B, bovine anterior tuitary cytosol. (Ogawa et al 1977).

TABLE 6

Enzyme	Concentration (µg/ml)	Specific binding of ^{125}I-labelled [Tyr^{1}]-somatostatin (% of control)	
		Rat liver cytosol	Bovine anterior pituitary cytosol
Control	-	100	100
Trypsin	50	37.9	27.5
	5	73.9	81.1
Ribonuclease	50	92.2	104.3
	5	106.2	108.6
Deoxyribonuclease I	50	101.9	101.4
	5	96.3	99.6
Phospholipase C	50	94.6	108.6
	5	106.9	99.5

e 6: Effect of enzyme treatment on SSBP activity. Cytosol fractions incubated at 37°C with different concentrations of enzyme for 15 min in same buffer used for binding studies. After the incubation period the tion tubes were chilled in ice. For trypsin digestion, 5 times as much ean trypsin inhibitor as trypsin was added. For controls, cytosol tions not exposed to enzymes, but incubated in the same buffer for the period were used. Determination of specific binding of [^{125}I]-iodo-^{1}]-somatostatin was identical with that described in Materials and Methods. shown are mean of triplicate determinations. (Ogawa et al 1977).

DISCUSSION

Properties of the binding of somatostatin binding protein

Somatostatin binding protein is found in cytosols (Table 1 and 2) of a variety of rat, bovine and human tissues, but not in the sera or cytosol of blood cells (Table 3). Therefore, the presence of somatostatin binding protein in many tissues is not due to contamination by a serum protein. There is some residual binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin to the membrane fraction of female rat liver after separation from the cytosol (Table 2). This binding is approximately 20% of that observed in the cytosol. Cytosol contamination of the pellet is not likely this great with techniques used. This means there may also be a membrane receptor for somatostatin.

The fact that EDTA in homogenization and incubation buffers augments binding indicates that trace amounts of divalent cations in the cytosol inhibit binding. The presence of 1 mM $CaCl_2$ in the same buffers inhibits binding further demonstrating the importance of divalent cations.

Ca^{+2} ions are also important to oestrogen receptors. Chamness and M^{c}Guire (1972) showed that the presence or absence of $CaCl_2$ altered sedimentation properties of the receptor drastically. Puca et al (1971) hypothesized that these changes were caused by a "receptor deforming agent" activated by Ca^{+2}. In our somatostatin binding system there may also be a Ca^{+2}-activated deforming factor or hydrolytic

enzyme which accounts for the importance of Ca^{+2} and EDTA in binding. However, if Ca^{+2} affects the conformation of of the binding protein, then this may inactivate the binding protein, accounting for the effect of Ca^{+2} and EDTA.

The assay system used shows optimal binding at pH's 8.0-8.5 (figure 2). Maximal binding occurs at 18h of incubation at 4°C (figure 3). For this reason, in our assay we used as a buffer, tris-HCl (50 mM) pH 8.0, 25 mM EDTA to maximize binding due to the Ca^{+2} effect and 0.5% BSA to decrease binding to glass. A direct relationship between cytosol protein concentration and specific binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin occurs between 3-50 ug/ml of protein in various rat tissues (figure 4). It is notable that such diverse tissues contain similar amounts of somatostatin binding activity per unit amount of cytosol protein.

Exposure of the somatostatin binding protein from rat liver and bovine anterior pituitary to 0.1 M acetic acid or NaOH at 24°C for 30 minutes decreases binding markedly. Heating for 1 minute on a boiling water bath completely destroys the activity. This is to be expected for a protein. Ethanol (85%) also decreases binding, but repetitive freezing and thawing had little effect on binding activity in the same tissues.

[^{125}I]-iodo-[Tyr^{1}]-somatostatin seems to bind almost irreversibly to the somatostatin binding protein since excess unlabelled somatostatin fails to displace it over 18h incubation at 4°C with only some displacement

after 24h at 4°C.

Table 5 shows that different reagents dissociated somatostatin from its binding protein with varying efficiencies. DTT and mercaptoethanol dissociate the binding of [^{125}I]-iodo-[Tyr^{1}]-somatostatin most efficiently and there is increased percent dissociation with increased pre-incubation time of hormone with binding protein. Conversely, 8M urea and 7M guanidine-HCl, while dissociating the hormone-binding protein complex well, dissociate it less well with increasing preincubation time. This indicates that there may be two steps in the formation of the hormone-binding protein complex. The first step of binding can be dissociated by guanidine-HCl and urea, but with time, a disulphide interaction takes place, making dissociation of the binding susceptible to DTT and mercaptoethanol but less so to guanidine-HCl and urea.

Many other reagents (Table 5) cause dissociation of [^{125}I]-iodo-[Tyr^{1}]-somatostatin from the somatostatin binding protein, but their ability to do so, decreases with increasing pre-incubation time. This strongly suggests the formation of an irreversible bond between the hormone and the binding protein because somatostatin itself cannot displace the tracer after it is bound.

Figure 5 shows specificity studies of [^{125}I]-iodo-[Tyr^{1}]-somatostatin. Competitive binding with the tracer occurs with synthetic ACTH (5%), p-glucagon (7%), [Ala^{3,14}]-somatostatin (10%), [D-Trp^{8}]-somatostatin (40%), and [Tyr^{1}]-somatostatin (150%) as compared to somatostatin displacement of 100%. The fact that [Tyr^{1}]-somatostatin displaces [^{125}I]-iodo-[Tyr^{1}]-somatostatin more readily than the native hormone is suprising since it has 25% (Vale et al 1975) or 100% (Ferland et al 1976) the biological potency of native somatostatin. [D-Trp^{8}]-somatostatin a cyclic analogue cross-reacts strongly (40%), but linear molecules such as [Ala^{3,14}]-somatostatin and p-glucagon cross-react weakly. Both p-glucagon and [Ala^{3,14}]-somatostatin have a four amino acid sequence (Thr-Phe-Thr-Ser) in common with native somatostatin which may explain why they seem to displace [^{125}I]-iodo-[Tyr^{1}]-somatostatin. Because they cannot form disulphide links with the receptor, because of lack of cysteine residues, the second step of somatostatin binding may be prevented explaining why full displacement is not seen.

Figure 6 and 7 show that somatostatin binding protein can greatly influence a radioimmunoassy for somatostatin giving spuriously high results if steps are not taken to completely destroy the binding protein activity (i.e. incubation at 100^{o}C).

