desorption; blocked active sites on the surface become free from blocking species; glycine in the solid phase becomes redissolved and available again for surface adsorption; and the surface concentration of glycine can be replenished during the next dehydration stage. The smaller, but real, enhancement associated with WDTF cycles in the presence of bentonite may result from a less efficient redistribution process for bentonite than for kaolinite (*8*).

On going from TF to WDTF cycles, total peptide yield increased, as did the proportion of large oligomers over small ones. In contrast, initial glycine-to-clay ratio had no apparent effect on the ultimate distribution of products among oligomers (experiments 3, 5 to 7, 17 to 20, and 23). Moreover, essentially no change occurs in oligomer composition after 11 cycles with either WDTF or TF cycles. With TF cycles, little or none is expected since there exists no redistribution mechanism other than slow surface diffusion. During WDTF cycles, however, redistribution is expected to occur. The fact that oligomer composition remains unchanged after many WDTF cycles is in accord with the attainment of a steady-state composition in which oligomer formation is balanced by oligomer decomposition (*9*). A plausible reaction scheme is shown in Fig. 2.

If the scheme that we propose is correct, and if reactions in the WDTF mode included a mixture of amino acids, then this model of a fluctuating system may produce nonrandom oligopeptides. The dynamic balance of peptide bond formation and destruction over a large number of cycles provides a mechanism for selective generation of oligomers with nonrandom sequences determined by factors characteristic of specific monomers and lower oligomers, such as strength of adsorption to clay, solubility, ease of condensation, stability to hydrolysis, and nearest neighbor interactions. Catalytic properties of such nonrandom oligomers would be of great interest.

We postulate that, in the prebiotic era when monomers were supplied at a reasonable rate by syntheses, the most favorable environment for condensation of amino acids, and perhaps for condensation reactions of other organic molecules, consisted of one in which diurnal fluctuations yielded a wet period during the night and a dry and hot period during the day. Prolonged dry periods would have inhibited further condensation, and incomplete dehydration would have led to low monomer surface concentrations and ineffective catalytic surfaces. The adsorptive and catalytic surface need not have been provided by clays or other inorganic substances (*10*); organic microstructures (*11*) may have served as well.

We have shown that condensation of glycine to oligomers can take place in a simple, geologic model system without implausible condensing agents, where changes in temperature and water content occur in cyclic fashion. Cycling is important because, in principle, the effective number of oligomer "generations" and, thus, the rate and extent of chemical evolution, can be increased simply by increasing the frequency and number of cycles (*12*).

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1. N. Lahav and S. Chang, *J. Mol. Evol.* **8**, 357 (1976).
2. See (*1*) for descriptions of the environments modeled in these experiments.
3. Consistent with glycine's low adsorbability [B. K. G. Theng, *The Chemistry of Clay-Organic Reactions* (Wiley, New York, 1973), p. 158] was our finding that more than 95 percent of ^{14}C-labeled glycine remained in the liquid phase of bentonite and kaolinite suspensions in the first wet period of the wetting-drying cycles. The recovery of diglycine from kaolinite was 93 percent.
4. The identity of diglycine was confirmed by combined gas chromatography–mass spectroscopy analysis of the *N*-trifluoroacetyl methyl ester [S. Chang, J. J. Flores, C. Ponnamperuma, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1011 (1969)]. The presence of glycine oligomers was verified by collecting the oligopeptide fraction eluted from the amino acid analyzer and comparing the chromatogram of a portion of the isolated sample with that of another portion that was hydrolyzed by acid. Di-, tri-, tetra-, and pentaglycine disappeared after the HCl treatment, and a concomitant release of glycine was detected.
5. For other examples of solid-state amino acid condensation at ≤100°C, see J. J. Flores and J. P. Leckie, *Nature (London)* **244**, 435 (1973); H. Sawai and L. E. Orgel, *J. Mol. Evol.* **6**, 185 (1975); H. Sawai, R. Lohrmann, L. E. Orgel, *ibid.*, p. 165; D. L. Rohlfing, *Science* **193**, 68 (1976).
6. We have assumed a surface area of 15 m²/g for kaolinite [R. E. Grim, *Clay Mineralogy* (McGraw-Hill, New York, 1968), p. 464]. The total external and interlayer surface area of bentonite ranges from 150 to 250 m²/g (unpublished measurements by N. Lahav) and is taken to be 200 ± 50 m²/g. This value is less than the theoretical value of 760 m²/g as a result of flocculation induced by aluminum hydroxide before bentonite was used in experiments.
7. A discussion of reciprocal surface density is found in (*1*).
8. Wetting of bentonite is a slow process [N. Lahav and A. Banin, *Isr. J. Chem.* **6**, 285 (1968)], and the hydration time may have been insufficient to complete wetting of the interlayer surfaces and to achieve effective redistribution.
9. Presumably, oligomers longer than pentapeptide were formed, but were not detectable with our analytical system.
10. For studies in which reactants or the reaction vessel provided the solid surface, see A. W. Schwartz, M. Veen, T. Bisseling, and G. J. F. Chittenden [*Biosystems* **5**, 119 (1973)] and E. Stephen-Sherwood, D. G. Odom, and J. Oro [*J. Mol. Evol.* **3**, 323 (1974)]; see also (*1*) and (*5*).
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12. The effect of precipitate accumulation on clay-mediated condensation reactions and the possibility of selective solute adsorption and leaching with natural and metal ion-exchanged clays remain to be evaluated.
13. Supported in part by NASA Ames–University Consortium Interchange NCA2-OR685-702. This work was performed at Ames by N.L. and D.W. as National Academy of Sciences senior research associate and National Science Foundation faculty research fellow, respectively.
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Histamine H_1 Receptor-Mediated Guanosine 3′,5′-Monophosphate Formation by Cultured Mouse Neuroblastoma Cells

