creased up to 7.01 by sucking (table 3). Furthermore, the administration of fatty acids with skim milk increased plasma unbound-bilirubin concentration (table 2). It would appear that the increase of plasma-free fatty acids was one of the causes which increased plasma unbound-bilirubin concentration by sucking in homozygous Gunn rat sucklings. The increase of plasma total bilirubin concentration by starvation in table 1 suggested an increased transfer of bilirubin from tissues to plasma due to the decrease of unbound-bilirubin concentration and a subsequent equilibrium change in bilirubin distribution[4]. No significant change in plasma total bilirubin concentration, however, was observed by milk administration (table 2, except for the case of a mixture of skim milk and fatty acids) in spite of the increase of unbound-bilirubin concentration. It seems possible, therefore, to ascribe the increase of total bilirubin concentration by starvation in table 1 to a decreased rate of hepatic clearance[16]. The reason why total bilirubin concentration increased by the administration of skim milk plus fatty acids remains unexplained.

Starinsky and Shafrir[17], and Thiessen et al.[15] suggested that plasma-free fatty acids in human newborns did not seem to reach concentrations effective for increase of unbound-bilirubin concentration. The discrepancy might be caused by the difference of species, e.g., lipid composition of milk. Since plasma-free fatty acid concentration increases transiently after lipid administration[13], the inconsistency might also derive from the difference in intervals between the last sucking and blood sampling.

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Inhibition in the rat of gastric acid secretion and cyclic AMP analogs accumulation in vitro by somatostatin

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Summary. Various somatostatin (S) analogs exhibited similar degree and similar, or shorter, duration of inhibition of basal gastric acid secretion as S in the unanesthetized rat and similar, or less, inhibition of the cyclic AMP accumulation induced by prostaglandin E_2 in the rat anterior pituitary in vitro. With the analogs examined, the gastrointestinal and pituitary receptors appear to exhibit generally similar recognition specificity with the differences within the gastrointestinal activities reflecting duration of availability rather than receptor affinity.

Somatostatin* inhibits basal gastric acid secretion in the rat[2] and pentagastrin-induced gastric acid secretion in the rat[2], dog[3] and cat[4]. Somatostatin decreases fasting plasma gastrin levels in normal subjects and prevents gastrin responses to a food stimulus in patients[5]. The elevated gastrin levels of patients with pernicious anemia and Zollinger-Ellison syndrome are decreased by somatostatin[5]. Somatostatin inhibits gastric acid secretion in normal subjects and in patients with Zollinger-Ellison syndrome[5].

Somatostatin also inhibits the release of various other hormones and the accumulation of cyclic AMP, which appears to be a mediator in the hormonal release. The basal[6,7] and prostaglandin-induced increase[6-8] in the cyclic AMP accumulation in the rat anterior pituitary in vitro are antagonized by somatostatin.

The structure-activity relationships for various somatostatin analogs have been reported with regard to their abilities to inhibit basal gastric acid secretion in the rat[2] and prostaglandin-induced cyclic AMP in the rat anterior pituitary in vitro[8]. The present studies with additional analogs further define these relationships.

Materials and methods. For the determination of the basal gastric acid secretion, male albino rats (Canadian Breeding Laboratories; 160–220 g) were chronically-implanted with two gastric cannulas as previously described[9]. The animals were fasted, stomachs perfused, and the acid in the gastric perfusate determined as previously described[2].

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

The method employed in the determination of the accumulation of cyclic AMP in the anterior pituitary was based upon that reported previously[10,11] and was carried out as previously described[8]. After a 60 min incubation, the incubation medium was replaced by fresh buffer and glucose, and the somatostatin analog was added; after a further 2 min incubation, 20 µl vehicle or PGE_2 (1×10^{-6} *M*) was added for the incubation period of 4 min. The vehicle employed for the PGE_2 was 0.1 ml ethanol, 0.1 ml sodium carbonate (1.8 mg/ml) and 0.8 ml water. For the assay of the cyclic AMP, the cyclic AMP was extracted from the tissues with 5% trichloracetic acid and measured by the receptor-binding assay[12] utilizing

