7. A relation between PV and GABA transmission per se is unlikely, although I. Ortega Blake, A. Lès, and S. Rybak [*J. Theor. Biol.* **104**, 571 (1983)] stressed the importance of calcium for GABA neurotransmission. In general, however, the calcium-dependent GABA release accounts for only 50 percent of the total released GABA and may be evoked from a different neuronal pool [J. Peris, R. A. Harris, N. R. Zahniser, *Soc. Neurosci. Abstr.* **11**, 283 (1985)]. The general distribution of PV was different from that of known GABA-receptor subtypes. Only the benzodiazepine binding sites associated functionally with the $GABA_A$ receptor [P. Schoch, *Nature (London)* **314**, 168 (1985)] were distributed similarly to PV in the cerebellum.
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16. This report is dedicated to Dr. Rafael Lorente de Nò on the occasion of his 83rd birthday. I thank L. Schärer and G. Weinbrenner for their excellent technical assistance; H. Haas for his continuous help and interest; P. Somogyi who kindly supplied me with preprints of his papers; P. Streit and C. Matute for suggestions concerning the cross-reaction tests; and C. W. Heizmann, J. M. Gillis, P. Sequela, and A. W. Norman for their gift of antisera and antigens. Supported by Swiss National Foundation 3.559.0.83 and Hartmann-Müller-, Mobiliar-, Rentenanstalt-, Sandoz- and EMDO-Stiftung.

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Tripeptide Structure of Bursin, a Selective B-Cell–Differentiating Hormone of the Bursa of Fabricius

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Differentiation of lymphoid precursor cells in a variety of species is induced by polypeptide hormones such as thymopoietin for T cells and bursin for B cells. In the present experiments, bursin isolated from the bursa of Fabricius of chicken was found to induce the phenotypic differentiation of mammalian and avian B precursor cells but not of T precursor cells in vitro. Similarly, bursin increased cyclic guanosine monophosphate in cells of the human B-cell line Daudi but not in cells of the human T-cell line CEM. These inducing properties of bursin are the reverse of the inducing properties of thymopoietin produced by the thymus and are appropriate to a physiological B-cell–inducing hormone. A tripeptide sequence (lysyl-histidyl-glycylamide) was determined for bursin and confirmed by synthesizing this proposed structure and demonstrating chemical identity of the natural and synthetic peptides. Similarity of biological action was indicated in induction assays by elevation of cyclic adenosine monophosphate and guanosine monophosphate in Daudi B cells but not in CEM T cells.

EARLY IN DEVELOPMENT, TWO SEPARATE differentiational pathways for lymphocytes are established, one for T (thymic) lymphocytes and the other for antibody-secreting B (bone marrow and bursal) lymphocytes. Throughout life, committed precursor T cells (prothymocytes) migrate from the bone marrow to the thymus, where they differentiate further under the influence of the thymic hormone thymopoietin (*1*). This induction of prothymocytes is assayed in vitro by measuring the appearance of thymocyte phenotypic markers in serum, which occurs within a few hours and is accompanied by elevation of intracellular adenosine 3′,5′-monophosphate (cyclic AMP) (*2*). Thymopoietin has later immunoregulatory actions [mediated by intracellular guanosine 3′,5′-monophosphate (cyclic GMP) elevation] on more mature T cells (*3*). These biological actions of thymopoietin are reproduced by the synthetic pentapeptide thymopentin (Arg-Lys-Asp-Val-Tyr) (*4*). Thymopoietin and thymopentin selectively induce differentiation of prothymocytes but not of precursor B cells (*2*). This inductive selectivity distinguishes thymopoietin from other prothymocyte inducers, which show neither this selectivity nor limitation of production to the thymus.