Trypsin treatment decreased binding markedly indicating the molecule is protein in nature (Table 6). The molecular weight of this binding protein in a variety of rat tissues was similar at approximately 77,000 daltons (figure 8) indicating that the molecule has a similar molecular weight in all tissues studied.

Isoelectricfocusing studies showed the binding protein to have an isoelectric point of approximately 4.8-5.0 (figure 9) in all tissues studied. Disc gel electrophoresis on a basic polyacrylamide gel gave an R_f of approximately .75 (figure 13) in all tissues studied. Tissue mixture studies were conducted with both isoelectricfocusing and disc gel electrophoresis. In these mixtures, rat liver cytosol binding protein was labelled with [^{131}I]-iodo-[Tyr^1]-somatostatin and mixed with one of a variety of rat tissue cytosol binding proteins bound to [^{125}I]-labelled hormone. This mixture was applied as one sample on electrophoretic and isoelectrofocusing gels. Patterns of distribution of radioactivity were identical in every case indicating all somatostatin binding proteins from various tissues are similar to that of liver and therefore to each other electrophoretically and by isoelectric point. (figure 11 and 15)

[^{125}I]-iodo-[Tyr^1]-somatostatin was also run on isoelectricfocusing and a peak of [^{125}I] activity was recorded at approximately pH 7.7. This indicates that the peak of [^{125}I] activity recorded during tracer-

labelled binding protein studies does not represent free tracer. In electrophoretic studies of tracer-labelled binding proteins, there were always two peaks, one corresponding to one of the peaks of free [Tyr^1]-somatostatin tracer and the other is sufficiently different from the two peaks of free somatostatin tracer, that it probably represents the binding protein at R_f=0.75 (figure 14).

Two protein bands occurred near an isoelectric point of pH 4.8-5.0 on an isoelectrofocusing gel after staining (figure 12). Differentially-labelled rat cytosol mixtures were run on isoelectrofocusing gels without BSA, stained for protein and the two protein bands at pH 5.0 were dissected out and counted in an automatic gamma counter. In every case, both [^{131}I] and [^{125}I] showed peak activity in the same band. This indicates great similarity of somatostatin binding proteins in the tissues studied. Labelled somatostatin binding protein was also run without BSA on electrophoresis and stained for protein. The protein band at R_f=0.75 was cut out and it coincided with the presence of somatostatin binding activity as indicated by the presence of a high gamma count in this band.

Somatostatin binding protein as a somatostatin receptor

In judging the somatostatin binding protein as a hormone receptor it is appropriate to compare properties of this binding protein with the criteria of Cuatrecasas (1975) for identification of a receptor.

The first criteria is specificity. This somatostatin binding protein is cross-reactive only with somatostatin

analogues, p-glucagon and synthetic ACTH to a small extent (figure 5). The cross-reactivity of p-glucagon may be explained in that it shares a common four amino acid sequence with somatostatin. Therefore the somatostatin binding protein appears not to be completely specific.

The criteria of saturability, indicating a limited number of binding sites, is obviously met, since [^{125}I]-iodo-[Tyr^{1}]-somatostatin competes with excess unlabelled somatostatin yielding a standard curve (figure 5). If the process were not saturable, competitive binding would not occur since there would be an excess of binding sites, enough for both tracer and excess cold hormone to bind.

The question of whether the somatostatin binding protein shows tissue specificity in accord with target organs of somatostatin is difficult to answer because the physiological actions of somatostatin have not been determined conclusively. Somatostatin has been found in the anterior pituitary, stomach, small intestine and brain (Table 2) by immunoassay (Arimura et al 1975a) and inhibits various hormone secretions in these tissues, indicating target organ specificity of the somatostatin binding protein. The binding protein was found in all tissues studied however, except blood cells and serum, so that unless somatostatin has some general action on all these tissue, target organ specificity is not achieved.

Conclusions are impossible however, since the true physiological actions of somatostatin are unknown.

A high affinity is necessary for a hormone-receptor complex according to Cuatrecasas (1975) so that it may be sensitive to low concentrations and fluctuations of the hormone in the blood stream. The affinity of the somatostatin binding protein is impossible to calculate since the binding appears to be irreversible and so is not amenable to Scatchard analysis. Such a calculation would however, be academic since no significant levels of somatostatin have been detected in the serum (Arimura et al 1975a) and so correlation of affinity with hormone levels is impossible.

Reversibility is the final criteria set by Cuatrecasas for a hormone receptor. This would be consistent with the observed termination of the hormone's effect when it is removed from media in _in vitro_ systems or administration is discontinued _in vivo_. The somatostatin binding protein seems to show irreversible binding. Reversibility as a criteria for hormone receptors is not necessary however, if the end result of somatostatin binding to the receptor is hormone degradation. Hormone degradtion would result in termination of somatostatin action on its removal from media _in vitro_. It is also possible that while the hormone-binding protein complex is irreversible to many chemical reagents, _in vivo_ it may be reversible upon reaction with an enzyme that catalyzes

dissociation.

There is other evidence in favour of somatostatin binding protein as a hormone receptor. Firstly, somatostatin binding protein has been found in human and bovine tissues as well as rats (Table 3) so that it is not just a phenomenon of one species. It also has similar molecular weights, isoelectric points, and electrophoretic mobilities in a number of tissues (figure 8 and 16).

Somatostatin decreases cyclic AMP accumulation in the pituitary and the pancreas at a point distal to cyclic AMP synthesis because it decreases cyclic AMP accumulation in these tissues due to theophylline stimulation (Borgeat et al 1974; Garcia et al 1976) and to exogenously administered dibutyryl cyclic AMP (Peracchi et al 1976; Garcia et al 1976). If somatostatin acts distal to cyclic AMP synthesis a cytosol receptor is much more likely, but does not exclude the possibility of mediation of hormone action via a membrane receptor.

Other polypeptide hormones may also act inside the cell since receptors for prolactin and melanocyte-stimulating hormone have been found in the Golgi apparatus (Posner and Bergeron 1975,1976; Varga et al 1976). This suggests an intracellular mode of action for peptide and polypeptide hormones.

It is of interest that disulphides are of importance in the binding of somatostatin to the binding protein.

Somatostatin analogues with blocked or [Ala]-substituted cysteine residues show low biological activity (<1.0%) (Rivier et al 1975b; Serantakis et al 1973). Likewise [$Ala^{3,14}$]-somatostatin shows only 10% of the cross-reactivity with [^{125}I]-iodo-[Tyr^{1}]-somatostatin of the native cyclic hormone in binding to the liver cytosol fraction.

