sive mortality in primarily rural counties in the North Central region (Minnesota, the Dakotas, Michigan, and Wisconsin). Concentrated in these areas are people of Russian, Austrian, Scandinavian, and German descent. In fact, the 306 counties with the highest rates (highest decile) have three times as many first and second generation Finns, Austrians, and Russians as expected, and 40 to 60 percent more Norwegians, Swedes, and Germans than expected on the basis of the national percentages for these ethnic groups (*12*). The possibility that these migrant groups are prone to stomach cancer is compatible with the high incidence of this tumor in their countries of origin (*13, 14*). The smaller cluster in New Mexico and Colorado seems consistent with reports of elevated stomach cancer rates among Spanish-Americans in this area (*15*). Thus, although urbanization and socioeconomic factors affect mortality from stomach cancer, ethnicity seems to be the major determinant of geographic variation within the United States.

A color atlas of U.S. cancer mortality by county for 35 cancer types has recently been published (*16*). For various cancers, the maps reveal a surprising number of clusters or "hot spots." In these areas, physicians, public health officials, county medical societies, occupational health groups, and others concerned with cancer may help to identify previously unrecognized causes of cancer and plan programs in cancer control.

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Somatostatin: Abundance of Immunoreactive Hormone in Rat Stomach and Pancreas

Abstract. *Growth hormone release–inhibiting hormone (somatostatin), a hypothalamic peptide that inhibits the release of growth hormone and also the secretion of insulin, glucagon, and gastrin, was found in the rat stomach and pancreas in a concentration similar to that in the hypothalamus, as measured by radioimmunoassay. Somatostatin was also found in the duodenum and jejunum, but in a smaller concentration. Gel filtration of the extracts of the pancreas and stomach on Sephadex G-25 yielded two immunoreactive peaks, one corresponding in each case to the somatostatin tetradecapeptide. The hormone was not detected in other viscera or the ovaries. The results imply that somatostatin may be synthesized in the pancreas and the stomach in addition to the brain, and may be involved in local regulatory mechanisms for pancreatic and gastric secretion as well as secretion of growth hormone.*

Growth hormone release–inhibiting hormone (somatostatin) was isolated from ovine hypothalamic tissue, and the structure was characterized as H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH (*1*) by Brazeau *et al.* (*2*). We found a similar peptide in porcine hypothalami (*3*). Both natural and synthetic somatostatin inhibit not only the release of growth hormone (GH) from the pituitary in vitro and in vivo, but also the release of thyrotropin and, in some cases, of prolactin (*4*). Furthermore, somatostatin suppresses the release of insulin, glucagon (*5*), and gastrin (*6*) by direct action on their respective secretory cells. However, whether the inhibitory effect of somatostatin on these nonpituitary cells is a true physiologic action or a reaction caused by pharmacologic doses of the tetradecapeptide remains obscure.

As judged from the extremely low level of other hypothalamic hormones, such as luteinizing hormone–releasing hormone, in the peripheral blood (*7*) and the requirement of a fairly large dose of somatostatin to suppress insulin, glucagon, and gastrin secretion (*5, 6*), it would be reasonable to assume that the hypothalamus is not the only source of somatostatin if this hormone is involved in the regulation of gastric and pancreatic secretion under physiologic conditions. Like substance P (a kinin-like undecapeptide), somatostatin may be present in gastrointestinal organs as well as in the brain, and it may play a physiologic role as both a hypophysiotrophic and gastrointestinal regulatory hormone. Accordingly, we have examined organs other than the brain for somatostatin content.

We have prepared an antiserum to synthetic somatostatin and developed a radioimmunoassay for the hormone (*8*). Since somatostatin lacks tyrosine and histidine, which can be iodinated, [Tyr^1]somatostatin was synthesized, labeled with ^{125}I, and used in the assay system. The binding of [^{125}I-Tyr^1]somatostatin with the antiserum was inhibited by the presence of unlabeled somatostatin in a dose-related manner in a range from 8 to 512 pg.

