and not well contrasted, often with several unspecific labelled structures (vessels, blood cells, melanin containing cells). The second step ($NaOH - H_2O_2$) allows the unmasking of the antigenic sites. The optimum time for $KMnO_4$ treatment was generally 20 min for thick Vibratome sections and also for deparaffinized sections. The same treatment can be applied on mounted cryostat sections (no longer than 20 min). Bleaching by Pal's solution for 2 min is often long enough to obtain 'white' sections. The time can be prolonged if necessary. For all tested antibodies except anti-Enk, this technique improves the immunostaining. These results confirm that these treatments are so efficient that they should be employed routinely when overfixed or fixed tissues are used in histochemical procedures.

1 Bouras, C., Magistretti, P. J., and Morrison, J. H., Human Neurobiol. *5* (1986) 213.
2 Perry, E. K., and Perry R. H., Life Sci. *33* (1983) 1733.
3 Rossor, M. N., in: Progress in Brain Research, vol. 65, p. 167. Elsevier Sci. Publ. 1986.
4 Rossor, M., Emson, P., Dawbarn, D., Dockray, G., Mountjoy, C., and Roth, M., in: Neuropeptides in Neurologic and Psychiatric Disease, p. 259. Raven Press, New York 1986.

0014-4754/89/020159-03$1.50 + 0.20/0

The hypothalamic somatostatinergic pathways mediate feeding behavior in the rat [1]

L. T. Ho, Y. F. Chern* and M. T. Lin**

*Department of Internal Medicine, Veterans General Hospital, Taipei (Taiwan), *Department of Physiology, China Medical College, Taichung (Taiwan), and **Department of Physiology, National Cheng Kung University Medical College, Tainan (Taiwan)*
Received 10 August 1988; accepted 25 October 1988

Summary. The level of somatostatin in the hypothalamus was higher in satiated rats than in hungry rats. Elevating hypothalamic somatostatin levels by administering somatostatin into the hypothalamus produced a decrease in food intake, whereas lowering hypothalamic somatostatin levels by administering cysteamine into the peritoneal cavity produced an increase in food intake in rats.
Key words. Hypothalamus; somatostatin; anorexia; food intake; starvation; cysteamine.

Somatostatins (SS) constitute a family of related molecules including the originally identified SS (designated SS-14), SS-28 and still larger forms [2, 3]. SS-like immunoreactivity has been identified in brain regions outside those involved in the regulation of growth hormone release, which suggests that these peptides may function as a neurotransmitter within the central nervous system [4, 5]. Concerning ingestive behavior, many investigators [6, 7] suggested that SS may play a physiological role in control of food intake. For example, Aponte et al. [6] reported that intracerebroventricular infusion of SS resulted in a reduction in food intake in fed rats. Our recent results also demonstrated that the hypothalamus is the most sensitive site for SS-induced anorexia in rats [7]. Here, we have attempted to assess the SS levels of different brain regions both in satiated rats and in hungry rats. In addition, we assessed the effects on food intake in rats of elevating hypothalamic SS-levels by administering SS-14 into the hypothalamus, or depleting hypothalamic SS-levels by administering cysteamine into the peritoneal cavity. Such experiments could indicate whether feeding behavior might be related to the SS-levels in the rat hypothalamus.

Materials and methods. Experiments were performed on male Sprague-Dawley rats weighing between 270 and 310 g. The animals were fed with a dry powder chow that is commonly used for chickens (Taiwan Sugar Co.). They were housed individually in wire-mesh cages in a room maintained at 22 ± 1.0 °C with a 12 h: 12 h light-dark cycle. For administration of SS-14 (Sigma Chemical Co., Saint Louis, MO, USA) or normal saline into the lateral hypothalamus, a cannula guide tube with trocars was implanted, using the stereotaxic atlas and coordinates of Paxinos and Watson [8], in animals under pentobarbital sodium (60 mg/kg, i.p.) anesthesia. After two self-tapping screws had been attached to the calvarium of the parietal bones, the cannula guide tubes were inserted to the desired depth through the craniotomy holes. They were anchored with dental cement to the cranial surface, which had been scraped clean of perioteum. A period of 2 weeks was allowed to permit the animals to recover from surgery. At the time of injection, the cannula insert was connected to a 10-µl Hamilton microsyringe by PE 10 polyethylene tubing. The volume of injection down each cannula was 1.0 µl. The animals were trained to consume regular meals within a period of 2 h. Food intake, water intake and body weight were measured between 10.00 and 12.00 h each day in a lighted room. Dry powder chow was dispensed from a special spillage-reducing cup and water from a graduated cylinder with spouts.

