4 Pereira, M.E.A., Loures, M.A., Villalta, F., and Andrade, A.F.B., J. exp. Med. *152* (1980) 1375.
5 Souto-Padrón, T.C.B., Carvalho, T.U., Chiari, E., and De Souza, W., Acta trop., *41* (1984) 215.
6 De Araújo Jorge, T.C., and De Souza, W., Acta trop. *41* (1984) 17.
7 Nogueira, N., Bianco, C., and Cohn, Z., J. exp. Med. *142* (1975) 224.
8 Confalonieri, A.N., Martin, N.F., Zingales, B., Colli, W., and Lederkremer, R.M., Biochem. Int. *2* (1983) 215.
9 Lederkremer, R.M., Zingales, B., Confalonieri, A.N., Couto, A.S., Martin, N.F., and Colli, W., Biochem. Int., in press (1984).
10 Andrews, N.W., and Colli, W., J. Protozool. *29* (1982) 264.
11 Zingales, B., Martin, N.F., Lederkremer, R.M., and Colli, W., FEBS Lett. *142* (1982) 238.
12 Ledeen, R.W., Yu, R.K., and Eng, L.F., J. Neurochem. *21* (1973) 829.
13 Bonner, W.M., and Stedman, J.D., Analyt. Biochem. *89* (1978) 247.
14 Hodges, L.C., Laine, R., and Chan, S.K., J. biol. Chem. *254* (1979) 8208.
15 Rosenberg, A., and Schengrund, C.L. (eds), Biological Roles of Sialic Acid 1. Plenum Press Publishing Corp., New York 1976.

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Effect of long acting somatostatin-analogue, SMS 201 995, on gut hormone secretion in normal subjects

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Summary. SMS 201 995 is a new long acting analogue of somatostatin. We have investigated its effect on basal and meal stimulated secretion of gut hormones and have shown that after a single s.c. injection of 50 µg it lowers significantly the basal plasma levels of pancreatic polypeptide, secretin, motilin, pancreatic glucagon and insulin, it also effectively suppresses the postprandial release of pancreatic polypeptide, gastrin, secretin, gastric inhibitory peptide, pancreatic glucagon and insulin. Except for the usual brief discomfort of an injection, no symptoms or untoward effects were observed.
Key words. Somatostatin analogue; gut hormones.

Somatostatin (SRIF), a tetradecapeptide initially isolated from the hypothalamus because of its inhibition of growth hormone secretion[1] has subsequently been shown to be present in the D cells throughout the gastrointestinal tract and pancreatic islets[2]. It has widespread actions including the suppression of growth hormone and TSH release from the pituitary[1,3], and of most hormones from the gut and pancreas[2]. It also potently inhibits gastric acid and pepsin secretion, pancreatic exocrine secretion, gut motility and nutrient absorption by mechanisms independent of its inhibition of hormone secretion[2,4].

Many somatostatin analogues have now been synthesized, and several of these have been reported to be more potent, and to have a different spectrum of action to natural somatostatin[5-7]. In the present study we have investigated the actions of a new somatostatin analogue

D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-NH-CH(CH_2OH)CHOHCH_3

(SMS 201 995 Sandoz), a long acting, cystine bridge octapeptide analogue of somatostatin. In vitro and in vivo studies have shown it to be more potent than the native hormone[8]. The present study evaluated the effect of a single s.c. injection of SMS 201 995 on test meal stimulated gut hormone secretion in healthy subjects.