The radioimmunoassay system appeared to be specific, as judged by the failure of various hormones to inhibit binding of the tracer with the antiserum. The hormones tested included thyrotropin-releasing hormone (Abbott); luteinizing hormone–releasing hormone (Sankyo); melanophore-stimulating hormone release–inhibiting hormone (Pro-Leu-Gly-NH_2) (*9*); a decapeptide formerly proposed as a GH-releasing hormone (Val-His-Leu-Ser-Ala-Glu-Glu-Lys-Glu-Ala) (Merck) (*10*); adrenocorticotrophic hormone (Cortisyn, Organon); arginine vasopressin; oxytocin (Syntocinon, Sandoz); ovine luteinizing hormone (H. Papkoff); rat follicle-stimulating hormone [rat FSH-I-1, National Institute of Arthritis, Metabolism, and Digestive Diseases (NIAMDD)]; rat GH (rat GH-I-1, NIAMDD); human GH (HS 1216C); luteinizing hormone (LER 960); follicle-stimulating hormone (LER 1366); thyrotropin [National Institutes of Health (NIH)]; bovine thyrotropin (TSH-B5, NIH); pig glucagon (Lilly); insulin (Iletin, Lilly); human gastrin 1 (Imperial Chemical Industries); substance P (N. Yanaihara); and the COOH-terminal fragment of the β chain of insulin [H-Glu-Arg-Gly-

Phe-Phe-Tyr-Thr-Pro-Lys (formyl)-Thr-OH] (N. Yanaihara).

Glucagon has a four-amino-acid sequence common with somatostatin, and the last two compounds in the list above contain Phe-Phe, as does somatostatin. On the other hand, linear somatostatin and the ring portion of the hormone (des-Ala[1], des-Gly[2]-somatostatin) gave 17 and 67 percent cross-reaction, respectively, in this radioimmunoassay system (*8*). The assay system was used for determining the somatostatin content of various regions of the rat brain (*11*), and the results were in agreement with those obtained by bioassays (*12*). This assay was then employed to determine somatostatin content of the various organs examined in the present study.

Unanesthetized adult female rats (Charles River, DC strain) were decapitated, and the lungs, heart, thymus, stomach, duodenum, jejunum (upper portion), pancreas, liver, spleen, kidneys, ovaries, and adrenals were removed and extracted with 2*N* acetic acid (*13*). The extracts were heated to boiling, centrifuged at 12,000 rev/min, and lyophilized (*13*). The residues were dissolved in 0.1 percent gelatin in phosphate-buffered saline containing 0.025*M* ethylenediaminetetraacetic acid, *p*H 7.0, and assayed for immunoreactive somatostatin. The protein in each sample was determined by the Lowry method (*14*). Table 1 shows the somatostatin concentration per microgram of protein and the total amounts in these organs. Values for the pancreas, stomach, duodenum, and jejunum are shown; the hormone was not detected in the other organs examined. The concentration of somatostatin in the pancreas was of the same order as that in the arcuate nucleus of the rat hypothalamus, which showed the highest concentration for any hypothalamic region other than the median eminence (*11*). The total amount of somatostatin in either the pancreas or the stomach was considerably greater than that in the hypothalamus. Assay of extracts of the pyloric antrum, fundus, and cardiac portions of the stomach and of the upper and lower portions of the duodenum (Table 1) revealed that the concentrations in the fundus and pyloric regions of the stomach were about the same. No somatostatin was detected in the cardiac portion of the stomach. The acid-secreting cells of the stomach are located mainly in the fundus and the gastrin-secreting cells are in the pyloric region; somatostatin suppresses the secretion of gastrin and reduces the acidity of the gastric juice (*6*). Both the duodenum and jejunum also contained immunoreactive somatostatin, but the concentrations were much smaller than in the pancreas and stomach. The ileum and large intestine were not examined.

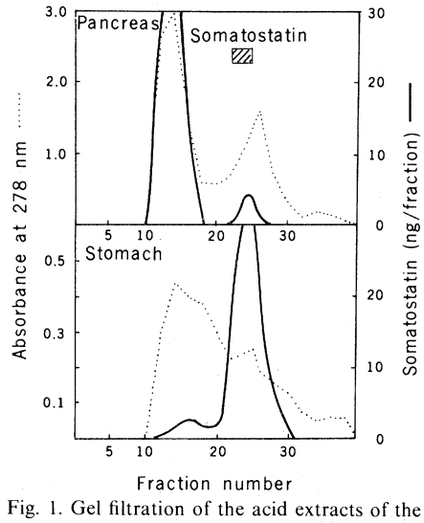

Fig. 1. Gel filtration of the acid extracts of the rat pancreas and stomach on Sephadex G-25 column (2 by 50 cm) in 0.2*M* acetic acid. The fraction volume was 4 ml. The extract of pancreas yielded two peaks of immunoreactive somatostatin, one eluted in the void volume and another in the fractions corresponding to synthetic somatostatin tetradecapeptide; this result suggests the presence of big and small somatostatin. The immunoreactive material of the stomach extract was eluted mainly in the fractions corresponding to small somatostatin. The cross-hatched bar indicates the position of synthetic somatostatin tetradecapeptide.