In the first series of experiments, both the satiated rats (rats that had eaten a full meal) and the hungry rats (rats that had been starved for 24 h) were sacrificed for brain SS assay. Rats were decapitated and the cortex, corpus striatum, hypothalamus, lower brain stem and cervical spinal cord removed. Each brain tissue was homogenized at room temperature for 15 min. For each mg of brain tissues 2 ml of 0.2 N HCl was added for extraction [9]. The mixture was then heated at 95 °C for 12 min, followed by centrifugation at 20,000 × g at 4 °C for 20 min. The supernatant was stored in 1:10 dilutions at − 20 °C until assayed. The radioimmunoassay procedures for SS have been described previously [10].

In the second series of experiments, the effects on food intake of intrahypothalamic administration of normal saline or SS-14, and also of i.p. administration of cysteamine, were assessed in rats. At the end of the experiments, the animals were killed with an overdose of pentobarbital sodium and the cerebral circulation was perfused with 0.9% saline, followed by 10% (v/v) formalin solution. Later, sections of the fixed brain were cut at 40 µm and stained with thionin so that the stereotaxic coordinates of the cannulae could be verified.

Results and discussion. Table 1 contains a summary of the means and standard error values for SS-14 concentration of

Table 1. The somatostatin concentration of different brain regions in satiated rats and in hungry rats

Groups of animals	Somatostatin concentration (ng/mg wet wt)				
	Cortex	Corpus striatum	Hypothalamus	Brain stem	Spinal cord
Hungry rats	62±7	65±10	215±17	89±9	81±11
Satiated rats	67±6	59±8	318±15*	83±7	89±9

The values are means±SEM of 8 rats for each group of animals. * Significantly different from corresponding control values, $p<0.05$ (Student's t-test).

Table 2. Effects of intrahypothalamic administration of normal saline or somatostatin-14, or intraperitoneal administration of cysteamine on both the food intake and the hypothalamic somatostatin content in the rat

Treatment	2-h food intake (g)			Hypothalamic somatostatin content (ng/mg)[c]
	Control	After drug injection	Difference	
0.9% Saline[a]	17.9±1.9(7)	18.4±1.6(7)	0.5±0.1(7)	302±14(7)
Somatostatin-14 1.5 µg[a]	17.2±1.8(8)	2.3±1.1(8)	−14.9±1.2(8)*	614±67(8)*
Cysteamine 60 mg/kg[b]	18.1±1.7(8)	27.5±2.2(8)	9.4±1.3(8)*	201±15(8)*

The values are means±SEM, followed by the numbers of rats in parentheses. [a] The food was presented to the animal about 1 min after an intrahypothalamic dose of normal saline or SS-14. [b] The food was presented to the animal about 30 min after an intraperitoneal dose of cysteamine. [c] At the end of the experiments (or 2 h after the food presentation), the animals were sacrificed for assay of somatostatin in the hypothalamus. * Significantly different from corresponding control values (saline group), $p<0.05$ (Student's t-test).

different brain regions both in satiated rats and in hungry rats. The animals of both groups were given free access to tap water. It can be seen from the table that, compared to the hungry rats, the satiated rats had a higher level of SS-14 in the hypothalamus. However, the SS levels in other brain regions (including cortex, corpus striatum, lower brain stem and cervical spinal cord) of the satiated animals were not different from those of the hungry animals.

