line, since 8-bromo-cyclic GMP is very resistant against degradation by phosphodiesterase activity[11] and the effect of this compound is rather limited by the cell membrane barrier. The enhancement by papaverine of the negative inotropic effect of acetylcholine is probably not due to the action of papaverine on the action potential, since the action potential is shortened by acetylcholine[12] and prolonged by papaverine[7]. Correspondingly, the shortening effect of acetylcholine on the action potential duration in rat atrial muscle is partially inhibited in the presence of papaverine, although the force of contraction is further depressed (figure 2).

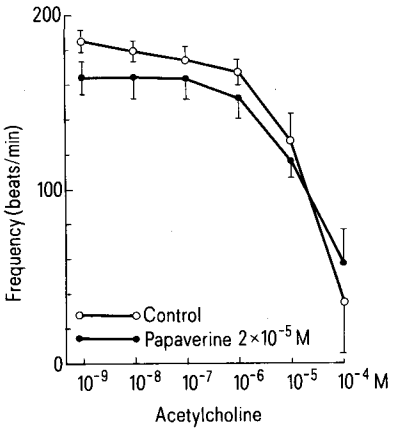

Fig. 3. Effects of acetylcholine on the frequency of spontaneously beating rat right auricles. ○, acetylcholine alone. ●, acetylcholine in the presence of papaverine 2×10^{-5} M. Symbols represent means±SE of 8 preparations. Experimental procedure as described in figure 1.

The pacemaker sensitivity of right auricles to acetylcholine was not significantly influenced in the presence of papaverine (figure 3). This result corresponds to the observation in rat hearts that 8-bromo-cyclic GMP did not influence the frequency of spontaneously beating right auricles[4] and would further suggest that cholinergic regulation of the pacemaker activity in rat right auricles is not modulated by cyclic GMP but rather by the effect of acetylcholine on the potassium permeability of the cell membrane[5].

1 I thank Lydia Paragnik for help. This research was supported by a grant from the Deutsche Forschungsgemeinschaft.
2 W.J. George, J.B. Polson, A.G. O'Toole and N.D. Goldberg, Proc. natn. Acad. Sci. USA *66*, 398 (1970).
3 W.J. George, L.J. Ignarro, R.J. Paddock, L. White and P.J. Kadowitz, J. cycl. Nucl. Res. *1*, 339 (1975).
4 H. Nawrath, Nature *262*, 509 (1976).
5 H. Nawrath, Nature *267*, 72 (1977).
6 C. Lugnier and J.-L. Stoclet, Biochem. Pharmac. *23*, 3071 (1974).
7 H. Nawrath and T. Meinertz, Naunyn-Schmiedebergs Arch. Pharmac. *299*, 253 (1977).
8 T. Meinertz, H. Nawrath and H. Scholz, Pflügers Arch. *333*, 197 (1972).
9 J. Koch-Weser and J.R. Blinks, Pharmac. Rev. *15*, 601 (1963).
10 W.F. Friedman, R.A. Buccino, E.H. Sonnenblick and E. Braunwald, Circulation Res. *21*, 573 (1967).
11 G. Michal, M. Nelboeck and G.Z. Weimann, Analyt. Chem. *252*, 189 (1970).
12 A.S.V. Burgen and K.G. Terroux, J. Physiol. (Lond.) *120*, 449 (1953).

Somatostatin reduces the release of colony-stimulating activity (CSA) from PHA-activated mouse spleen lymphocytes

W. Hinterberger, C. Cerny, H. Kinast, H. Pointner and K.H. Tragl[1]

1st Dept. of Medicine, University of Vienna, Lazarettgasse 14, A-1090 Wien (Austria), 7 November 1977

Summary. PHA-activated lymphocytes release colony-stimulating activity (CSA) for macrophage-granulocyte precursor cells (colony forming units, CFU_C) in the culture medium. Somatostatin, known to interfer with ribosomal protein synthesis, was demonstrated to reduce the release of CSA from PHA-treated mouse spleen lymphocytes.

Unspecific stimulation of lymphocytes induces blastogenesis, division and the release of lymphokines. The activity of a particular type of lymphokine, the colony-stimulating factor (CSF) is estimated by adding culture supernatants to human or mouse bone marrow agar cultures. The number of growing colony-forming cells (CFU_C) correlates with the colony-stimulating activity (reviewed by Metcalf[2]). The use of these techniques has produced evidence for different susceptibility for both DNA synthesis and release of CSF from mitogen-treated lymphocytes. Cytosin-arabinosid and vinblastin have been found to block DNA synthesis withouth blocking CSF release, while cycloheximide and puromycin inhibits CSF release without reduction of DNA synthesis[3].

