Effect of HMG on tissue lipids of atherosclerotic rats (mean ± SE)

Lipids	Group I	HMG-treated groups			
		Group II		Group III	
Serum (mg/100 ml)					
Cholesterol	225 ± 9	215 ± 8 (4)[a]	—	140 ± 9 (38)	$p < 0.01$
Triglyceride	265 ± 12	202 ± 10 (24)	$p < 0.05$	159 ± 13 (40)	$p < 0.01$
Phospholipid	300 ± 14	240 ± 8 (20)	$p < 0.05$	225 ± 11 (25)	$p < 0.05$
Liver (mg/g)					
Cholesterol	3.5 ± 0.2	3.2 ± 0.1 (9)		2.5 ± 0.1 (30)	$p < 0.01$
Triglyceride	8.9 ± 0.8	8.1 ± 0.8 (8)		5.6 ± 0.7 (37)	$p < 0.01$
Phospholipid	15.8 ± 1.3	15.9 ± 1.0 —		13.2 ± 1.1 (17)	$p < 0.05$
Aorta (mg/g)					
Cholesterol	2.6 ± 0.2	2.5 ± 0.2 (4)		2.0 ± 0.1 (22)	$p < 0.02$
Triglyceride	6.3 ± 0.3	5.9 ± 0.6 (6)		4.7 ± 0.2 (26)	$p < 0.05$
Phospholipid	10.5 ± 1.2	10.1 ± 1.6 (4)		8.9 ± 2.5 (15)	—
Serum (mg/100 ml)					
β-lipoproteins	175 ± 8	135 ± 6 (23)	$p < 0.05$	110 ± 6 (37)	$p < 0.01$

[a] The values in parenthesis indicate percent change with respect to group I (control values).

produce hyperlipidemic condition in serum and aorta but also formed atheromatous plaque in rats. Except in the case of cholesterol, 25 mg HMG/kg significantly lowers serum triglyceride and phospholipid levels. There was little or no lipid lowering effect of HMG observed in liver and aorta. All serum, liver and aorta lipids except aortic phospholipids were significantly decreased by administration of 50 mg HMG/kg. Serum β-lipoprotein levels were also significantly decreased at both concentrations of HMG.

On gross examination, the animals of group I (control) had visible atheromatous lesions scattered throughout the aorta, but prominently at aortic arch (++++). The atheromatous arterial lesions regressed most effectively in group III rats treated with 50 mg HMG/kg (+). However, the animals of group II had visible lesions much closer to group I (++++). The animals remained active throughout the experimental period. The weights of treated animals were same as that of control animals.

Discussion. The results obtained show that HMG at 50 mg/kg concentration effectively counteracts the lipemic and atherosclerotic response of massive doses of vitamin D_2. Animals receiving 25 mg HMG/kg significantly decreased serum triglyceride and phospholipid levels to the extent of 24 and 20% respectively. Serum β-lipoproteins were also significantly decreased at both concentration of HMG. The level of lipid parameters did not return to normal value (unpublished observation) even with 50 mg HMG/kg. This could be due to short duration of treatment. The serum cholesterol, liver and aorta lipid did not record any significant change on 25 mg HMG/kg treatment. This could be attributed to lesser dose of HMG.

HMG inhibits biosynthesis of cholesterol[13,14]. The maximum decrease in triglyceride and cholesterol levels of HMG treatment could be due to decrease in VLDL triglyceride and cholesterol levels as a significant decrease was observed in serum β-lipoprotein levels.

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Antagonism of Prostaglandin-Induced Cyclic AMP Accumulation in the Rat Anterior Pituitary in vitro by Somatostatin Analogues

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Summary. The PGE_2-induced cyclic AMP accumulation in the rat anterior pituitary in vitro is inhibited by [desamino[1]]-, [desamino[1]] [descarboxy[14]]- and [D-Lys[4]]-somatostatin similarly to somatostatin, while the [descarboxy[14]]-somatostatin exhibits reduced activity; [D-Lys[9]]-somatostatin is ineffective at a higher concentration.

