SOMATOSTATIN : ITS INHIBITING EFFECT ON THE RELEASE OF HORMONES AND IgG FROM CLONAL CELLS STRAINS ITS Ca-INFLUX DEPENDENCE

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Summary: In order to investigate the inhibitory effect of somatostatin on the release of various hormones and IgG, several clonal strains or cell lines were used in this study. Results showed that in somatostatin-responsive cells, the entry of Ca^{++} into the cells triggered the releasing response and, further, that somatostatin exhibits its inhibitory effect by blocking the entry of Ca^{++} into the cells.

Somatostatin(1), the growth hormone-releasing inhibiting hormone (GH-RIH), has been shown to inhibit the release not only of GH(2,3,4,5) but also of a number of other hormones in vivo and in vitro. These hormones include glucagon(6,7), insulin(2,8,9), gastrin(10,11), thyroid stimulating hormone(TSH)(12) and prolactin(13). Somatostatin has also been shown to inhibit pancreatic exocrine secretion(14) and pepsin secretion(11). However, the effect of somatostatin on the release of adrenocorticotrophic hormone(ACTH) is still controversial. Some investigators have reported that somatostatin is effective, while other researchers have found somatostatin ineffective in inhibiting the release of ACTH in vivo(15,16). Taminato et al.(17) demonstrated that the inhibitory action may involve a calcium mobilization process. The present study was undertaken to investigate more fully the inhibitory effect of somatostatin on the release of various hormones. Of paticular consideration was whether or not the inhibitory action of somatostatin relates to the calcium mobilization process. Clonal cell strains, which require continuous cell growth, proliferation and organ-specific function(constant hormone production) in the culture

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system were used. The effect of somatostatin on the release of IgG from the clonal cell strain of plasmacytoma was also examined.

Materials and Methods: The following clonal cell strains were used in the present experiment: Neuroblastoma cell(IMR 32 clonal strain), pituitary tumor cells(GH 1, GH 3, and AtT 20 clonal strains), plasmacytoma cell (MOPC-31-C clonal strain), Lydig tumor cell(1-10 clonal strain), adrenal cortex tumor cell(Y-1 clonal strain) obtained from the American Type Culture Collection, Rockville, M.D.. Rat anterior pituitary cell(NRAP-P 1, primary culture) derived from 10-day old rats, human thyroid epitherial cell(NHT 3 cell line) derived from a 5-month-old foetus(18), carcinoid tumor cell (EAtT 1) derived from a human intestinal carcinoid(19), ACTH cell(NRAPA 1 clonal strain) derived from normal rat anterior pituitary cells(20).

The above cell strains and cell lines were cultured in our laboratory. The IMR 32 clonal strain was grown in Ham's F10 medium supplemented with 10% foetal calf serum(Flow laboratory) and with 10% non-essential amino acids. The GH 1, GH 3, AtT 20, 1-10, and EAtT 1 strains were cultured in Ham's F 10 medium supplemented with 2.5 % foetal calf serum and 12.5 % horse serum (Gibco). The MOPC-31-C clonal strain was cultured in Leibovitz's medium L-15. NHAP 1 and NHT 3 cell lines were cultured in Eagle's MEM supplemented with 10 % foetal calf serum(Gibco). All cultures were grown routinely in Falcon dishes(60 x 15 mm, Falcon #3002) using a humidified atomosphere of 5 % O_2, 5 % CO_2, and 90 % air at 37 C. Prior to the experiments, cultures were washed three times with fresh medium and then incubated in somatostatin (0.2, 0.8, and 1.60 ug of somatostatin/ml)-containing medium and somatostatin-free medium(control) for 15 minutes, respectively.

Hormone contents liberated in a 15 min-incubation were determined by the following radioimmunoassay methods after centrifugation(10 min at 1,500 rpm) of conditioned medium. GH was assayed by the method of Ohtsuka et al(21). ACTH was measured by that of Berson and Yallow(22). FSH, LH and testosterone were assayed by the method of Gupta et al(23). Prolactin

Table 1. The effect of somatostatin on the release of various hormones and IgG from the cultures. The amounts (mean ±S.E.,6 dishes) are shown in terms of μg/mg cell protein. The following abbreviations are used, GH:growth hormone, ACTH:adrenocorticotropic hormone, LH:luteinizing hormone, FSH:follicle stimulating hormone, TSH:Thyroid stimulating hormone, IgG:immunoglobulin G.