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10 μg of protein of the inhibitor (P-5636, Sigma Chemical Co.) and 1 μg of receptor preparation (P-5511, Sigma Chemical Co.). [8-^{3}H]-Cyclic AMP (Schwarz-Mann Co.; 28 Ci/mole) was employed at a final concentration of 40 n*M*. Unlabelled cyclic AMP was obtained from Calbiochem Co. Assays were performed in triplicate. After filtration, the filters were dried and 10 ml toluene-phosphor [0.4% 2,5-diphenyloxazole and 0.005% 1,4-*bis*(5-phenyloxazole-2-yl)benzene] employed for scintillation counting.

In the calculation of the acid output, the area under the curve was determined. The data were analyzed statistically utilizing Student's *t*-test. Somatostatin and the somatostatin analogs were prepared by Drs H. U. IMMER, K. SESTANJ, V. R. NELSON, N. A. ABRAHAM and M. GÖTZ[8,13,14]. PGE_2 was obtained from Ono Pharmaceutical Co.

Results. Somatostatin at 0.6 μmole/kg, s.c., decreased the basal gastric acid output to a rate of about 10 μEq/15 min (from about 60 μEq/15 min) for a period of 15 min with a maximal inhibition occurring by 30 min. The rate of output returned to the initial rate during a subsequent 45 min period (Fig. A). At 0.2 μmole/kg the duration of

Table 1. Effect of somatostatin analogs on basal gastric acid secretion

Compound	Dose (μmole/kg, s.c.)	No. of animals	Duration of inhibitory effect (min ± S.E.)	Inhibition of total acid output (percent ± S.E.)
S[a]	0.6	20	86 ± 7	71 ± 2
Di-Gly-S	0.6	11	79 ± 9	71 ± 2
Leu-di-Gly-S	0.6	11	86 ± 9	66 ± 3
Leu-di-Gly[descarboxy^{14}]-S	0.6	8	41 ± 6[b]	72 ± 3
S	0.6	7	90 ± 7	59 ± 2
[Des-Ala^{1}Gly^2][desamino^3] [descarboxy^{14}]-S	0.6	8	39 ± 3[b]	60 ± 5
S	0.6	13	74 ± 6	76 ± 4
	0.2	8	53 ± 6[c]	75 ± 3
[Des-Ala^{1}Gly^2][acetyl^3] [descarboxy^{14}]-S	0.6	7	43 ± 5[b]	72 ± 6

[a] Somatostatin, [b] $p<0.001$, [c] $p<0.05$ vs somatostatin-treated (0.6 μmole/kg)

Table 2. Effect of somatostatin analogs on PGE_2-induced accumulation of cyclic AMP in anterior pituitary in vitro

Addition	Final concentration ($M \times 10^{-7}$)	N	pmoles Cyclic AMP/anterior pituitary ± S. E.	Percent inhibition
None		16	28.3 ± 3.2	
S[a]	1.0	16	15.5 ± 1.3[b]	45
	0.2	16	24.5 ± 1.9	13
di-Gly-S	1.0	5	17.9 ± 2.4[c]	37
	0.2	6	26.2 ± 2.8	7
tri-Gly-S	1.0	5	16.0 ± 3.1[c]	43
	0.2	5	22.5 ± 3.6	20
[Des-Ala^{1}Gly^2][acetyl^3] [descarboxy^{14}]-S	1.0	5	12.8 ± 1.1[b]	55
	0.2	5	27.3 ± 5.9	4
None		8	68.0 ± 4.1	
S	5.0	8	38.3 ± 2.4[b]	44
	1.0	8	43.0 ± 2.9[b]	37
	0.2	8	61.4 ± 4.8	10
[Des-Ala^{1}Gly^2][desamino^3] [descarboxy^{14}]-S	5.0	7	29.3 ± 3.5[b]	57
	1.0	8	35.4 ± 2.6[b]	48
	0.2	7	55.8 ± 2.8[b]	18
None		6	55.2 ± 8.0	
[Des-Ala^{1}Gly^2][desamino^3] [descarboxy^{14}]-S	1.0	6	25.8 ± 3.6[c]	53
	0.2	5	48.8 ± 6.2	12
[Des-Ala^{1}Gly^2][desamino^3] [descarboxy^{14}]-retroenantio-S	1000	6	40.2 ± 3.1	27
	10	5	49.2 ± 4.3	11
None		5	55.4 ± 5.4	
S	1.0	5	34.2 ± 3.4[d]	38
	0.2	5	43.7 ± 3.7	21
[Desamino^1][descarboxy^{14}] retro-enantio-S	10,000	5	36.1 ± 4.4[c]	35
	1000	5	52.5 ± 6.0	5