Prostaglandins cause release of growth hormone in the rat[2–5] and bovine[6,7] anterior pituitaries in vitro. In vivo the plasma growth hormone levels are increased by administration of prostaglandin in the rat[8], sheep[9] and man[10]. Cyclic AMP appears to be a mediator in the release as prostaglandins increase both the accumulation of cyclic AMP and the release of growth hormone in the rat[5] and bovine[7] anterior pituitaries in vitro. Somatostatin, a tetradecapeptide (H-Ala-Glys-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH) inhibits the release of immunoreactive growth hormone in vitro and in vivo[11,12].

Recently, this peptide was demonstrated to inhibit the basal increase in cyclic AMP accumulation and the accompanying growth hormone release in the rat anterior pituitary in vitro[13,14]. Somatostatin also antagonizes the prostaglandin-induced increase in the cyclic AMP accumulation[13,14] and the accompanying growth hormone release in the rat anterior pituitary in vitro[14]. The basal, prostaglandin-induced and N^6-monobutyryl cyclic AMP-induced releases of growth hormone from rat anterior pituitary cells in monolayer culture are inhibited by somatostatin[15].

In the present studies, the inhibitory effects of various somatostatin analogues on prostaglandin-induced cyclic AMP accumulation in the rat anterior pituitary in vitro have been determined.

Materials and methods. The syntheses of the analogues utilized in the studies were achieved by following the method established for the preparation of somatostatin[16]. The optical rotations of the analogues ($[\alpha]_D^{22}$, c = 1 in 1% acetic acid) were: [desamino[1]]-somatostatin (−32.5°), [descarboxy[14]]-somatostatin (−26.3°), [desamino[1]][descarboxy[14]]-somatostatin (−27.9°), [D-Lys[4]]-somatostatin (−30.5°) and [D-Lys[9]]-somatostatin (−20.7°). PGE_2 was obtained from ONO Pharmaceutical Co.

The method employed in the determination of the accumulation of cyclic AMP in the anterior pituitary was based upon that described previously[17,18]. Anterior pituitaries from male Sprague Dawley rats (180–200 g)

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Effects of somatostatin analogues on PGE_2-induced accumulation of cyclic AMP in rat anterior pituitary in vitro

Addition	Final concentration ($M \times 10^{-7}$)	pmoles cyclic AMP/ anterior Pituitary ± SE	Inhibition (%)
None		31.6 ± 3.8	
Somatostatin	1.0	16.8 ± 2.4[a]	47
	0.2	25.4 ± 1.9	20
[Desamino[1]]-somatostatin	1.0	14.8 ± 2.3[a]	53
	0.2	20.0 ± 1.6[b]	37
	0.04	26.6 ± 3.8	16
None		37.0 ± 4.0	
Somatostatin	1.0	18.9 ± 2.6[a]	49
	0.2	25.6 ± 2.9[b]	31
[Descarboxy[14]]-somatostatin	5.0	22.1 ± 2.6[b]	40
	1.0	26.3 ± 3.7	29
	0.2	28.4 ± 2.9	23
None		54.3 ± 7.3	
Somatostatin	1.0	26.9 ± 3.5[a]	51
	0.2	32.1 ± 3.5[b]	41
[Desamino[1]]	1.0	26.0 ± 2.4[a]	52
[descarboxy[14]]-somatostatin	0.2	34.7 ± 1.8[b]	36
None		34.8 ± 4.0	
Somatostatin	1.0	17.4 ± 2.9[a]	50
	0.2	27.1 ± 2.0	22
[D-Lys[4]]-somatostatin	1.0	18.6 ± 1.9[a]	47
	0.2	26.2 ± 5.5	25
None		41.4 ± 2.9	
Somatostatin	1.0	15.2 ± 1.5[a]	63
	0.2	24.4 ± 4.1[a]	41
[D-Lys[9]]-somatostatin	5.0	46.0 ± 5.1	
	1.0	41.9 ± 2.7	
	0.2	43.6 ± 3.3	

[a] $p < 0.01$; [b] $p < 0.05$.

were utilized. The experiments were initiated between 08.15–09.15 h. The anterior pituitaries, from which the posterior and intermediary lobes were removed, were separated into identical halves. 3 pituitary halves were used in each group and there were 4–6 groups in each determination.