Abstract. *Incubation of cultured mouse neuroblastoma cells with histamine caused a rapid and marked increase in the formation of guanosine 3′,5′-monophosphate (cyclic GMP) by these cells. Receptor agonists for H_1, but not H_2, caused this effect which was reduced by H_1 but not by H_2 or muscarinic acetylcholine receptor antagonists. These results indicate that activation of H_1 receptors in these cultured nerve cells stimulated cyclic GMP formation.*

Histamine has many different effects on various cell types, and may serve as a neurotransmitter (*1*). This biogenic amine apparently brings about its effects by activation of two different receptors (H_1 and H_2) which are distinguished by their differential sensitivities to agonists (for example, 2-methylhistamine for H_1 and 4-methylhistamine for H_2 receptors) and to antagonists (for example, pyrilamine for H_1 and metiamide for H_2 receptors).

As do many neurotransmitters, histamine stimulates the formation of adenosine 3′,5′-monophosphate (cyclic AMP) in nervous and other tissues (*2*). This effect is mediated by both H_1 and H_2 receptors. Recently, tricyclic antidepressants were reported to be potent competitive inhibitors of the H_2 receptor-mediated response (*3*).

In the course of experiments on muscarinic receptor-mediated cyclic GMP (guanosine 3′,5′-monophosphate) formation in cultured mouse neuroblastoma cells (*4*), we found that histamine also

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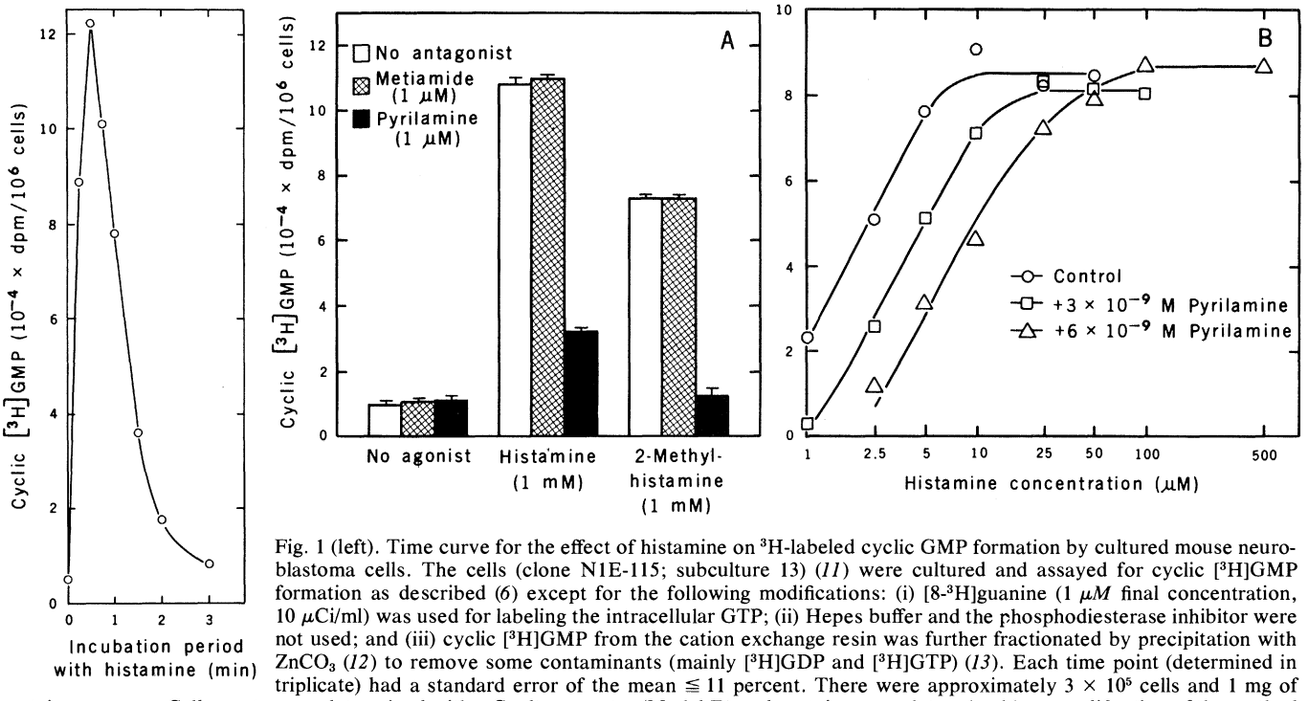