With cytosol oestrogen receptors, variation of Ca^{+2} concetrations affect receptor structure greatly (Chamness and M^{c}Guire 1972). If somatostatin binding protein is a hormone receptor like that of oestrogens, it would account for the dramatic effect of Ca^{+2} on somatostatin binding (figure 1). Somatostatin binding protein is also sensitive to SH-blocking reagents as are intracellular androgen receptors (Hansson et al 1974; Naess et al 1975). These similarities, along with the fact that steroid receptors are also present in the cytosol, indicates that somatostatin may have a cytosol hormone receptor like that of steroids.

There is also considerable evidence that somatostatin binding protein is not a hormone receptor. In tissue preparations (Table 2) there is residual binding in the membrane fraction after separation from the cytosol. This membrane binding is approximately 20% that found in the cytosol, much more than possible cytosol contamination could account for, considering the methods used. The existence of a membrane binding site for somatostatin, would not exclude somatostatin

binding protein as being physiologically important, but its exact role is unclear. A membrane receptor for somatostatin would not be surprising since the two other hypothalamic hormones isolated, LH-RH and TRH have membrane receptors (Borgeat et al 1972; Grant et al 1972; Wilber and Seibel 1973).

Finally, evidence exists for somatostatin as a neurotransmitter since it is found in synaptosomes in various neurons in the brain (Brownstein et al 1975; Alpert et al 1976). Known neurotransmitters such as acetylcholine and catecholamines use membrane receptors to exert their effect. If somatostatin is a neurotransmitter it would be unique among them in having an intracellular receptor such as the somatostatin binding protein.

Acetylcholine and catecholamines are bound to lipoprotein complexes in synaptosomes (Green 1962; Smith and Winkler 1967; Banks et al 1969). Oxytocin and vasopressin are also bound to neurophysins in neuro-secretory granules of the neurohypophysis (Dean et al 1968). This somatostatin binding protein may simply be a binding protein used to store somatostatin in synaptosomes. Somatostatin binding protein is also found in other tissue besides nervous tissue however, and even in tissues without somatostatin stores. This storage role for the somatostatin binding protein would not therefore explain its presence in other tissues.

Somatostatin binding protein and theories of the mechanism of action of somatostatin

There are various theories of the mechanism of action of somatostatin as explained in the Introduction. The physiological significance of this cytosol somatostatin binding protein would also be determined by how well properties of this possible intracellular site of action of somatostatin fits these theories. Therefore, the possible role of such a receptor in the various theories will be discussed.

Because somatostatin's action is independent of protein and RNA synthesis (Vale et al 1973; Vale et al 1974), it may act by modulating an enzyme. Somatostatin decreases cyclic AMP levels in many tissues. Most importantly, it decreases cyclic AMP accumulation in pituitaries and pancreatic islets due to theophylline stimulation (Borgeat et al 1974; Garcia et al 1976) and exogenously administered dibutyryl cyclic AMP (Peracchi et al 1976; Garcia et al 1976). This indicates somatostatin acts distally to cyclic AMP synthesis to decrease intracellular cyclic AMP levels. This action may involve stimulation of degradation of cyclic AMP which therefore would overcome the effect of theophylline in inhibiting phosphodiesterase. To exert this effect, somatostatin may enter, binding to a cyclic AMP degratory enzyme like phosphodiesterase, increasing its activity, or it may act indirectly to exert its effect.

A possible indirect mechanism of affecting cyclic AMP levels was shown by Kenako et al (1974) who found that somatostatin decreases cyclic AMP and increases cyclic GMP levels in rat pituitaries. Since cyclic GMP is produced by a cytosol enzyme guanylate cyclase unlike cyclic AMP (White and Aurbach, 1969; Hardman and Sutherland 1969; Schultz et al 1969) and cGMP enhances hydrolysis of cyclic AMP by way of phosphodiesterase (Hardman et al 1971; Klotz et al 1972), somatostatin may decrease intracellular cyclic AMP levels by acting on a cytosol enzyme.

Somatostatin may also act by a membrane receptor because a membrane bound enzyme comparable to adenyl cyclase could be stimulated by somatostatin binding to a membrane receptor, causing for example, a series of reactions ending in a stimulation of phosphodiesterase and hence to decreased cyclic AMP levels.

The importance of Ca^{+2} fluxes into the cell has also been shown in the secretion of insulin. Insulin secretion and other secretory processes are dependent on the presence of Ca^{+2} (Curry et al 1968b; Douglas and Poisner 1963). Somatostatin inhibits insulin secretion and cyclic AMP accumulation in pancreatic islet cells (Efendic et al 1975; Gerich et al 1974b; Borgeat 1974). Somatostatin's inhibitory effect is overridden if extracellular Ca^{+2} is increased (Curry and Bennett 1974b) or if Ca^{+2} fluxes into the cell are increased by addition

of ionophore A23187 to the media (Fujimoto and Ensinck 1975) which is specific for calcium (Wolheim et al 1975). Since Ca^{+2} is necessary for insulin release (Curry et al 1968b) somatostatin may inhibit insulin release by inhibiting a plasma membrane calcium carrier system, so reducing Ca^{+2} influx. Because membrane carrier systems are located in the plasma membrane, the somatostatin receptor may be membrane bound. It is notable that high Ca^{+2} levels inhibit both somatostatin binding activity (figure 1) and the effects of somatostatin on pancreatic cells. This is evidence, if only circumstantial, that the actions of somatostatin may be mediated by the somatostatin binding protein.

Somatostatin and the α-adrenergic receptor

Somatostatin is contained in certain central and peripheral neurons (Pelletier et al 1974), so somatostatin may be a neurotransmitter. Phentolamine (an α-adrenergic blocker) abolishes somatostatin's inhibition of insulin release. This means somatostatin may act by the α-adrenergic receptor. α-Adrenergic receptor activation causes inhibition of insulin release elicited by most secretagogues except arginine and secretin (Malaisse 1972). Somatostatin inhibits insulin secretion due to arginine and secretin stimulation as well. Somatostatin's action therefore is not due solely to activation of α-adrenergic receptor activation. This is evidence that another receptor, possibly a cytosol receptor may be necessary.

If somatostatin is a neurotransmitter, as indicated by its presence in synaptosomes (Alpert et al 1976) in various parts of the brain, a membrane receptor seems the most likely type of receptor. This is because other neurotransmitters, such as acetylcholine and catecholamines have membrane receptors. LH-RH and TRH, which have proposed neurotransmitter actions, have membrane receptors (Grant et al 1972; Wilber and Seibel 1973; Borgeat et al 1972).

It is impossible to make conclusions from the above evidence, but a case exists for both a membrane and a cytosol receptor for somatostatin.