The tissues were incubated, with shaking, for 60 min at 37 °C in an atmosphere of 5% CO_2–95% O_2 in 1.0 ml of Krebs Ringer bicarbonate buffer containing 11 m*M* D-glucose[17]. The incubation medium was then replaced by fresh buffer and glucose and the somatostatin analogue was added as indicated in the Table. In the present studies, 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% trichloroacetic acid and measured by the receptor-binding assay of GILMAN[19] utilizing 10 µg of protein of the inhibitor 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.

Results and discussion. Somatostatin, 1×10^{-7} *M*, inhibited by 50% the adenohypophyseal cyclic AMP accumulation induced by PGE_2; at the lower level of 0.2×10^{-7} *M* the inhibition was decreased (Table). Similar inhibitory activities at these concentrations were observed with the [desamino[1]]-, [desamino[1]][descarboxy[14]]- and [D-Lys[4]]-somatostatin analogues; the [descarboxy[14]]-somatostatin was effective at 5×10^{-7} *M*. The [D-Lys[9]]-somatostatin analogue did not cause an alteration in the cyclic AMP accumulation even at 5×10^{-7} *M*.

Somatostatin at 1×10^{-7} *M* did not cause any appreciable change in the basal cyclic AMP accumulation nor did any of the above analogues at 5×10^{-7} *M* (e.g., control: 5.7 ± 0.7; somatostatin: 5.0 ± 0.8; D-Lys[4]-somatostatin: 5.2 ± 0.3 pmoles cyclic AMP/anterior pituitary ± SE).

These results indicate that, in regard to the structure-activity relationship of the analogues, the terminal amino group does not appear to be of importance for the inhibitory activity on the PGE_2-induced cyclic AMP accumulation; elimination of the terminal carboxyl group results in an analogue exhibiting reduced activity. Both groups may be eliminated without altering the inhibitory activity. Further, the configuration at the asymmetric center of the lysyl moiety at position 4 is irrelevant since the [D-Lys[4]]-analogue exhibited a similar activity. In contrast, the configuration at the asymmetric center of the lysyl moiety at position 9 is of importance since the [D-Lys[9]]-analogue did not exhibit the inhibitory activity even at a higher level.

Somatostatin exhibits a wide spectrum of suppressor action on hormonal release in various species. In addition to an effect on growth hormone and thyrotropin, somatostatin also shows extrapituitary actions in inhibiting the release of insulin, glucagon and gastrin[20]. In view of the present findings, the effects of the somatostatin analogues on the release of growth hormone, and other hormones, are of interest. In this regard generally similar relative activities as found in the present study have been observed with the analogues with respect to their abilities to inhibit basal gastric acid secretion in the unanesthetized rat[21].

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The Sedative Effects of Nicotinamide on Gerbil Wheel-Running Activity[1]

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Summary. The activity of 5 groups of gerbils was monitored over 22 days. 3 of the groups received daily injections of nicotinamide (125, 250 or 500 mg/kg) and a 4th group received saline. The 5th group was untreated. The results indicated that both the 250 and 500 mg/kg nicotinamide administrations greatly reduced the activity levels of the gerbils.

BEATON et al.[2] have previously reported that the administration of 250 mg/kg nicotinamide to mice resulted in a significant increase in the amount of paradoxical or rapid eye movement sleep. WOOLLEY[3] had observed that nicotinamide administration appeared to induce sedation in mice. This was later confirmed[4] in rats using the Animex motilitymeter, however the dosage used in these studies was 1 g/kg. The present study describes the effects of lower doses of nicotinamide on gerbil activity.

Methods. 25 adult, male gerbils weighing between 55 and 65 g were used. The gerbils were housed singly in a sound-attenuated room which had a reverse 12 h light/dark cycle. The lights were off from 06.00–18.00 h. Each animal was given access to a Lafayette Instrument Company Running Wheel, with a circumference of 1.1 meters, for 30 min on 2 consecutive habituation sessions on which no data were recorded. The animals were given a further 6 pre-study sessions to determine a baseline level of running wheel activity. These sessions were on the Monday, Wednesday and Friday of the 2 weeks preceeding the study. The number of wheel revolutions per animal per session was noted each day. The total number of wheel revolutions per animal was calculated over all 6 pre-study sessions. On the basis of these data the gerbils were divided into 5 equal groups which were matched on the level of wheel running activity.

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