Strains or cell lines	Secretory hormones and IgG	Dose of somatostatin(μg/ml) non(control)	0.2	0.8	1.6
GH 1	GH[2]	74.2 ± 3.2*	39.6 ± 4.9	12.6 ± 0.9	8.3 ± 2.2
	Prolactin[3]	75.0 ± 4.7	48.5 ± 5.2	22.4 ± 1.6	7.1 ± 1.9
GH 3	GH[2]	98.1 ± 6.4	26.1 ± 3.6	5.5 ± 0.7	4.9 ± 0.5
	Prolactin[3]	84.0 ± 5.3	22.8 ± 2.7	8.9 ± 0.9	6.2 ± 0.4
AtT 20	ACTH[1]	138.0 ± 27.2	112.3 ± 23.5	35.2 ± 4.3	16.0 ± 1.1
EAtT 1	ACTH[1]	96.3 ± 12.1	74.9 ± 8.3	10.5 ± 2.0	6.2 ± 0.7
NRAPA 1	ACTH[1]	72.0 ± 0.7	15.6 ± 2.5	7.8 ± 0.6	5.8 ± 0.8
NRAP-P 1	GH[2]	326.0 ± 25.4	36.7 ± 4.5	15.8 ± 2.6	13.3 ± 0.2
	ACTH[1]	120.3 ± 13.1	52.8 ± 6.1	18.4 ± 2.3	13.0 ± 0.3
	Prolactin[3]	248.4 ± 31.2	84.6 ± 7.5	21.6 ± 2.7	15.0 ± 2.0
	FSH[4]	131.0 ± 17.1	50.2 ± 6.6	7.8 ± 0.2	1.4 ± 0.1
	LH[5]	156.6 ± 19.5	47.8 ± 7.1	11.2 ± 3.1	12.0 ± 1.1
	TSH[6]	13.3 ± 0.7	10.7 ± 1.2	2.5 ± 0.1	n.d.
1-10	Testosterone[7]	74.0 ± 3.1	51.6 ± 6.3	11.7 ± 0.4	3.2 ± 0.2
Y-1	Corticosterone[8]	118.0 ± 27.4	68.9 ± 7.4	21.2 ± 1.9	11.2 ± 2.5
MOPC-31-C	IgG[9]	35.) ± 0.3	27.3 ± 1.8	8.5 ± 0.7	3.1 ± 2.5
IMR 32	Norepinephrine[10]	127.3 ± 20.1	108.2 ± 11.7	34.2 ± 4.5	24.2 ± 0.5
NHT 3	Thyroxine[11]	32.4 ± 3.1	21.3 ± 3.7	7.2 ± 0.6	4.9 ± 0.5

* Mean ± S.E.(6 dishes). All hormones and IgG are shown ng/mg cell protein. 1: In terms of corticotropin A; 2: In terms of NIAMDD-R-GH-RP 1 ; 3: NIAMDD-R-Prol-RP 1; 4: NIAMD-R-FSH-RP 1; 5: NIH-LH-S 17; 6: NIAMD-R-TSH-RP 1; 7: Sigma T 1875; 8: Sigma C2505; 9: Sigma; 10: Sigma A7257; 11: Sigma T2376.

Table 2. The effect of somatostatin on ^{45}Ca content in clonal cell strains. Concentration (mean ± S.E., n=6) are in CPM/mg cell protein. The clonal cell strains were incubated for 15 minutes at 37 C with somatostatin and 0.4 uCi of ^{45}Ca/ml of medium.

Strains	Dose of somatostatin(μg/ml)			
or cell lines	non(control)	0.2	0.8	1.6
GH 1	8253 ± 531*	3151 ± 113	1653 ± 87	82 ± 3.5
GH 3	9346 ± 438	2649 ± 105	1475 ± 91	91 ± 4.3
AtT 20	11642 ± 508	2754 ± 142	1386 ± 112	111 ± 3.2
EAtT 1	9965 ± 362	2864 ± 132	1506 ± 82	86 ± 2.7
NRAPA 1	10820 ± 381	2641 ± 118	1320 ± 77	97 ± 4.5
NRAP-P 1	11429 ± 283	2753 ± 107	1446 ± 73	74 ± 2.1
1-10	10537 ± 428	2284 ± 192	1158 ± 69	95 ± 3.6
Y-1	9764 ± 392	2074 ± 115	1074 ± 82	84 ± 2.4
MOPC-31-C	11431 ± 554	2858 ± 127	1533 ± 90	94 ± 1.1
IMR 32	9243 ± 286	2759 ± 151	1432 ± 112	84 ± 3.5
NHT 3	10868 ± 552	3753 ± 63	1821 ± 64	86 ± 2.7

* Mean ± S.E.(6 dishes). The values are shown in terms of CPM/mg cell protein. The clonal cell strains and cell lines were incubated for 15 minutes at 37 C with various doses of somatostatin and 0.4 μCi of ^{45}Ca/ml in medium.

was assayed by the technique of mallampati and Johnson(24). TSH content was determined by the technique of Kieffer et al(25). Corticosterone was assayed by that of Kowal and Fiedler(26). Thyroxine was measured by Clopra's method(27). Norepinephrine was assayed by the method of Cryer et al(28). IgG was determined by the technique of Mancini et al(29). Ca-influx was measured by Rogas and Taytor's method(30). The hormone content and amount of IgG in the media were calculated in terms of μg of cell culture protein. The cells were digested with 1 N NaOH overnight at 37 C and the total protein content was determined(24).

Results and Discussion: Table 1 shows the mean hormone content and the amount of IgG in control media and somatostatin-containing media. These results indicate that somatostatin was effective in suppressing the spontaneous release of all hormones; GH, ACTH, prolactin, TSH, LH, FSH, norepinephrine, thyroxine, corticosterone and testosterone. Somatostatin

also suppressed the release of IgG from the clonal cell strain of plasmacytoma. The concentration of somatostatin necessary to completely suppress the release of hormones and IgG was approximately 1.60 μg per ml of medium.

Researchers have argued recently that the general mechanism of secretion, including the release of hormones, may be Ca-dependent(31,32,33). It occurred to us that somatostatin may exert its inhibitory effect through the suppression of the Ca-activated process of secretion. With this view in mind, the present experiment was undertaken to observe the effect of somatostatin on Ca-influx in various types of hormone- and IgG-producing clonal cell strains. As shown in Table 2, the spontaneous influx of Ca^{++} in various clonal cell strains was completely suppressed by increasing the somatostatin concentration in the medium from 0.20 to 1.60 μg/ml, a sufficient concentration to suppress the release of hormones and IgG.

These results, suggest that in somatostatin-responsive cells, the entry of Ca^{++} into the cells triggered the releasing response and further, that somatostatin exerts its inhibitory effect by blocking the entry of Ca^{++} into the cells.

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