Possible identities and roles for somatostatin binding protein

Somatostatin binding protein could also be a cytosol receptor protein for somatostatin like those found for steroids because it has some properties in common with steroid receptors (Chamness and M^{c}Guire 1972; Hansson et al 1974; Naess et al 1975). Overall, the evidence for this cytosol somatostatin binding protein being a receptor is inconclusive.

If somatostatin binding protein is not a hormone receptor, it may have other roles. It may have a storage role, protecting somatostatin from degradation. This however, seem unlikely since somatostatin binding protein is found in nearly all tissues studied, while somatostatin

stores are found by immunoassay, in only certain cells in the pancreas, gastrointestinal tract and brain (Arimura et al 1975a).

Another role for somatostatin binding protein is that of a first step in the degradation of somatostatin. This might explain its widespread distribution, since other hormone degra tory enzyme systems such as catecholamine-O-methyl transferase are found in many tissues (Axelrod et al 1959).

There is little evidence for any of these other functions for somatostatin binding protein. If it is physiologically important, a receptor function is most likely. Somatostatin should alter some enzyme function to exert its effect since somatostatin's action is not dependent on new protein synthesis (Vale et al 1973a; Vale et al 1974). For this reason a variety of enzymes were considered as possible hormone receptors for somatostatin in order to explain its actions.

Somatostatin acts distal to cyclic AMP synthesis to decrease cyclic AMP accumulation (Borgeat et al 1974; Garcia et al 1976; Peracchi et al 1976) so this cytosol receptor may be phosphodiesterase. Somatostatin could stimulate phospodiesterase activity to decrease cyclic AMP accumulation. Phosphodiesterase is an unlikely receptor however, since rat liver phosphodiesterase has a molecular weight of 400,000 (Terasaki et al 1973) while that of somatostatin binding protein is 77,000.

Kenako et al (1974) showed somatostatin enhances cyclic GMP synthesis in rat pituitary. This means guanylate cyclase could be the somatostatin cytosol receptor, but again it's molecular weight of 300,000 (White and Aurbach 1969) is too large to be the somatostatin binding protein.

A survey of various metabolic enzymes was also done to determine those whose proerties correlated well with those of the somatostatin binding protein. The monomer of glycogen synthetase has a similar molecular weight (M^{C}Verry and Kim 1974), electrophoretic properties (Lin and Segal 1973) and a widespread tissue distribution (Larner and Villar-Palasi 1971) to the somatostatin binding protein. However, modulation of glycogen synthetase could not account for all the actions of somatostatin.

A more interesting observation is that the regulatory subunit of mammalian cyclic AMP-dependent cytosol protein kinase (R) has properties similar to somatostatin binding protein. Cyclic AMP-dependent protein kinases are found in all tissues examined (Kuo and Greengard 1969). While most protein kinase activity is located in the cytosol, (Chen and Walsh (1971) there is also some membrane bound activity (Maeno et al 1971) which could account for some somatostatin binding activity in the particulate fraction during tissue preparation (Table 1). Binding of cyclic AMP to the regulatory unit of the protein kinase causes

dissociation of regulatory and catalytic subunits, which activates the catalytic unit. This catalytic unit may then phosphorylate any of a variety of enzymes, activating or deactivating them (Walsh and Krebs 1973). Phosphorylase kinase, for example, is activated after phosphorylation by a cyclic AMP-dependent protein kinase (Walsh and Krebs 1973). If somatostatin binds to a cyclic AMP-dependent protein kinase and alters its activity it could then exert its effect by altering the activity of any of a variety of intracellular enzymes by effecting the rate at which they are phosphorylated.

The regulatory subunit of the cyclic AMP-dependent protein kinase has a molecular weight of approximately 80,000 daltons in various mammalian tissues (Walsh and Krebs 1973) including rat liver cytosol (Kumon et al 1972). The isoelectric point is approximately 4.5 which is similar to somatostatin binding protein (Kumon et al 1972, Chen and Walsh 1971). Protein kinases have similar properties not only from tissue to tissue but in various species as well (Walsh and Krebs 1973) like somatostatin binding protein. Finally Ca^{+2} is a potent inhibitor of protein kinase activity (Chambaut et al 1971, Labrie et al 1971, Hoffman and Sold 1971) as it is of somatostatin binding activity. This suggests that protein kinase may be the site of action of somatostatin.

Further evidence that somatostatin may alter protein kinase activity is that Walsh et al (1971) purified a peptide of molecular weight 2,600 in rabbit skeletal muscle that inhibits protein kinase activity. While somatostatin has a molecular weight of only 1,800, the fact that one peptide inhibits protein kinase activity, means other peptides, like somatostatin, may also affect the enzyme's activity.

FURTHER STUDIES

The similarity of somatostatin binding protein to the regulatory subunit of protein kinase is the most promising area for future research, especially since preliminary data indicates purified mammalian protein kinase has high specific binding for somatostatin.

Firstly, rat and other mammalian protein kinase regulatory subunits should be shown by immunological and biochemical techniques to be identical to the somatostatin binding protein. The binding properties of somatostatin binding protein should also be compared to that of protein kinases. This would establish whether somatostatin binding protein is indeed the regulatory subunit of protein kinases.

Confirmation of the above, would permit study of somatostatin's mechanism of action, by allowing study of its receptors. Since its receptor would be a much studied enzyme,previous research on protein kinases would be helpful. The effect of somatostatin on protein kinase activity could then be determined as well as what role this protein kinase activity has in eliciting the final action of somatostatin. Cyclic AMP also binds to the regulatory subunit of the cyclic AMP-dependent protein kinase. It is of interest to determine if somatostatin and cyclic AMP are competing for the same site.

Because there is residual binding of somatostatin in particulate fractions, it must be determined if there is a membrane receptor somatostatin. While there may be a

different receptor on the membrane, cyclic AMP-dependent protein kinase can also be membrane bound (Maeno et al 1971). The presence of a plasma membrane receptor for somatostatin would lead to further studies of the mechanism of action of somatostatin mediated by a membrane receptor.

Ultimately, from such studies, a greater insight into the role of somatostatin in vivo and its mechanism of action would be obtained.

REFERENCES

Alberti K.G., Christensen H.J., Christensen S.E., Hansen A.P., Iversen J., Lundback K., Seyer-Hansen K., and Orskov H. (1973). Lancet 2:1299

Alpert L.C., Brawer, J.R., Patel, Y.C. and Reichlin, S. (1976). Endocrinol. 98:255

Alpert L.C., Brawer, J.R., Patel, Y.C. and Reichlin, S. (1975). Fed. Proc. 34:238.