In mammals, no B-cell–differentiating organ equivalent to the T-cell–differentiating thymus has been defined, and the existence of a mammalian B-cell–differentiating hormone equivalent to thymopoietin has not been unequivocally demonstrated. In birds, however, B-cell differentiation occurs within the bursa of Fabricius, a dorsal diverticulum of the cloaca (*5*). Bursal extracts contain the low molecular weight inducing agent bursin (formerly termed bursopoietin) (*6*). Bursin selectively induces avian B cells, but not avian T cells, from their precursors in vitro (*6*). Elevation of intracellular cAMP accompanies this inductive process in the chicken, as in the mammalian lymphocyte induction assays. In both the mouse and chicken, avian bursin selectively induces B cells but not T cells (*7*). We now report that bursin elevates cyclic AMP and cyclic GMP in human cells of the Daudi line, derived from a Burkitt's lymphoma, and we have used this observation as an assay for bursin. For tests of inductive selectivity we used the inducible human T-cell line CEM in parallel.

Frozen chicken bursa (80 g, Pel-Freeze Biologicals) was homogenized with 800 ml of 1*N* acetic acid at 4°C in a Waring blender. After centrifugation at 14,000*g* for 60 minutes at 4°C, the supernatant was removed and recentrifuged. This supernatant was evaporated in a Rotavapor-RE and lyophilized, and the lyophilizate was dissolved in 1*N* acetic acid at room temperature for 1 hour and centrifuged at 12,000*g* for 20 minutes. This supernatant was further frac-

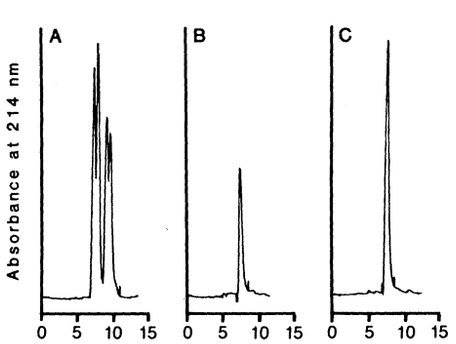

Fig. 1. Chromatographic comparisons of native bursin and four synthetic tripeptides. HPLC was performed on an Analyst series 7800 HPLC system with a Constametric III pump and Zorbax octadecyl silane analytical column. Peptides were eluted with 200 m*M* perchloric acid at 0.5 ml/min and the elution was monitored at 214 nm. (A) Coinjection of Lys-His-Gly-NH_2 (7.3 minutes), Gly-His-Lys-NH_2 (7.9 minutes), Lys-His-Gly (9.1 minutes), and Gly-His-Lys (9.6 minutes). (B) Injection of native bursin (7.3 minutes). (C) Coinjection of equal amounts of native bursin and Lys-His-Gly-NH_2, showing a single peak with a retention time of 7.3 minutes.

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tionated by molecular sieve chromatography on a 2.5 by 90 cm column of G-50 Sephadex equilibrated with 1*N* acetic acid. Fractions constituting the internal volume were pooled, evaporated in a Rotavapor-RE, and lyophilized. This lyophilizate was dissolved in 20 ml of 50 m*M* ammonium bicarbonate and centrifuged at 7000*g* for 15 minutes.

The supernatant was mixed with 100 ml of a biphasic solvent mixture (ethyl acetate:1-propanol:H_2O; 3:2:4 by volume) in a separatory funnel and mixed gently by inversion for 30 minutes. The upper layer, containing 85 percent of the biological activity, was collected, evaporated under reduced pressure overnight, and lyophilized. The lyophilized material was dissolved in a minimal amount of 1-propanol and crystallized by the addition of diethyl ether. After the crystals were washed several times with dry ether, the material was dried in a desiccator under reduced pressure.