Somatostatin, known to inhibit the release from the synthesizing cell of a spectrum of proteins was used in experiments instead of cycloheximide/puromycine. It was shown that activated mouse spleen lymphocytes cultured with somatostatin released less CSF into the culture medium than controls. Furthermore, DNA synthesis, as measured by 3H-TdR incorporation, was unaffected. The results described in this paper offer indirect evidence for interference of somatostatin with either the production or the release of CSF from PHA-activated mouse spleen lymphocytes.

Materials and methods. Spleen and bone marrow cells from C 57 bl mice (2-3 months old, both sexes, inbred) were used in all experiments. 3 mice were killed for each culture run and their femora were rinsed with Hank's solution. After 3 washes, bone marrow cells were suspended in McCoy 5 A medium containing 20% fetal calf serum. Spleen cells were suspended in cold RPMI 1640 medium following gentle dispersion through stainless steel sieves. Suspensions of mononuclear cells were obtained by Isopaque Ficoll gradient centrifugation (sp.wt 1077[4]). Adherent cells were removed by using the method of Messner[5]. Spleen cells were incubated in petri dishes for 1 h at 37 °C. Nonadherent cells were pipetted off and counted.

Table 1. Recovery of somatostatin in lymphocyte culture supernatants at various intervals. Mean values from 5 pooled supernatants (percentage of amount added)

1 h	24 h	48 h	72 h	96 h
68%	7%	6%	2%	0%

Table 2. Colony-stimulating activity in lymphocyte culture supernatants on days 0, 2 and 5. 9 experiments

	Day of culture 0		2		5	
With/without 5 µg/ml somatostatin	With	Without	With	Without	With	Without
Number of colonies/ml supernatant	0	0	30±18	42±23	46±35	62±27
			$p<0.05$		$p<0.01$	

Lymphocyte cultures: Nonadherent spleen cells were cultured in RPMI 1640 medium, enriched with 5% heat-inactivated fetal calf serum in a concentration of 2×10^6 cells/ml in loosely capped plastic tubes at 37 °C in 7.5% CO_2 (v/v). A total of 1 µg/ml PHA was added to each tube. Somatostatin (cyclic, Serono) was added at various concentrations approximately 2 min after PHA to each 2 or 4 culture tubes.

Assay for 3H-TdR incorporation: 48 h after PHA, 1 µCi 3H-TdR (sp.act. 21 Ci/mmole, Radiochem. Centre, Amersham) per ml culture medium was added. Then, 6 h later, cell pellets were spun down by centrifugation ans washed twice in cold Hanks solution. Acid-soluble material was precipitated in 10% trichloroacetic acid at 4 °C. The precipitate was dried, dissolved in 4 ml soluene/ml culture medium and counted in a liquid scintillation spectrometer.

Assay for colony-stimulating factor: On days 0 (1 h after PHA), 2 and 5, supernatants were removed from lymphocyte cultures and assayed for their content of CSF. Culture medium (McCoy 5 A medium, containing fetal calf serum at 20%, amino acids, vitamins[6], penicillin/streptomycin and agar at 0.3% final concentration) containing 7.5×10^4 mouse bone marrow cells per 0.9 ml was mixed with 0.1 ml supernatant. After gelation of the plates, a one-week culture period followed (37 °C, 7.5% CO_2). Colony counts were performed with an inverted microscope at ×25. Aggregates of 40 or more cells were scored as colonies. CSF activities were expressed as number of colonies obtained from 7.5×10^4 bone marrow cells. Somatostatin in the lymphocyte culture supernatants was measured on day 0 (i.e. 1 h after somatostatin was added) and days 1, 2, 3 and 4 by use of a radioimmunoassay. These investigations were kindly performed in the laboratory of Dr Arimura, Tulane University, New Orleans.

Results and discussion: The amount of somatostatin, as measured by Ria, decreased considerably during the lymphocyte culture period (table 1). It is uncertain whether somatostatin binds on the lymphocyte surface or is degraded. Specific binding of somatostatin on the lymphocyte surface would strongly facilitate the development of a radio receptor assay. Our experiments do not allow us to decide whether the remaining activity of somatostatin is sufficient to maintain the effects described below, or whether these effects are mediated during the first few h of culture.

Effect of somatostatin on lymphocyte DNA synthesis: 2 experiments were performed with and without somatostatin (figure 1). It is shown that somatostatin in concentrations up to 100 µg/ml does not affect 3H-TdR incorporation.