Arimura A., Smith W.D. and Schally A.V. (1976). Endocrinol. 98:540

Arimura A., Sato H., Coy D.H. and Schally A.V. (1975a). Proc. Soc. Exptl. Biol. Med. 148:784

Arimura A., Sato H., Dupont A., Nozomu N. and Schally A.V. (1975b). Science 189:1007

Arimura A., Sato H., Kumasaka T., Worobel R.B., Debeljuk L., Dunn J. and Schally A.V. (1973). Endocrinol. 93:1092

Axelrod J., Albers W. and Clements C.D. (1959). J. Neurochem. 5:68

Baker B.L. and Yen Y.-Y. (1976). Proc. Soc. Exptl. Biol. Med. 151:599

Banks P., Helle K.B. and Mayor D. (1969). Molecular Pharmacol. 5:210

Bar H.G. and Hechter O. (1969). Biochem. Biophys. Res. Commun. 35:681

Barnea A., Ben-Jonathan N., Porter J.C. (1975). Fed. Proc. 34:239

Bélanger A., Labrie F., Borgeat P., Savary M., Côté T. and Drouin J. (1974). Mol. Cell. Endocrinol. 1:329

Besser G.M., Paxton A.M., Johnson S.A., Moddy E.J., Mortimer C.H., Hall R., Gomez-Pan A., Kastin A.J., Schally A.V. and Coy D.H. (1975). Lancet 2:1168

Besser G.M., Mortimer C.H., Carr D., Schally A.V., Coy D.H., Evered D., Kastin A.J., Tunbridge W.H.G., Thorner M.D. and Hall R. (1974a). Brit. J. Med. 1:352

Besser G.M., Mortimer C.H., McNeilly A.S., Thorner M.D., Batistoni G.A., Bloom S.R., Kastrup K.W., Hanssen K.F., Hall R., Coy D.H., Kastin A.J. and Schally A.V. (1974b). Br. Med. J. 4:622

Besses G.S., Burrow G.N., Spaulding S.W., Donakedian R.K. (1975). J. Clin. Endocrinol. Metab. 41:985

Bloom S.R., Barraclough M.A., Jamieson C.A., Clement-Jones V., Wiley K., Polak J.M., Besser G.M., Hall R., Coy D.H., Kastin A.J. and Schally A.V. (1975). Proc. Endocrine Sect. of Royal Soc. Med. London

Bloom S.R., Mortimer D.H., Thorner M.O., Besser G.M., Hall R., Gomez-Pan P., Roy U.M., Russell R.C.G., Coy D.H., Kastin A.J. and Schally A.V. (1974). Lancet 2:1106

Boden G., Switz M.C., Owen O.E., Essa-Koumar N., London J.H. (1975). Science 188:163

Borgeat P., Garneau P. and Labrie F. (1975). Mol. and Cell. Endocrinol. 2:117

Borgeat P., Labrie F., Drouin J., Bélanger A., Immer H., Sestasy K., Nelson V., Gotz M., Schally A.V., Coy D.H. and Coy E.J. (1974). Bioch. Biophys. Res. Commun. 56:1052

Borgeat P., Chavancy G., Dupont A., Labrie F., Arimura A. and Schally A.V. (1972). Proc. Natl. Acad. Sci. USA 69:2677

Brazeau P., Vale W., Burgus R. and Guillemin R. (1974a). Can. J. Biochem. 52:1067

Brazeau P., Vale W., Rivier J. and Guillemin R. (1974b). Bioch. Biophys. Res. Commun. 60:1202

Brazeau P., Rivier J., Vale W. and Guillemin R. (1974c). Endocrinol. 94:184

Brazeau P., Vale W., Burgus R., Ling N., Butcher M., Rivier J. and Guillemin R. (1973). Science 179:77

Breese G.R., Cott J.M., Cooper B.R., Prange A.J., Lipton M.A. and Plotnikoff N.P. (1975). J. Pharmacol. Exptl. Therap. 193:11

Brown M. and Vale W. (1975). Endocrinol. 96:1333

Brownstein M. Arimura A., Sato H., Schally A.V. and Kizer J.S. (1975). Endocrinol. 96:1456

Burt D.R. and Snyder S.H. (1975). Brain Res. 93:309

Carr D., Gomez-Pan A., Weightman D.R., Roy V.C.M., Hall R., Besser G.M., Thorner M.O., McNeilly A.S., Schally A.V., Kastin A.J. and Coy D.H. (1975). Brit. Med. J. 3:67

Chambaut A.M., Lefray F. and Hansun J. (1971). Febs. Letters 15:328

Chamness G.C. and McGuire W.L. (1972). Bioch. 11:2466.

Chen L.J. and Walsh D.A. (1971). Biochem. 10:3614

Chideckel F., Palmer J., Koerker D., Ensinck J., Davidson M. and Goodner C. (1975). J. Clin. Invest. 55:754

Christensen S.E., Prange-Hansen A., Iversen J., Lundback K., Orskov H. and Seyer-Hansen K. (1974). Scand. J. Clin. Lab. Invest. 34:321

Copinschi G., LeClercq-Meyer V., Virasoro E., L'Hermite M., Vanhaelst L., Goldstein J., Le Clercq R. and Fery-Fand Robeyn C. (1976). Horm. Metab. Res. 8:226

Creutzfeldt W., Lankisch P.G. and Falsch V.R. (1975). Dtsch. Med. Wschr. 100:1135

Cuatrecasas P., Hollenberg M.D., Chang K.-J. and Bennett V. (1975). In "Recent Progress in Hormone Research". (Rod.) Greep ed. Acad. Press (London) p. 37

Cuatrecasas P. (1974). Ann. Rev. Biochem. 43:169

Curry D.L. and Bennett L.L. (1976). Proc. Natl. Acad. Sci. USA 73:248

Curry D.L., Bennett L.L. and Li C.H. (1974a). Bioch. Biophys. Res. Commun. 58:885

Curry D.L. and Bennett L.L. (1974b). Bioch. Biophys. Res. Commun. 60:1015

Curry D.L., Bennett L.L. and Grodsky G.M. (1968a) Endocrinol. 83:572

Curry D.L., Bennett L.L. and Grodsky G.M. (1968b) Am. J. Physiol. 214:174

Dannies P.S. and Tashjian A.H., Jr. (1976a). Nature 261:707

Dannies P.S. and Tashjian A.H., Jr. (1976b). Biochem. Biophys. Res. Commun. 70:1180

Dannies P.S. and Tashjian A.H., Jr. (1976c). J. Biol. Chem. 248:6174

Dannies, P.S., Gautvik K.M. and Tashjian A.H., Jr. (1976). Endocrinol. 98:1147

Davies B.J. (1964). Ann. N.Y. Acad. Sci. 121:404

Dean C.R., Hope D.B. and Kazic T. (1968). Br. J. Pharmacol. 34:192P

Devane G.W., Siler T.M. and Yen S.-S. (1974). J. Clin. Endocrinol. Metab. 38:913

Dobbs R., Sakurai H., Sakaki H., Faloona G., Valverde I., Baetens D., Orci C. and Unger R. (1975). Science 187:544

Douglas W.W. and Poisner A.M. (1963). J. Physiol. (London) 165:529.