The acid extracts of the stomach and pancreas were purified on a Sephadex G-25 column and eluted with 0.2*N* acetic acid. Each fraction was assayed for immunoreactive somatostatin. Two immunoreactive peaks were eluted, one in the void volume and another in the fractions corresponding to the somatostatin tetradecapeptide, a result suggesting the presence of a big and small somatostatin (Fig. 1). The pancreas contained two peaks, but most of the somatostatin consisted of the big form. On the other hand, the stomach contained mainly the small form. Both big and small somatostatin, as well as the crude extracts of pancreas and stomach, displaced the tracer in a manner parallel to that of synthetic somatostatin, which indicates that these gastric and pancreatic somatostatin-like substances are immunologically indistinguishable from the somatostatin tetradecapeptide. Porcine hypothalamus was also found to contain three types of immunoreactive somatostatin with different molecular weights (*3*). It is not known whether big somatostatin is a prohormone or the physiologic hormone itself.

A recent immunohistochemical study with the same somatostatin antiserum used in the present experiment showed that hormone immunoreactivity was present in certain islet cells of guinea pig and rat pancreas (*15*). The distribution patterns of the somatostatin-positive cells indicate that these may belong to the α-cell system, known to produce glucagon. However, this immunoreactivity is not thought to be due to glucagon, since no cross-reaction with glucagon was demonstrated in the assay system for somatostatin.

The high concentrations of somatostatin in the stomach and pancreas, as well as the immunohistochemical study, suggest that these organs may synthesize somatostatin or a similar substance that might play a role in the regulation of the secretory activities of these organs, but further studies are

Table 1. Somatostatin content in various rat organs. Four or five animals were used for determinations of mean wet organ weight. Protein concentration was determined with bovine serum albumin as standard.

Organ	Mean wet weight (mg)	Somatostatin (picograms per microgram of protein)	Somatostatin (nanograms per organ)
Heart	775	<0.026	
Lung	1000	<0.001	
Thymus	350	<0.016	
Liver	8035	<0.026	
Pancreas	775	33.8	110.9
Stomach	1175	11.7	199.0
Spleen	575	<0.016	
Duodenum	500	1.6	12.8
Jejunum (upper portion)	1200	1.6	36.3
Kidneys (two)	1750	<0.02	
Adrenals (two)	106	<0.024	
Ovaries (two)	130	<0.022	
Stomach			
Cardiac portion	163	<0.01	
Fundus	477	9.5	221.4
Pyloric antrum	204	9.7	86.9
Duodenum			
Upper portion	144	1.6	4.8
Lower portion	145	1.3	3.1

needed for confirmation of this possibility. We think it unlikely that the somatostatin in the upper gastrointestinal tract and pancreas is of hypothalamic origin because of the large quantities found, but this possibility has not been excluded.

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References and Notes

1. Amino acid abbreviations are as follows: Ala, alanine; Gly, glycine; Cys, cysteine, Lys, lysine; Asn, asparagine; Phe, phenylalanine; Trp, tryptophan, Thr, threonine; Ser, serine; Glu, glutamic acid; Arg, arginine; Tyr, tyrosine; Pro, proline; Leu, leucine; Val, valine; and His, histidine.
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Diphenylhydantoin: Action of a Common Anticonvulsant on Bursting Pacemaker Cells in *Aplysia*

Abstract. *A commonly used anticonvulsant, diphenylhydantoin (Dilantin), decreases the bursting pacemaker activity in certain cells of* Aplysia. *Dilantin decreases this bursting activity whether it is endogenous to the cell or induced by a convulsant agent. The sodium-dependent negative resistance characteristic which is essential for bursting behavior is reduced in the presence of Dilantin.*

Although the anticonvulsant action of diphenylhydantoin (Dilantin) has been studied extensively for several years (*1*), the mechanisms involved remain somewhat obscure. Several theories have been proposed to explain the action of Dilantin. One hypothesis has been that Dilantin increases the adenosine triphosphatase–dependent Na-K active transport (*1, 2*). This theory, however, has been brought into serious question by recent data on the crayfish stretch receptor and other preparations (*3, 4*). Other studies, instead, have indicated that Dilantin decreases the downhill flow of sodium ions during the action potential (*5*) and the sodium-dependent excitatory postsynaptic potentials (EPSP's) by a postsynaptic mechanism (*6*).