Effect of somatostatin on CSF release: 9 experiments were performed, 3 of them with various doses of somatostatin. Using 5 µg/ml somatostatin, a significant reduction in the release of CSA on days 2 and 5 was observed ($p < 0.05$, day 2, $p < 0.01$, day 5, Students t-test for paired data; figure 2, table 2). Various concentrations of somatostatin (0.5/5/50 µg/ml) gave a dose-dependent reduction in the release of CSF (table 3).

Effect of somatostatin on mouse bone marrow colony formation: 7.5×10^4 bone marrow cells were activated with 0.1 ml human lung conditioned medium (HLCM), which

Table 3. Effect of various concentrations of somatostatin on CSF release from PHA treated lymphocytes. Supernatants were assayed for CSF on day 5. 3 experiments

		Number of colonies		
Without somatostatin		61	42	107
Somatostatin	0.5 µg/ml	51	32	92
	5.0 µg/ml	44	26	86
	50.0 µg/ml	36	22	62

Table 4. HLCM-activated bone marrow colony growth. Number of colonies without and with somatostatin at various concentrations. 3 experiments

		Number of colonies		
HLCM 1:16		64	58	79
Somatostatin	0.05 µg/ml	65	62	84
	0.5 µg/ml	67	61	76
	5.0 µg/ml	61	64	79
	50 µg/ml	42	54	68

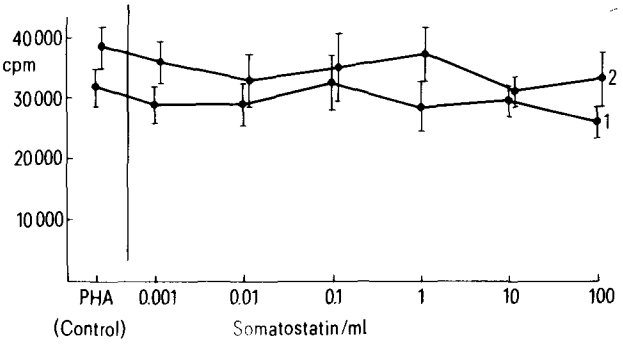

Fig. 1. Effect of various concentrations of somatostatin on lymphocyte 3H-TdR incorporation. 2 experiments.

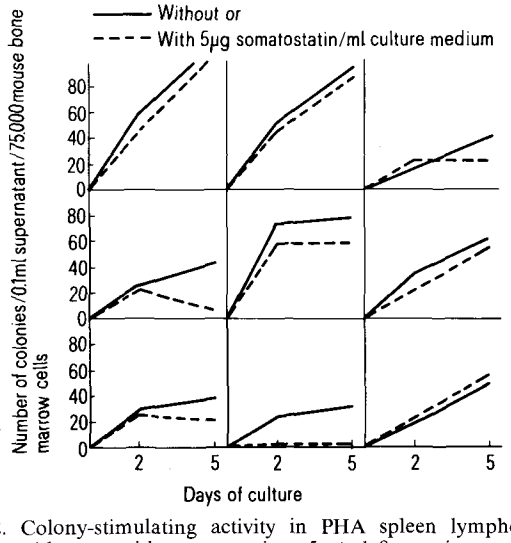

Fig. 2. Colony-stimulating activity in PHA spleen lymphocyte cultures without or with somatostatin at 5 g/ml. 9 experiments.

serves as standard preparation of CSF in our laboratory[7]. HLCM was diluted at 1:16 to avoid excessive stimulation which might mask inhibition by somatostatin. Somatostatin at 0.05/0.5/5 µg/ml did not influence colony growth; 50 µg/ml however reduced colony formation (table 4). Since lymphocyte culture supernatants are diluted at 1:10 by transfer into the bone marrow culture medium, the remaining activity of somatostatin is far too low to cause the reduced colony growth observed with somatostatin-containing lymphocyte culture supernatants.

Since these experiments exclude direct suppression of somatostatin on CFU_C, reduced production or impaired release of CSF from PHA-treated lymphocytes must be responsible for our observations. Thus, somatostatin interferes with CSF elaboration, but does not affect DNA synthesis. The possibility however exists that assays for CSF release and DNA synthesis relate to different lymphocyte subpopulations. In this case, somatostatin would preferentially inhibit the CSF-releasing subpopulation.

It has been demonstrated that CSF production from mitogen-treated lymphocytes requires active protein synthesis[8]. Somatostatin is believed to inhibit ribosomal protein synthesis by competing with C-AMP, which activates protein-kinases by phosphorylation[9]. Accordingly, the inhibitory effect of somatostatin on rat liver protein synthesis becomes more pronounced by elevating the extracellular concentration of C-AMP in the cultures[10]. The demonstration of this effect in lymphocyte cultures, however, is impossible, since the addition of C-AMP to the culture medium markedly abolishes the PHA activation (Hirschorn[11] and own observations), probably due to loss of binding of PHA on the lymphocyte surface.