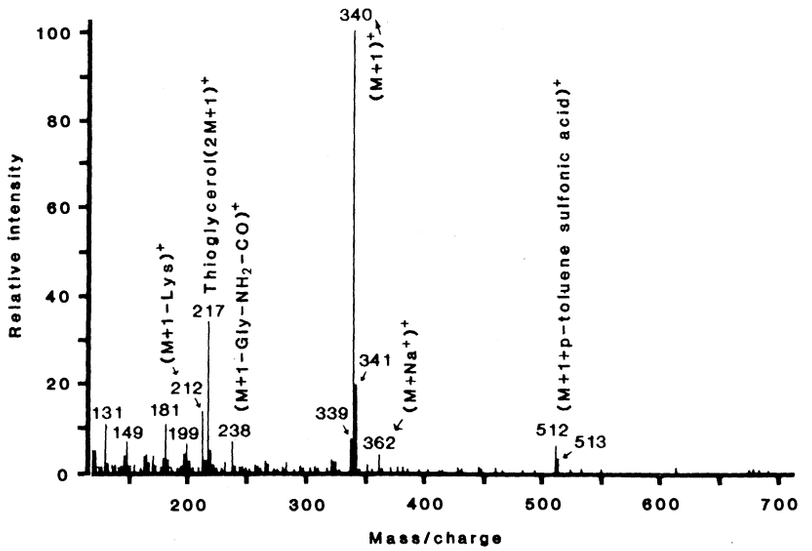

Fig. 2. Mass spectrograph obtained from positive-ion fast atom bombardment of bursin on a Finnegan MAT 8230 mass spectrometer. The fast atom gun was operated at 7 keV with xenon gas. The sample was applied in methanol; a matrix of thioglycerol with 0.01 *p*-toluene sulfonic acid was then added. The mass spectrograph distinguishes the sequence Lys-His-Gly-NH_2, showing a major peak of 340 corresponding to $(M + 1)^+$ and peaks corresponding to fragments at 238 $(M + 1 - Gly\text{-}NH_2 - CO)^+$ and 212 $(M + 1 - Lys)^+$.

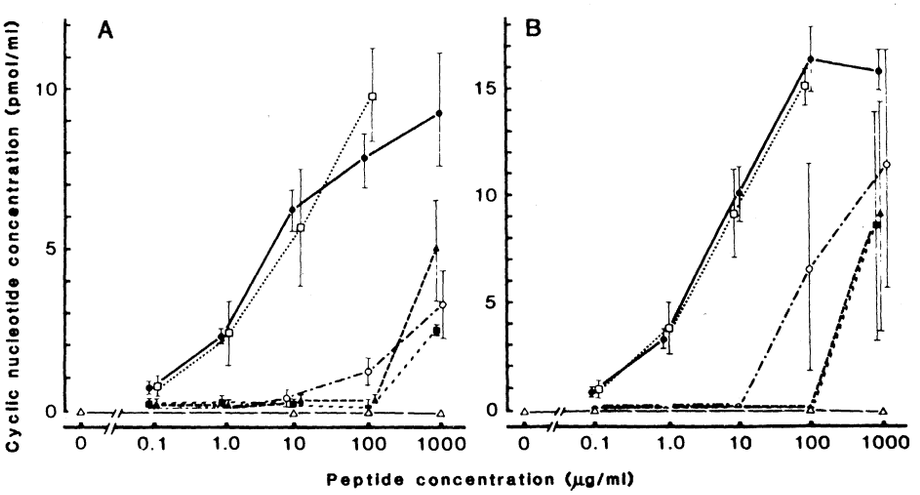

Fig. 3. Intracellular cAMP (A) and cGMP (B) in Daudi cells after incubation with native bursin (□), Lys-His-Gly-NH_2 (●), Gly-His-Lys-NH_2 (▲), Gly-His-Lys (○), Lys-His-Gly (■), or no peptide or thymopentin (Arg-Lys-Asp-Val-Tyr) (△). Daudi cells were transferred to fresh medium at 5×10^5 per milliliter and incubated for 5 days before being used for assay. The cells were washed three times with RPMI 1640 medium and placed into test tubes at a concentration of 1.0×10^7 per milliliter (1 ml per tube). The tubes were allowed to warm to 37°C in a water bath for 30 minutes. Compounds to be tested were prepared at 40 times final concentration and 25 μl was added to each tube. Cells were incubated at 37°C with the compounds for exactly 2 minutes and the reaction was terminated with 1 ml of cold 10 percent trichloroacetic acid. Samples were then frozen and thawed three times, extracted with hydrated ether, and lyophilized. Cyclic nucleotide levels were determined (*11*) with a commercial radioimmunoassay kit. Native bursin and Lys-His-Gly-NH_2 had similar potencies, whereas the other variants of this tripeptide were active only at very high concentrations. Data are means and ranges for two or three experiments.