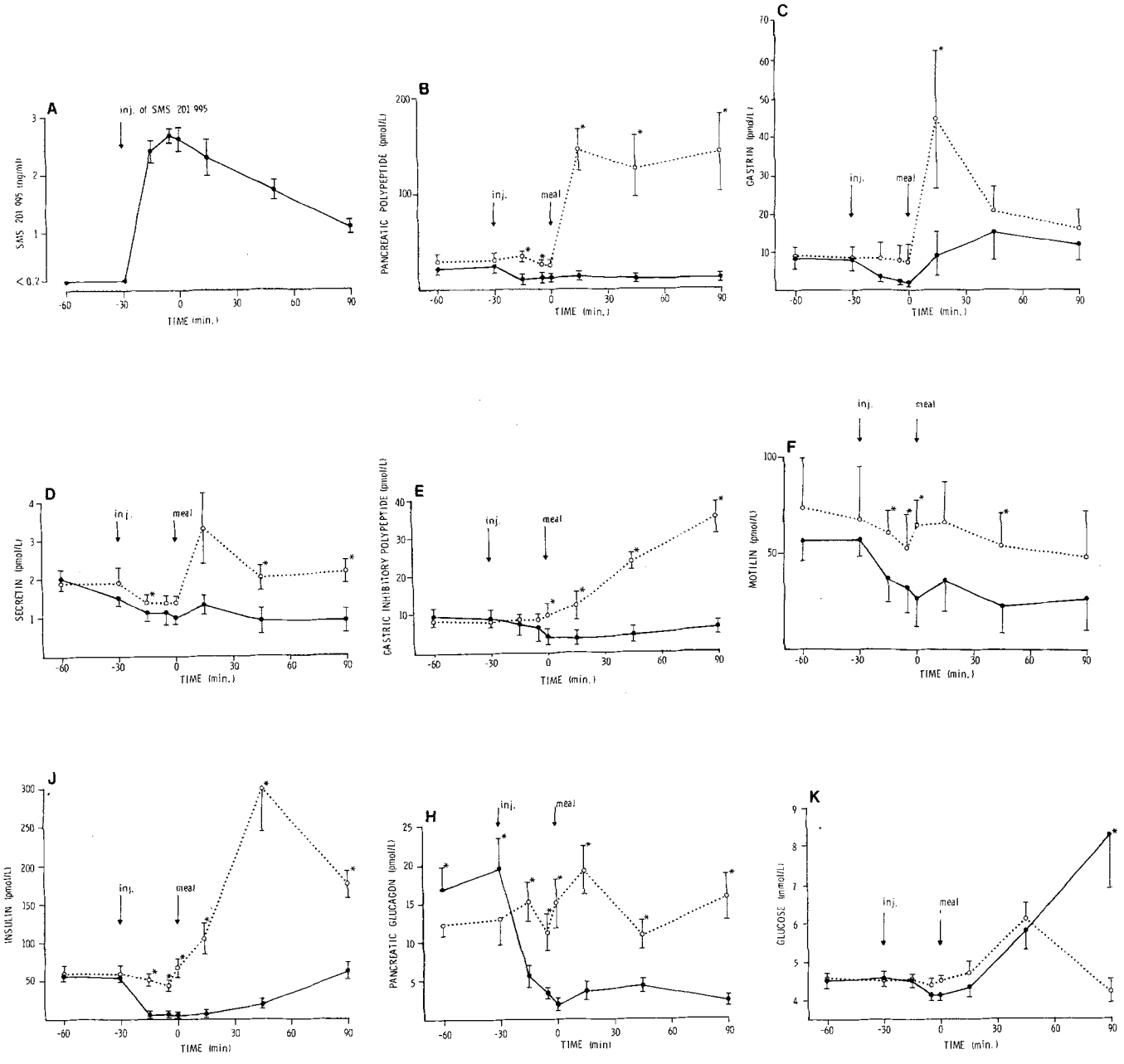
Subjects and methods. Five healthy male subjects (aged 21–27 years) were studied. The test protocol was approved by the Royal Postgraduate Medical School Ethical Committee and informed consent was obtained from all subjects. Following an overnight fast the subjects were given a s.c. injection of SMS 201 995 (50 µg) or placebo in random order. At time 0, 30 min after injection, a standard breakfast was given (60 g white bread, 35 g jam, 10 g butter, 150 ml unsweetened orange juice, 2 eggs; protein 20 g, fat 22 g, carbohydrate 67 g, 530 kcal). Blood samples were taken at −60, −30, −15, −5, 0, +15, +45, and +90 min into heparinized tubes containing 400 KIU aprotinin (Trasylol) per ml blood, centrifuged and the plasma deep frozen within 15 min of sampling. Pulse and blood pressure were measured at the same time intervals. Insulin, pancreatic polypeptide (PP), gastrin, gastric inhibitory polypeptide (GIP), pancreatic glucagon, motilin, cholecystokinin (CCK8), secretin and SMS 201 995 plasma levels were measured using previously described radioimmunoassay[8,9]. The glucose was measured using a standard glucose oxidase method adapted for autoanalyzer. Results are expressed as mean ± SEM. Statistical analysis was carried out using the paired Student t-test. p-value of less than 0.05 was regarded as significant.

Results. The mean peak plasma concentration of SMS 201 995 (2.7 ± 0.12 ng/ml) was reached 15–30 min after its s.c. injection, and then fell slowly over the subsequent 90 min to a value of 1.1 ± 0.1 ng/ml (fig., A).

The effect of SMS 201 995 on gut hormone secretion is shown in the panels of the figure. 15 min after injection there was a significant difference between basal levels of PP for placebo and SMS 201 995 respectively (35.6 ± 5.2 pmoles/l and 10.2 ± 3 pmoles/l, $p < 0.01$). The postprandial release of PP was completely suppressed throughout by SMS 201 995; on placebo the peak concentration 15 min after test meal was 145.2 ± 14.2 pmoles/l, on SMS 201 995 it remained in the same range as the basal values (13 ± 1.5 pmoles/l, $p < 0.005$).