1 The technical assistance of Ulrike Wallner and Paulette Bais is thankfully acknowledged.
2 D. Metcalf, J. Cell Physiol. *76*, 89 (1970).
3 F. Ruscetti and P.A. Chervenick, J. Immun. *114*, 1513 (1975).
4 A. Boyum, Scand. J. Lab. Invest. *21*, suppl., 97 (1968).
5 H. Messner, J. Till and E. McCullough, Blood *44*, 671 (1974).
6 D. Metcalf and M.A.S. Moore, in: Haemopoietic Cells, p. 35. North Holland Publ. Company, 1971.
7 W. Hinterberger and W.R. Paukovits, IRCS *5*, 137 (1977).
8 J.W. Parker and D. Metcalf, J. Immun. *112*, 502 (1974).
9 P.R. Ogida, P.V.N. Murthy and J.M. McKenzie, Biochemistry *10*, 711 (1971).
10 K.H. Tragl and H. Kinast, Klin. Wschr. *55*, 707 (1977).
11 R. Hirschorn, in: Cyclic AMP, Cell growth and the Immune Response, p. 45. Ed. Braun, Lichtenstein & Parker. Springer, Berlin/Heidelberg/New York 1974.

Electrical activity of the pectoral muscles during gliding and flapping flight in the herring gull (*Larus argentatus*)[1]

G. Goldspink[2], C. Mills and K. Schmidt-Nielsen

Department of Zoology, Duke University, Durham (North Carolina, USA), 14 November 1977

Summary. Electromyographic recording from the pectoral muscles of the herring gull during flight showed that very little muscle activity is associated with gliding flight. However, the integrated gliding potentials could be increased very considerably by loading the bird. The muscle activity during gliding and flapping flight are in accordance with the known energy requirements for these 2 types of flight.

It is well known that certain birds can glide for long periods of time. Birds such as gulls and storks can remain airborne for hours, or in the case of the albatross presumably for months, without landing or without having to resort to flapping flight. Some birds, such as African vultures, travel long distances by alternately soaring on up-drafts and gliding between up-drafts[3]. Gliding flight has been assumed to require considerably less energy than flapping flight. However, the only quantitative measurements of the energy requirements for gliding flight are those of Baudinette and Schmidt-Nielsen[4]. These workers found that the rate of oxygen consumption by the herring gull during gliding was about 2 times its resting level, whereas in level flapping flight of the laughing gull Tucker[5] found it to be 6–8 times the resting level. This present study was undertaken to see how much muscular activity is necessary for gliding flight, and to relate this to the known metabolic requirements of the bird.

Materials and method. The electrical activity of the pectoralis major muscle was measured during gliding and flapping flight of the herring gull (*Larus argentatus*). Measurements were carried out mainly on 1 gull (herring gull No. 72, 748 g) which had been trained for flying in a wind tunnel. Such training is very time-consuming and consists of h-long daily sessions for a period of several weeks. In order to obtain gliding flight the wind tunnel was tilted at an angle of 7° below the horizontal with a wind speed of 11.5 m sec^{-1}. Measurements were carried out on other birds, (laughing gull, *Larus atricilla,* and North American black vulture, *Coragyps atratus*) which also had been trained to fly in the wind tunnel. The results were essentially the same as for the herring gull.

EMG recordings were made from 3 insulated copper electrodes inserted into the muscle in a triangular configuration. Each copper wire (0.1 mm diameter) was first pushed through a syringe needle, and after removing the end 2 mm of the insulation, the tip of the wire was bent over to form a barb. The needle was inserted into the muscle and immediately withdrawn, leaving the wire electrode in the desired position. The distance between the 3 wires in the muscle was approximately 6 mm. These were connected to a small preamplifier (weight 23 g) which was mounted on the back of the bird with 1 of the wires connected to ground. The signal was further amplified to give an overall amplification of 1.3×10^5. Following this it was processed to give a rectified compressed signal plus a rectified linear signal. The former was obtained by putting it through a square rooter. The latter was obtained by putting it through the square rooter followed by a squarer, followed by a limiter (figure 1). This additional circuitry was necessary because a gain that was suitable for recording gliding potentials was not suitable for recording flapping potentials. In particular, the limiter was necessary to prevent overdriving of the recording system should the bird suddenly change from gliding to flapping flight.

The processed signals, the linear and the compressed, were recorded on separate channels of an fm tape recorder. A 3rd channel was used to mark the periods of stable gliding. The signals were recorded at a tape speed of 38 cm sec^{-1}, and eventually played back and recorded on paper using a