The yields of protein and bursin during the isolation (total from two batches, which were very similar) are shown in Table 1. Recrystallized bursin (1.3 mg) was obtained from 160 g of chicken bursa (0.0008 percent). Purity was determined by high-performance liquid chromatography (HPLC) (Fig. 1) and thin-layer chromatography (TLC), which was performed on precoated silica gel 60-F-254 sheets (0.2 mm thick) with several solvent systems: (i) 1-propanol:NH_4OH (1:2), (ii) 1-propanol:dimethylsulfoxide:NH_4OH:H_2O (4:4:2:1), and (iii) butanol:acetic acid:H_2O:pyridine (4:2:3:1). After chromatography, the sheets were dried in air and exposed to iodine vapor for 10 minutes. Purified bursin yielded a single spot in each system. Amino acid composition was determined after acid hydrolysis with a Beckman 119 CL amino acid analyzer (*8*). Lysine, histidine, glycine, and ammonia were found in a molar ratio of 1.0:0.9:1.0:0.8, consistent with bursin being an amidated tripeptide. The amino acid sequence of bursin was determined to be Lys-His-Gly-NH_2 by positive-ion fast atom bombardment (Fig. 2).

Lys-His-Gly-NH_2 and control peptides Lys-His-Gly, Gly-His-Lys-NH_2, and Gly-His-Lys were synthesized by solid-phase methodology. Each had the correct amino acid composition and was homogeneous by both TLC and HPLC. Different retention times were obtained on HPLC for all four peptides, with native bursin having a retention time identical to that of Lys-His-Gly-NH_2 and coeluting with this peptide when the two were injected together (Fig. 1). All four synthetic peptides were resolved on TLC with 1-propanol:NH_4OH (1:2). Native bursin had an R_F value identical to that of Lys-His-Gly-NH_2 and comigrated with this peptide. The mass spectrograph of Lys-His-Gly-NH_2 was identical to that of native bursin.

The biological activity of purified natural bursin and of the four synthetic tripeptides was assayed in vitro by comparing the cAMP and cGMP responses of Daudi B cells with those of CEM T cells in the standard induction assay. In Daudi cells incubated with natural or synthetic bursin (Lys-His-

Table 1. Purification of bursin from the chicken bursa of Fabricius.

Purification step	Total bursin (mg)	Total protein (mg)	Bursin (%)	Purification (fold)	Recovery (%)
Chicken bursa (wet weight, 180 g)	6.5	7986	0.08	1	100
Gel filtration, Sephadex G-50	2.9	25.5	11.4	142	45
Solvent partitioning, upper phase	1.54	1.87	82	1012	24
Crystallization	1.36	1.41	96.5	1185	21