N = number of groups of hemipituitaries in each determination; 3 pituitary halves per group. [a] Somatostatin, [b] $p< 0.001$, [c] $p < 0.05$, [d] $p < 0.01$.

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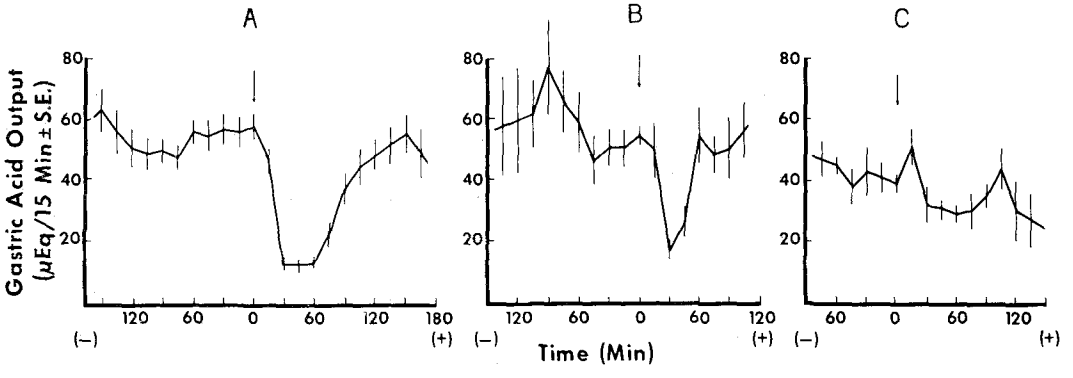

Effect of somatostatin analogs on basal gastric acid secretion. The test compounds (0.6 μmole/kg, s.c., in 0.5 ml): *A* Somatostatin; *B* di-Gly-somatostatin; *C* Leu-di-Gly-descarboxy^{14}-somatostatin were administered at time shown by arrow designation. There were 20, 11 and 7 animals employed, respectively.

inhibition was shorter (53 min vs 74 min) with the other characteristics being similar (Table 1).

Various analogs of somatostatin were examined under similar conditions at 0.6 μmole/kg, s.c. Di-Gly-S and Leu-di-Gly-S exhibited activities, with respect to the duration and degree of inhibition, similar to somatostatin (86 min; 71%) (Table 1). Leu-di-Gly-[descarboxy^{14}]-S was shorter in duration of action (41 min) while being as effective in degree of inhibition. [Des-Ala^{1}Gly^2][desamino^3][descarboxy^{14}]-S was shorter in duration of action than somatostatin (39 min vs 90 min) but exhibited a similar degree of inhibition (60% vs 59%); similar activities were exhibited by [Des-Ala^{1}Gly^2][acetyl^3][descarboxy^{14}]-S (43 min vs 74 min; 72% vs 75%). Each of the analogs was similar to somatostatin with respect to the maximal inhibitory effect and time in reaching this effect with the difference being that the less active analog exhibited a more rapid rate of return to control secretory levels than somatostatin (Fig. B, C). The less active analogs exhibited about one-half the duration of action as somatostatin.