Drouin J., DeLéan A., Rainville D., LaChance R. and Labrie F. (1976). Endocrinol. 98:514

Dubois M. (1975). Proc. Natl. Acad. Sci. USA 70:684

Duckworth W.C., Chiang T.M., Beachey F.H. and Kang A.H. (1975). Proc. 57th Ann. Meeting Endocrine Soc. N.Y. p. 128

Efendic S., Grill V. and Luft R. (1975) Febs. Letters 55:31

Efendic S., Luft R. and Grill V. (1974). Febs. Letters 42:169

Endroczi E. and Hilliard J. (1965). Endocrinol. 77:667

Enoch M.R. and Johnson L.R. (1975). Gastroenterology 68:889

Evered D.C., Gomez-Pan A., Tunbridge W.M.G., Hall R., Lind T., Besser G.M., Mortimer C.M., Thorner M.O., Schally A.V., Kastin A.J. and Coy D.H. (1975). Lancet 1:1250

Fehm H.L., Voigt K.H., Lang R., Beinert K.E., Raptis S. and Pfeiffer E.E. (1976). Klin. Woch. 54:173

Feldman D. (1975). Ann. Rev. Med. 26:83

Feldman D., Funder J.W. and Edelman S. (1972). Am. J. Med. 53:547

Ferland L., Labrie F., Coy D.H., Arimura A. and Schally A.V. (1976). Mol. Cell. Endocrinol.

Fujimoto W.Y. (1975). Endocrinol. 97:1494

Fujimoto W.Y. and Ensinck J.W. (1975). Endocrinol. 98:259

Fujimoto W.W., Ensinck J.W. and Williams R.H. (1974). Life Sci. 15:1999

Gabrys B.F., Alair S.M., Uyhiro L.M. and Bombeck C. (1973). Gastroenterology 64:730

Garcia S.D., Jarrousse C. and Rosselin G. (1976). J. Clin. Invest. 57:230

Gerich J.E., Lovinger M., Schneider V., Karam J.H., Rivier J., Guillemin R. and Forsham P. (1974a). New Engl. J. Med. 291:544

Gerich J.E., Lorenzi M., Schneider V. and Forsham P.H. (1974b). J. Clin. Endocrinol. Metab. 39:1057

Gerich J.E., Lovinger R. and Grodsky G. (1975). Endocrinol. 96:749

Goldsmith P.C., Rose J.C., Arimura A. and Ganong W.F. (1975). Endocrinol. 97:1061

Gomez-Pan A., Inthuprapa M., Schardt C.W., Hall R., Besser G.M., Mortimer C.H., Thorner M.O. and Schally A.V. (1976a). in press

Gomez-Pan A., Carr D., Evered D.C., Tunbridge W.M.G., Hall R., Thorner M.O., Mortimer C.H., Besser G.M., Coy D.H., Kastin A.J. and Schally A.V. (1976b). in press

Gomez-Pan A., Snow M.H., Piercy D.A., Robon V., Wilkinson R., Hall R. and Evered P.C., Besser G.M., Schally A.V., Kastin A.J. and Coy D.H. (1976c). J. Clin. Endocrinol. Metab. 43:240

Gomez-Pan A., Hall R., Lind T., Evered D.C., Bloom S.R., Ratchliffe J.G., Child D.H., Kastin A.J. and Schally A.V. (1975). Lancet 1:888

Grant G., Yale W. and Guillemin R. (1972). Bioch. Biophys. Res. Commun. 46:28

Green J.P. (1962). Adv. Pharmacol. 1:349

Hall R. and Gomez-Pan A. (1976). Adv. Clin. Chem. 18:173

Hall R., Besser G.M., Schally A.V., Coy D.H., Evered D., Goldie D.J., Kastin A.J., McNeilly A.S., Mortimer C.H., Phenekos C., Tunbridge W.M.G. and Weightman D. (1973). Lancet 2:581

Hansen A.P., Orskov H., Seyer-Hansen K., Lundback K. (1973). Bri. Med. J. 2:523

Hansson V., Trygstad O., French F.S., McLean W.S., Smith A.A., Tindall D.J., Weddington S.C., Petrusz P., Nayfeh S.N. and Retzen E.M. (1974). Nature 250:387

Hardman J.G., Blavo J.A., Gray J.P., Chrisman T.D., Patterson W.D., Sutherland E.W. (1971). Ann. N.Y. Acad. Sci. 185:27

Harris M.C. and Singhera M. (1974). Brain Res. 81:401

Hardman J.G. and Sutherland E.W. (1969). J. Biol. Chem. 244:6363

Hinkleman P.M. and Tashjian A.H., Jr. (1975). Biochem. 14:3845

Hinkleman P.M. and Tashjian A.H., Jr. (1973). J. Biol. Chem. 248:6180

Hoffman F. and Sold G. (1972). Biochem. Biophys. Res. Commun. 49:1100

Hokfelt D., Efendic S., Johansson O., Luft R. and Arimura A. (1974). Brain Res. 80:165

Iversen J. (1974). Scand. J. Clin. Lab. Invest. 33:125

Jackson M.D. and Reichlin S. (1975). Prog. Ann. Meeting Endocrine Soc. 57th N.Y. p. 96

Jackson M.D. and Reichlin S. (1974a). Life. Sci. 14:2259

Jackson M.D. and Reichlin S. (1974b). Endocrinol. 95:854

Johnson D.G., Ensinck J.W., Koerker D., Palmer J. and Goodner C.J. (1975). Endocrinol. 96:370

Johnson P.N. and Inesi G. (1969). J. Pharmacol and Exptl. Therap. 169:308

Kaneko T., Oka H., Munemura M., Suzuki S., Yasuda H., Oda T. and Yanaimaia N. (1974). Bioch. Biophys. Res. Commun. 61:53