The basal gastrin levels did not differ significantly between SMS and placebo. The postprandial peak on placebo was 44.9 ± 18 pmoles/l, this was significantly suppressed by SMS 201 995 (9.4 ± 5.8 pmoles/l, $p < 0.05$) which was followed by a slight rise (15.3 ± 7.6 pmoles/l) at 45 min.

The postprandial secretin response was significantly suppressed (0.9 ± 0.3 pmoles/l compared with 2.0 ± 0.3 pmoles/l at 45 min, $p < 0.05$). Basal GIP levels were significantly lower 30 min after SMS 201 995 than on placebo (4.0 ± 1.9 pmoles/l v. 9.8 ± 2.3 pmoles/l, $p < 0.05$), and postprandial release of GIP was significantly suppressed throughout. SMS significantly lowered basal motilin levels (27 ± 14 v. 64 ± 12 pmoles/l, $p < 0.01$); there was no postprandial rise in motilin. SMS 201 995 suppressed basal secretion of pancreatic glucagon (1.8 ± 0.6 v. 14.8 ± 3 pmoles/l, $p < 0.01$; 30 min after injection) and inhibited its postprandial release ($p < 0.01$). Basal insulin was markedly suppressed by SMS 201 995 (5.0 ± 0.3 v. 57.6 ± 13 pmoles, 30 min



Mean plasma concentration of circulating SMS 201995, hormonal peptides and glucose after 50 μg s.c. SMS 201995 (closed circles) compared to placebo (open circles) in five subjects after ingestion of a meal. A: SMS 201995, B: pancreatic polypeptide, C: gastrin, D: secretin, E: gastric inhibitory polypeptide, F: motilin, J: insulin, H: pancreatic glucagon, K: glucose. Results are expressed as the mean ± SEM. * represents a statistically significant difference between the values obtained on SMS 201995 and placebo at a given time.

after injection, $p < 0.01$) as was its postprandial release ($p < 0.01$). As would be expected from these figures there was mild hyperglycemia on SMS 201995, 90 min after the test meal: plasma glucose on SMS 201995 was 8.3 ± 1.36 mmoles/l and on placebo 4.2 ± 0.26 mmoles/l ($p < 0.02$).

After the injection of SMS 201995 mild discomfort occurred at the site of the injection, lasting for a few min; no other symptoms or signs or untoward effects were observed. Blood pressure, pulse rate, hematological and biochemical parameters remained unchanged.

Discussion. Pharmacokinetic studies and studies of the time course of inhibition of hormone secretion have demonstrated SRIF to have a very short half-life (1.1–3.0 min) and a rapid plasma disappearence time, necessitating i.v. administration of the hormone[11,12]. Previously synthetized SRIF analogues which were used in clinical trials also exhibited a short duration of action. Thus on stopping i.v. infusion plasma hormone concentrations rapidly return to basal values and, in patients, a rapid return or even rebound of symptoms related to the hormone secretion was observed, e.g. with endocrine pancreatic tumors or carcinoid syndrome[13,14]. Only the SRIF analogue Des $AA^{1,2,4,5,12,13}$ D try[8], given s.c., exhibited, a prolonged action, due to slow uptake from the site of injection which appears to be related to the extremely hydrophobic nature of its structure[14]. In the present study we found that SMS 201995 is quite quickly absorbed from the s.c. tissue as the peak plasma concentration occurred 15–30 min after injection with a subsequent slow decay, and a half-life of 70.6 ± 6.3 min was observed.

In this study SMS 201 995 lowers the basal levels of PP, secretin, motilin, pancreatic glucagon and insulin and also effectively suppresses the postprandial release of PP, gastrin, secretin, GIP, pancreatic glucagon and insulin. Hormone suppression was prolonged. SMS 201 995 was found to have a similar pattern of suppression of gastrointestinal hormones, compared to that previously reported for other analogues[15].

SRIF and its analogues have been shown to suppress hormonal hypersecretion in patients with insulinomas, glucagonomas, gastrinomas and VIPomas[14,15] and to inhibit pentagastrin, noradrenaline and alcohol stimulated flushing in patients with carcinoid syndrome, associated with good symptomatic remission[13,16]. However, a major problem in its therapeutic use is its short half-life. In view of the longer duration of action of SMS 201 995 it may prove to be more useful than previous analogues and valuable as an adjunct to surgery or chemotherapy in the treatment of pancreatic endocrine tumors. Ongoing investigations in our department on eight patients with pancreatic endocrine tumors show that SMS 201 995 administered s.c. twice per day suppresses peptide secretion from these tumors resulting in dramatic remission of symptoms[17].

In conclusion the new somatostatin analogues SMS 201 995 is an effective inhibitor of the release of gastroenteropancreatic hormones. Because of its longer duration of action it is likely to be more useful therapeutically in patients with endocrine pancreatic tumors.