Somatostatin at 1×10^{-7} *M* inhibited by about 50% the adenohypophyseal cyclic AMP accumulation induced by PGE_2 (Table 2). At the lower concentration of 0.2×10^{-7} *M*, somatostatin did not cause a significant inhibition. Similar inhibitory activities at these concentrations were exhibited by the di-Gly-, tri-Gly- and [Des-Ala^{1}Gly^2][acetyl^3][descarboxy^{14}]-S and [Des-Ala^{1}Gly^2][desamino^3][descarboxy^{14}]-S analogs. The retro-enantio-derivative of the latter analog was ineffective even at 1×10^{-4} *M* as was [desamino^1][descarboxy^{14}]-retro-enantio-S; the latter analog exhibited a significant decrease at 1×10^{-3} *M*.

Discussion. The findings show that for inhibition of basal gastric acid secretion elongation of somatostatin at the amine terminal by a di-Gly or Leu-di-Gly does not prolong the action. The terminal carboxyl group in the elongated analog, i.e., Leu-di-Gly-S, is of importance for duration of action since the analog lacking this group exhibited a shorter duration of activity. This latter observation is consistent with the findings under similar conditions that the somatostatin analog lacking the terminal carboxyl group and also the somatostatin analog lacking the terminal carboxyl and amino groups were also shorter in duration of action than somatostatin[2]. The ring portion of somatostatin, i.e., the ring peptide chain containing the 2 linked cysteine moieties, is sufficient for maximal inhibitory activity, but not for the duration of action, as this analog exhibited a shorter duration of action; the duration of action is not enhanced to that of somatostatin by the presence of an acetyl group on the amine terminal.

The results obtained indicate that for inhibition of cyclic AMP accumulation elongation of somatostatin at the amine terminal by a di-Gly or tri-Gly does not decrease the inhibitory activity; further, the activity is not increased under the conditions examined. In similar activity studies the terminal amino group was also shown to be unessential for the inhibitory activity[8]. The ring portion of somatostatin is sufficient for maximal activity. The nature of the amine terminal in the latter type of analog does not appear to be of importance as the presence of an acetyl group does not alter the activity; this is also consistent with the above observations. In the ring portion of somatostatin reversal of the direction of the peptide bond and inversion of all asymmetric centers, i.e., the retroenantio isomer of the ring portion, causes loss of the activity even at much higher concentrations. In accord are the findings that the analog lacking the terminal amino and carboxyl groups is effective[8] whereas the retroenantio isomer is relatively ineffective (present studies).

With the somatostatin analogs examined in the present and previous studies[2,8], it appears that the pituitary and gastrointestinal receptors exhibit similar recognition specificity. Differences in the gastrointestinal activities of the analogs appears to reflect differences in the duration of availability of the analogs rather than receptor affinity.

Pimozide and pyrogen-induced fever in rabbits

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Summary. Pimozide, a selective blocker of DA receptors, partly inhibits but does not suppress either the DA-induced hyperthermia or the LPS-induced fever in rabbits. This suggests that a common DA-related mechanism could be at least partly involved in both responses.

Pimozide, a selective blocker of dopamine (DA) receptors[3] has been reputed to inhibit the hyperthermic effects of apomorphine, amphetamine and L-dopa in rabbits[4-6], and for this reason it was suggested that dopamine was involved in these effects. Since there is no data on the possible interference of pimozide with bacterial pyrogen-induced fever, we investigated the effects of this substance when administered to rabbits by different routes alone and before the injection of either DA or E. coli lipopolysaccharide (LPS).

Materials and methods. The experiments were performed on male mongrel rabbits, with a mean body weight of 2.5 kg. They were kept in a temperature-controlled environment (20° ± 1) for a week and during the whole experiment. All the material used were made pyrogen-free by treating at 180 °C for 3 h. Pimozide was dissolved in 100 μl glacial acetic acid and then diluted to 2.5 ml in the pyrogen-free 0.9% NaCl solution (pH = 3.5). The pyrogen solution (E. coli LPS, DIFCO W.E. Coli 0127 B8) and dopamine (HCl) were also made up in pyrogen-free 0.9% NaCl solution. Injections were made either i.p. or into the lateral ventricle (i.c.v.). For cannulation of the lateral ventricle in rabbits, the method was that described by Hasselblatt and Sproull[7]. The sequence of

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