Table 2 contains a summary of the means and standard error values for food intake both before and after administration of normal saline, SS-14 or cysteamine in rats. Both the food and the water were presented to the animals either 1 min after an intrahypothalamic dose of saline or SS-14, or 30 min after an intraperitoneal dose of cysteamine. Intrahypothalamic injection of SS-14 produced a reduction in food intake in the rat. On the other hand, i.p. administration of cysteamine led to an increase in food intake. At the end of the experiments, the animals were sacrificed for assay of hypothalamic SS content. The data are summarized in table 2. It was found that exogenous administration of SS-14 into the hypothalamic region elevated hypothalamic SS-levels, whereas cysteamine administration lowered hypothalamic SS-levels.

It has been shown that administration of SS into the rat's hypothalamus caused increased metabolism[11] and hyperglycemia[7]. Alternatively, lowering hypothalamic SS-levels produced by systemic injection of cysteamine caused decreased metabolism in the rat[12]. In addition, according to the review of Morley[13], feeding behavior is related to the levels of glucose, amino acids, fatty acids and other metabolic factors in the blood. Therefore, it might be feasible to design an experiment in which a somatostatin antiserum would be applied intracerebroventricularly, to neutralize the hypothalamic SS-increase after feeding and interrupt possible feedback control mechanisms.

Evidence has accumulated to indicate that other peptides in the central nervous system, such as cholecystokinin[14,15], thyrotropin-releasing hormone[16,17] or bombesin[18] can also suppress feeding in rats. Furthermore, the roles played by the monoamines in the hypothalamus in appetite control have been vigorously investigated in the rat[13,17]. Therefore, it would be worthwhile in future studies to assess the possible monoaminergic mechanisms within the hypothalamus underlying the SS-induced anorexia.

1 This work was supported by grants from the National Science Council (Taipei, Taiwan, Republic of China).

2 Richard, I., and Richter, D., Brain Res. *292* (1984) 93.
3 Benoit, R., Bohlen, P., Ling, N., Briskin, A., Esch, F., Brazeau, P., Ying, S. Y., and Guillemin, R., Proc. natl Acad. Sci. USA *79* (1982) 917.
4 Kobayashi, R. M., Brown, M., and Vale, W., Brain Res. *126* (1977) 584.
5 Sawchenko, P. E., and Swanson, L. M., J. comp. Neurol. *205* (1982) 260.
6 Aponte, G., Leung, P., Gross, D., and Yamada, T., Life Sci. *35* (1984) 741.
7 Lin, M. T., Chen, J. J., and Ho, L. T., Neuroendocrinology *45* (1987) 62.
8 Paxinos, G., and Watson, C., The rat brain, in: Stereotaxic Coordinates. Academic Press, Sydney 1982.
9 Benoit, R., Bohlen, P., Brazeau, P., Ling, N., and Guillemin, R., Endocrinology *107* (1980) 2127.
10 Ho, L. T., Lam, H. C., Wang, J. T., Tang, K. T., Perng, J. C., Liu, Y. F., and Chen, R. L., Chinese J. Physiol. *29* (1986) 91.
11 Lin, M. T., Adv. Biosci. *38* (1982) 229.
12 Brown, M. R., Fisher, L., Mason, R. T., Rivier, J., and Vale, W., Fedn Proc. *44* (1985) 2556.
13 Morley, J. E., Life Sci. *27* (1980) 355.
14 Gibbs, J., Young, R. C., and Smith, G. P., J. comp. Physiol. Psychol. *84* (1973) 488.
15 Tsai, S. H., Passaro, E. Jr, and Lin, M. T., Neuropharmacology *23* (1984) 1351.
16 Suzuki, T., Kohno, H., Sakurada, T., Tadano, T., and Kisara, K., Pharmac. Biochem. Behav. *17* (1982) 249.
17 Shian, L. R., Wu, M. H., Lin, M. T., and Ho, L. T., Pharmacology *30* (1985) 259.
18 Stuckey, J. A., and Gibbs, J., Soc. Neurosci. Abstr. *275* (1981) 4.

0014-4754/89/020161-02$1.50 + 0.20/0