Kaneko T., Saito S., Oka H., Oda T., Yanaihara N. (1973). In "Hypothalamic Hypophysiotrophic Hormones "(C. Gual and E. Rosenberg eds.) Int. Congr. Ser. No. 263 Excerpta Medica, Found. Amsterdam p. 198

Kato Y., Chichasa K., Ohgo S. and Imura H. (1974). J. Endocrinol 62:687

Klotz V. and Stock K. (1972). Arch. Pharmaco. 274:54

Koerker D.J. (1975). J. Amer. Med. Assn. 231:391

Koerker D.I., Ruch W., Chideckel E., Palmer J., Goodner C.J., Ensinck J. and Galk D.D. (1974). Science 184:482

Konturek S.J., Tasler J., Cieszkowski M., Coy D.H. and Schally A.V. (1976). J. Clin. Invest. 58:1

Krulich L., Dharival A.P.S. and McCann S.M. (1968). Endocrinol. 83:783

Kumon A., Nishiyama K., Yamamura H. and Neshizuko Y. (1972). J. Biol. Chem. 247:3726

Kuo J.F. and Greengard P. (1969). Proc. Natl. Acad. Sci. U.S. 64:1349

Labrie F., Borgeat P., LeMay A., LeMaire S., Borden N., Brouin J., Lemaire I., Jolicoeur P. and Bélanger A. (1975). Adv. Cyclic Nucleotide Res. 5:790

Labrie F., LeMaire S. and Courte C. (1971). J. Biol. Chem. 246:7293

Larner J. and Villor-Palasi C. (1971). Curr. Top. Cll. Regul. 3:195

Leftkowitz, R.J., Roth J. and Pastan I. (1970). Nature 228:864

Lin D.C. and Segal H.L. (1973). J. Biol. Chem. 248:7007

Maeno H., Johnson E.M. and Greengard P.J. (1971). J. Biol. Chem. 246:134

Malaisse W.J. (1972). In "Handbook of Physiology" (Sternes D.F. and Freinkel K. eds). Sect. 7., Vol. 8, Waverly, Baltimore p. 237

Malaisse W.J., Malaisse-Lagal F. and Mayhew D. (1967). J. Clin. Invest. 46:1724

Marks N., Stern F. and Benuck M. (1976). Nature 261:511

Marks N. and Stern F. (1975). Febs. Letters 55:220

Marshall J.C., Shakespear R.A. and Odell W.D. (1975). J. Endocrinol. 67(2) p. 38

Martin J.B. (1974). Endocrinol. 94:497

McVerry P.H. and Kim K.-H. (1974). Biochem. 13:3505

Montague C.H.W. and Cook J.R. (1971). Biochem. J. 122:115

Mortimer C.H., Carr D., Lind T., Bloom S.R., Mallinson C.N., Schally A.V., Tunbridge W.M.G., Yeomans L., Coy D.H., Kastin A., Besser G.M. and Hall R. (1974). Lancet 1:697

Naess O., Attramadal A. and Aakvaag A. (1975). Endocrinol. 96:1

Nickerson M. and Hollenberg N.K. (1967). In "Physiological Pharmacology" (Root W.F. and Hoff F.G. eds.) Vol IV. Acad. Press N.Y. p. 243

O'Malley B.W. (1971). New Engl. J. Med. 284:370

Ogawa N., Thompson T., Friesen H.G., Martin J.B. and Brazeau P. (1977). Biochem. J. in press

Parker D.C., Rossman L.G., Siler T.M., Rivier J., Yen S.-S. and Guillemin R. (1974). J. Clin. Endocrinol. Metab. 38:469

Patel Y.C., Weir G.C. and Reichlin S. (1975a). Proc. Ann. Meeting Endocrine Soc. 57th N.Y. p. 127

Patel Y.C., Rao K. and Reichlin S. (1975b). Clin. Res. 23:389A

Patrick J. and Lindstrom J. (1973). Science 180:871

Pearse A.G.E. and Polak J.M. (1975). Histochem. 41:373

Pelletier G., Labrie F., Arimura A. and Schally A.V. (1974). Am. J. Anat. 440:445

Perracchi M., Reschini F., Cantalamessa L., Catoma A. and Guistina G. (1976). Metab. 25:323

Piacsek B.E. and Meites J. (1966). Endocrinol. 79:432

Pimstone B.L., Becker D. and Krosheim S. (1975). J. Clin. Endocrinol. Metab. 40:168

Plotnikoff N.P., Kastin A.J., Anderson M.S. and Schally A.V. (1971). Life Sci. 10:1279

Polak J., Pearse A., Grimelus L., Bloom S. and Arimura A. (1975). Lancet 1:1220

Porte D. Jr. and Robertson R. (1973). Fed. Proc. 32:1792

Posner B. and Bergeron J.J.M. (1975a). Clin. Res. 23:A617

Posner B. and Bergeron J.J.M. (1975b). Progr. 58th Meeting Endocrine Soc. Abstr. No. 218

Posner B.I., Kelly P.A., Shiu R.P.C. and Friesen H.G. (1974). Endocrinol. 95:521

Powell D.W., Farris R.K. and Carbonetto S.T. (1974). Am. J. Physiol. 227:1428

Prange A.J. Jr., Breeze G.R., Cott J.M., Martin B.R., Cooper B.R., Wilson I.C. and Plotnikoff N.P. (1974). Life Sci. 14:447

Puca G.A., Nala E. and Bresciane F. (1970). In "Res. on Steriods" Vol. IV (Finklestern M., Klopper A., Conti C., and Cassini C. eds.) N.Y. Pergamanon Press, p. 319

Redding T.W. and Schally A.V. (1971). Endocrinol. 89:1075

Reed P.W. and Lardy H.A. (1972). J. Biol. Chem. 247:6970

Reichlin S. (1975). Arch. Int. Med. 135:1350

Reichlen S., Saperstein R., Jackson M.D., Boyd A.E. and Patel Y. (1976). Ann. Rev. Physiol. 38:389

Renaud L.P. (1976). Brain Res. 105:59

Renaud L.P. and Martin J.B. (1975). Brain Res. 93:145

Renaud L.P. and Martin J.B. (1974). Proc. Can. Fed. Biol. Soc. 17:96

Rezek M., Havlìcek V., Hughes K.R. and Friesen H.G. (1976a). Pharmacol. Biochem. and Behaviour 5:73

Rezek M., Havlìcek V., Hughes K.R. and Frìesen H.G. (1976b). Neuropharmacol. 15:499