1 Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R., Science *179* (1973) 77.
2 Schusdziarra, V., Horm. Metab. Res. *12* (1980) 563.
3 Siler, T.M., Yen, S.S.C., Vale, W., and Guillemin, R., Clin. Endocr. *38* (1974) 742.
4 Bloom. S.R., Mortimer, C.H., Thorner, M.O., Besser, G.M., Hall, R., Gomez-Pan, A., Roy, V.M., Russel, R.C.G., Coy, D.H., Kastin, A.J., and Schally, A., Lancet *1* (1974) 1106.
5 Rivier, J., Brown, M., and Vale, W., Biochem. biophys. Res. Commun. *65* (1975) 746.
6 Brown, M., Rivier, J., and Vale, W., Science *196* (1977) 1467.
7 Vale, W., Rivier. J., Ling, N., and Brown, N., Metabolism *27*, suppl. 1 (1978) 1391.
8 Bauer, W., Briner, U., Doeptner, W., Haller, R., Huguenin, R., Marbach, P., Petcher, T.J., and Pless, J., Life Sci. *31* (1982) 1133.
9 Bloom, S.R., and Long. R.G., Radioimmunoassay of gut regulatory peptides. W.B. Saunders Co. Ltd, London 1982.
10 Sheppard, M., Shapiro, B., Primstone, B., Kronheim, S., Berelowitz, M., and Gregory, M., J. clin. Endocr. Metab. *43* (1979) 50.
11 Vale, W., Rivier, C., Brazeau, P., and Guillemin, R., Endocrinology *95* (1974) 968.
12 Redding, T.W., and Coy, D.H., 56th Meeting of the American Endocrine Society, Atlanta, 1984 (Abstract 198).
13 Long, R.G., Peters, J.R., Bloom, S.R., Brown, M.R., Vale, W., Rivier, J.E., and Grahame-Smith, D.G., Gut *22* (1981) 549.
14 Long, R.G., Peters, J.R., Bloom, S.R., Brown, M.R., Rivier, J.E., Barnes, A.J., Mallinson, C.N., Vale, W., and Christofides, N.D., Lancet *2* (1979) 764.
15 Adrian, T.E., Barnes, A.J., Long. R.G., O'Shaughnessy, D.J., Brown, M., Rivier, J., Vale, W., Blackburn, A.M., and Bloom, S.R., J. clin. Endocr. Metab. *53* (1981) 675.
16 Frolich, J.C., Bloomgarden, Z.T., Oates, J.A., McGuigon, J.E., and Rabinowitz, D., N. Engl. J. Med. *299* (1978) 1055.
17 Wood, S.M., Kraenzlin, M.E., and Bloom, S.R., Gut, in press (1985).

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Ecdysteroids: possible candidates for the hormone which triggers salivary gland degeneration in the ixodid tick *Amblyomma hebraeum**

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Summary. Following engorgement, female ixodid ticks secrete a 'tick salivary gland degeneration factor' (TSGDF) into the hemolymph. Here we show that the arthropod ecdysteroid hormones, ecdysone and 20-hydroxyecdysone, induce degeneration of tick salivary glands maintained in organ culture. The effective dose range in vitro is 30–300 ng/ml, a range reported to be physiological for this species following repletion. In addition, infusion of 20-hydroxyecdysone in vivo induces salivary gland degeneration. We therefore propose that TSGDF may be an ecdysteroid.
Key words. Ecdysone; 20-hydroxyecdysone; ticks; salivary gland degeneration.

Female ixodid ticks commonly increase their b.wt 100-fold or more by imbibing blood of the mammalian host[3]. The blood meal is hyposmotic to tick body fluids (290 mOsm vs 360 mOsm[4]); thus, osmoregulation during this period is accomplished by the secretion of a copious, slightly hyposmotic saliva back into the host's circulation[4,5]. The nutrient portion of the blood meal is also concentrated as a result of salivary fluid secretion.

Following detachment of the tick from the host, secretory ability of the salivary glands is reduced[6]. This loss of function is due to autolysis[7] and is triggered by a blood-borne factor which we have called 'tick salivary gland degeneration factor' (TSGDF)[6]. Salivary gland degeneration will not proceed in female *Amblyomma hebraeum* unless they have fed beyond a weight of approximately 0.30–0.40 g[8]. Degeneration is blocked by severing the opisthosomal nerves; we have thus suggested that stretch receptors in the abdomen signal the CNS to initiate salivary gland degeneration, once a critical weight has been attained[8]. The majority of females will not feed to the critical weight unless they have mated. However, those virgin ticks which do feed beyond the critical level nevertheless fail to resorb their salivary glands[9]. The latter suggests that, in addition to promoting full engorgement, mating exerts a direct influence on salivary gland degeneration. We recently showed that a chemical factor, distinct from TSGDF, must be transferred from the male to the female during copulation in order for salivary gland degeneration to proceed[8]. The identity of the male factor is, as yet, unknown.