Gly-NH_2), cAMP and cGMP were increased and the dose-response curves were similar (Fig. 3). The threshold concentrations were 0.1 to 1.0 μg/ml, with the maximal response at 100 μg/ml. The three control synthetic tripeptides increased cAMP and cGMP only at concentrations 1×10^3 to 1×10^4 higher than those for natural and synthetic bursin. Thymopentin did not increase cAMP or cGMP in Daudi cells but elevated cGMP in CEM T cells (*9*). Bursin did not affect cGMP in CEM or three other T-cell lines, the erythropoietic stem cell line K562, and five other B-cell lines, although it did increase cGMP in the murine B-cell line MOPC 315 (American Type Culture Collection TIB 23). Natural and synthetic bursin also induced selective phenotypic differentiation of murine precursor B cells but not precursor T cells, in conformity with previous avian and mammalian inductive studies with partially purified bursal extracts (*6, 7*). Thus bursin and thymopoietin display the reciprocal inductive selectivity for B versus T cells that seems appropriate to physiological inducers for these two cell lineages. Furthermore, preliminary immunohistological studies in the chicken with antibodies to bursin indicate that bursin production is restricted to the bursa of Fabricius.

Gly-His-Lys has been detected in extracts of rat liver and is reported to be a growth factor (*10*). The activity of this tripeptide and of Gly-His-Lys-NH_2 and Lys-His-Gly in the bursin assays is provocative and may imply a tertiary structure distantly resembling that of bursin, but the weak inductive capacity, 1×10^3 to 1×10^4 lower than that of bursin, suggests that this activity is physiologically irrelevant.

We conclude that the structure of chicken bursin is Lys-His-Gly-NH_2 and that this tripeptide avian hormone has similar actions in birds and mammals, including humans. The presence of receptors for avian bursin on murine and human cells would suggest that the tripeptide structure of bursin has been conserved in evolution, but the occurrence and structure of the mammalian equivalent of bursin and its site of production remain to be determined.

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Hyperacuity in Cat Retinal Ganglion Cells

ROBERT SHAPLEY AND JONATHAN VICTOR

Cat X retinal ganglion cells that can resolve sine gratings of only 2.5 cycles per degree can nevertheless respond reliably to displacements of a grating of approximately 1 minute of arc. This is a form of hyperacuity comparable in magnitude to that seen in human vision. A theoretical analysis of this form of hyperacuity reveals it to be a result of the high gain and low noise of ganglion cells. The hyperacuity expected for the best retinal ganglion cells is substantially better than that observed in behavioral experiments. Thus the brain, rather than improving on the retinal signal-to-noise ratio by pooling signals from many ganglion cells, is unable to make use of all the hyperacuity information present in single ganglion cell responses.

HUMAN OBSERVERS CAN DETECT the change in position of a grating when the displacement is only 10 seconds of arc, which is the visual angle subtended by 1 cm at a distance of 200 m. In other spatial localization tasks, the threshold for displacement is also a small fraction of the interreceptor spacing in the fovea (equivalent to about 30 seconds). Such performance, in which the threshold for detecting positional displacements is much smaller than the inverse of the spatial frequency resolution, has been called hyperacuity (*1*). We have studied spatial localization acuity of cat retinal ganglion cells and found that single X ganglion cells (*2*) can respond reliably to displacements more than an order of magnitude smaller than the radius of the receptive field center. This retinal hyperacuity is a consequence of the high gain and low noise of the receptive field center mechanism.

Visual stimuli were produced on a Tektronix 608 CRT monitor with a raster display by means of an electronic display instrument (*3*). A computer sent control signals to the instrument and measured the times when nerve impulses occurred. The stimuli were spatial sine gratings on a mean luminance of 140 cd/m². In the grating displacement experiments, spatial phase of the grating was shifted back and forth a fixed displacement every 474 msec; the displacement was repeated 64 times and the displacement responses averaged. In these displacement experiments, the contrast of the grating—$(L_{max} - L_{min})/(L_{max} + L_{min})$—was held constant at 0.5. In other experiments gratings were not displaced; instead, the contrast of stationary gratings was varied sinusoidally in time at a frequency of approximately 1 Hz. Responses to 32 cycles of modulation were averaged in these runs. Single-unit recordings of optic tract fibers were obtained with Ringer-filled glass micropipettes. Details of our procedure for recording from anesthetized and paralyzed cats are given in (*4*). Ganglion cells were classified as X or Y on the basis of a modified null test (*2*).

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