Rivier J. (1974). J. Amer. Chem. Soc. 96:2986

Rivier J., Brown M. and Vale W. (1975a). Biochem. Biophys. Res. Commun. 65:746

Rivier J., Brazeau P., Vale W. and Guillemin R. (1975b). J. Med. Chem. 18:123

Rivier J., Brazeau P., Vale W., Ling N., Burgus R., Gilon C., Yardley J. and Guillemin R. (1973). Comptes Rendus Hebd. Seances Acad. Sci. 276:2737

Robberecht P., Deschodt-Lanckman M., DeNeef P. and Christophe J. (1975). Biochem. Biophys. Res. Commun. 67:315

Robberecht P., Deschodt-Linckman M., DeNeef P., Borgeat P. and Christophe T. (1974). Febs. Letters 43:139

Sachs, H., Goodman R., Osinchak J., McKelvy J. (1971). Proc. Natl. Acad. Sci. USA 68:2782

Sakurai N., Dobbs R. and Unger R.H. (1974). J. Clin. Invest. 54:1395

Sayers G., Beall R.J. and Seeling S. (1972). Science 175:1113

Schally A.V., Dupont A., Arimura A., Redding T.W. and Linthicun G.L. and Schlesinger D.H. (1976). Biochem. 15:509

Schally A.V., Dupont A., Arimura A., Redding T.W. and Linthicium G.L. (1975). Fed. Proc. Fed. Am. Soc. Exptl. Biol. 34:584

Schultz G., Bohme F. and Munski K. (1969). Life Sci. 8:1323

Scuro L.A., LoCascio V., Adami S., Galvanni G., Brianchi I., Cominacini L. and Corgnati A. (1976). Metab. 25:603

Serantakis D., McKinley W.A. and Grant N.H. (1973). Biochem. Biophys. Res. Commun. 55:538

Setalo G., Vigh S., Schally A.S., Arimura A., and Flerko B. (1975). Brain Res. 90:352

Shiu R.P.C. and Friesen H.G. (1976). Science 192:259

Shiu R.P.C., Kelly P.A. and Friesen H.G. (1973). Science 180:968

Siler T.M., Yen S.S., Vale W. and Guillemin R. (1974). J. Clin. Endocrinol. Metab. 38:742

Siler T.M., Vandenberg G., Yen S.-S., Brazeau P., Vale W. and Guillemin R. (1973). J. Clin. Endocrinol. Metab. 37:632

Singer S.J. and Nicholson G.L. (1972). Science 175:720

Smith A.D. and Winkler H. (1967). Biochem. J. 103:483

Smith P.H., Woods S.C.and Porte D. Jr. (1976). Endocrinol. 98:1073

Spona J. (1973). Febs. Letters 35:59

Steiner D.F., Kemmler W., Clark J.L., Oyer P.E. and Rubenstein A.H. (1972). Handbook Physiol. 1:175

Sutherland E.W. and Rall T.W. (1976). Pharmacol. Rev. 12:265

Szabo M. and Frohman L.A. (1975). Endocrinol. 96:955

Szentagothal J., Flerko B., Mess B. and Halasz B. (1968). In "Hypothalamic Control of the Anterior Pituitary" Akademiai Kiado Budapest p. 22

Taminoto T., Seino N., Coto Y., Iniura H. (1975). Bioch. Biophys. Res. Commun. 66:928

Terasaki W.L., Russell T.R. and Appleman M.M. (1973). In "Methods in Enzymology" (Hardman J.G. and O'Malley B.W. eds.) Vol. 38, pt. C, p. 257

Terry L.C., Willoughby J., Brazeau P., Martin J.B., Patel Y. (1976). Science 192:565

Thorell J.I. and Johannson B.G. (1971). Biochim. Biophys. Acta 251:363

Tsang D., Tan S., Martin J.B., Lab S., Renaud L.P. and Brazeau P. (1975). Neurosci. Abstr. 1:450

Tyrrell J.B., Lorenzi M., Forsham P.H. and Geri Med. Clin. N. Am. 58:1359

Vale W., Brazeau P., Rivier G., Brown M., Boss B., Rivier J., Burgus R., Ling N. and Guillemin R. (1975). Rec. Prog. Horm. Res. 31:365

Vale W. and Grant C. (1974). In "Methods in Enzmology" (B.W. O'Malley and J.G. Hardman eds.) Acad. Press N.Y. Vol. 37, Chapt. 15, p. 82

Vale W., Rivier C., Brazeau P. and Guillemin R. (1974). Endocrinol. 95:968

Vale W., Brazeau P., Rivier C., Rivier J. and Guillemin R. (1973). In "Advances in Growth Hormone Research" NIH p. 159

Vale W., Grant G., Amoss M., Blackwell R. and Guillemin R. (1972a). Endocrionl. 91:562

Vale W., Grant G., Rivier J., Monahan M., Amoss M., Blackwell R., Burgus R. and Guillemin R. (1972b) Science 176:933

Vale W., Brazeau P., Grant C., Nussey A., Burgus K., Rivier J., Ling N. and Guillemin R.G. (1972c). C.R. Acad. Sci. 275:2913

Varga J.M., Moellman G., Fritsh P., Godawska E. and Lerner A.B. (1976). Proc. Natl. Acad. Sci. USA 73:559

Walsh D.A. and Krebs E.G. (1973). Enzymes 8:555

Walsh D.A., Ashley C.D., Gonzalez C., Calkins D., Fisher E. and Krebs E.G. (1971). J. Biol. Chem. 296:1977

Walsh D.A., Perkins J.P. and Krebs E.G. (1968). J. Biol. Chem. 243:3763

Weir G.C., Knowltoz S.D. and Martin D.B. (1974). Endocrinol. 95:1744

Welbourn R.B., Pearse A.C.E., Polak J.M., Bloom S.R., Joffe S.N. (1974). Med. Clin. N. Am. 58:1359

White A.A. and Aurbach G.D. (1969). Biochim. Biophys. Acta 191:686

Wilber J.G. and Seibel M.J. (1973). Endocrinol. 92:888

Wilber J.F., Montoya E., Plotnikoff N.P., White W.F., Gendrich R., Renaud L. and Martin J.B. (1976). Rec. Prog. Horm. Res. 32:117

Wolheim C.B., Blondel B., Truehart P.A., Renaud A.E. and Sharp C.W.G. (1975). J. Biol. Chem. 250:1354

Woods S. and Porte D. Jr. (1974). Physiol. Rev. 54:598

Yen S.-S., Siler T.M. and DeVane G.W. (1974). N. Engl. J. Med. 299:935