Recently, considerable information has become available on the mechanisms underlying bursting properties in the bursting pacemaker cells of *Aplysia* (*7, 8*). In a normal bursting pacemaker cell, a region of negative slope resistance (*8*) exists in the steady-state current-voltage (*I-V*) curve in the range of the potential oscillations. The negative resistance characteristic (NRC) has been shown to be due to a regenerative inward sodium current (*9*). The hyperpolarizing phase of the oscillation is produced by an outward potassium current which is activated during the depolarizing

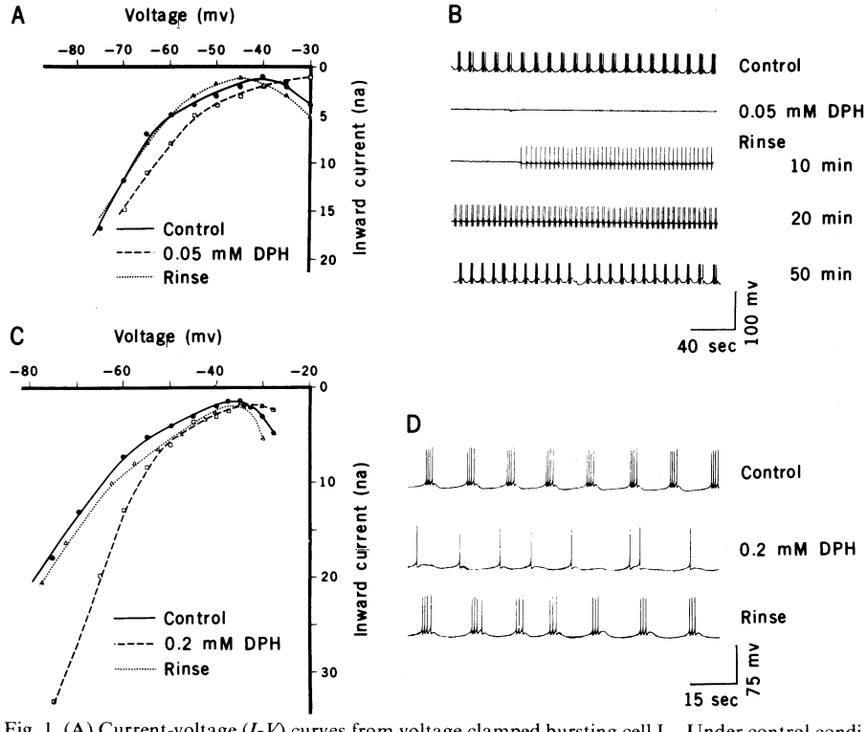

Fig. 1. (A) Current-voltage (*I-V*) curves from voltage clamped bursting cell L_6. Under control conditions there is a region of negative slope resistance in the *I-V* curve in the range of the potential oscillation. After 30 minutes of perfusion with 0.05 m*M* Dilantin this NRC has disappeared along with the bursting activity. The region of negative resistance returns with rinse. (B) Effects of Dilantin on bursting pacemaker cell. Cell L_6 is bursting regularly during control. After 30 minutes of perfusion with 0.05 m*M* Dilantin bursting activity has disappeared. The cell slowly returns to control condition after 10, 20, and 50 minutes of rinse with normal seawater. (The true amplitude of the action potentials cannot be taken from this figure because the spikes are being clipped by the pen recorder.) (C) Current-voltage curves from voltage clamped bursting cell L_6. The bursting decreases but does not fully disappear after more than 60 minutes of perfusion with 0.2 m*M* Dilantin. The NRC in the *I-V* curve decreases but is still present with Dilantin. (D) Dilantin (0.2 m*M*) is applied to cell L_6 from another ganglion. The bursting pattern is nearly abolished after 60 minutes of perfusion. The cell, however, is still firing spontaneously but at irregular intervals. Rinsing returns the cell to the control level of activity.