Fig. 1 (left). Time curve for the effect of histamine on ^{3}H-labeled cyclic GMP formation by cultured mouse neuroblastoma cells. The cells (clone N1E-115; subculture 13) (*11*) were cultured and assayed for cyclic [^{3}H]GMP formation as described (*6*) except for the following modifications: (i) [8-^{3}H]guanine (1 μM final concentration, 10 μCi/ml) was used for labeling the intracellular GTP; (ii) Hepes buffer and the phosphodiesterase inhibitor were not used; and (iii) cyclic [^{3}H]GMP from the cation exchange resin was further fractionated by precipitation with $ZnCO_3$ (*12*) to remove some contaminants (mainly [^{3}H]GDP and [^{3}H]GTP) (*13*). Each time point (determined in triplicate) had a standard error of the mean $\leq$ 11 percent. There were approximately 3 $\times$ 10^5 cells and 1 mg of protein per assay. Cell counts were determined with a Coulter counter (Model Z_f) and proteins were determined by a modification of the method of Lowry *et al.* (*14*) with bovine serum albumin as standard. Fig. 2 (right). (A) Effects of histamine receptor antagonists on cyclic [^{3}H]GMP formation by cultured nerve cells stimulated by histamine and 2-methyhistamine. Mouse neuroblastoma cells (clone N1E-115; subculture 14) were assayed for cyclic [^{3}H]GMP formation as described in Fig. 1 except that the precipitation step was not used. In addition, cells were incubated for 30 minutes with antagonists prior to the addition of agonists (*15*) for 30 seconds. There were approximately 3 $\times$ 10^5 cells and 1 mg of protein per assay. Each condition was determined in triplicate. Vertical bars represent standard error of the mean. (B) Effect of pyrilamine on the histamine dose-response curve. The cells (clone N1E-115; subculture 13) were assayed for cyclic [^{3}H]GMP formation as described in (A). The radioactivity recovered from the cation exchange resin in the absence of histamine (average = 1.4 $\times$ 10^4 dpm per 10^6 cells; range = 1.2 to 1.7 $\times$ 10^4 dpm per 10^6 cells) were subtracted from all values. Each point represents the average of duplicate samples. There were approximately 2 $\times$ 10^5 cells and 600 μg of protein per assay.

stimulated cyclic GMP formation by these cells. Here we describe some of the characteristics of this histamine effect which is apparently mediated by H_1 receptors.

Cyclic GMP formation by intact mouse neuroblastoma cells (clone N1E-115) was determined by an assay technique which involves radioactively labeling the intracellular stores of guanosine triphosphate (GTP) and isolating the radioactively labeled cyclic GMP that was formed. The results with this assay technique are comparable to those obtained with radioimmunoassay (*5*).

Histamine caused a rapid and marked increase in cyclic [^{3}H]GMP formation by mouse neuroblastoma cells with a peak around 30 seconds (Fig. 1). This time course was very similar to that found for muscarinic receptor-mediated cyclic GMP formation by this clone in the absence of a phosphodiesterase inhibitor (*6*). At maximum concentrations of histamine, there was a 7- to 50-fold increase over the basal rate of cyclic [^{3}H]GMP formation (25-fold at the peak in Fig. 1). The ED_{50} (median effective dose) for histamine ranged from 2 to 5 μM. Like the stimulation of cyclic GMP by carbamylcholine (*5*), the stimulation of cyclic [^{3}H]GMP formation by histamine was markedly dependent on external Ca^{2+}.

To determine what type of histamine receptor participated in this response, we tested some H_1 and H_2 receptor agonists and antagonists for their effects on cyclic [^{3}H]GMP formation by N1E-115 cells (Fig. 2, A and B). The synthesis of cyclic [^{3}H]GMP stimulated by 1 mM histamine was not altered by 1 μM metiamide but was markedly reduced (80 percent) by 1 μM pyrilamine. In dose-response curves for histamine 10 μM metiamide had no effect but pyrilamine (Fig. 2B) was a competitive inhibitor with an apparent K_d (equilibrium dissociation constant) of 2 $\times$ $10^{-9} M$ (*7*). 2-Methylhistamine (1 mM) was less potent than histamine (1 mM) in stimulating cyclic [^{3}H]GMP formation (Fig. 2A). In addition, the H_1 antagonist completely abolished this stimulation. 4-Methylhistamine (1 mM) had no effect on cyclic [^{3}H]GMP formation. These data indicate that H_1 receptors mediated the cyclic GMP response by these cells.

Tricyclic antidepressants were also potent competitive inhibitors of this histamine response, with equilibrium dissociation constants in the range of 10^{-10} to $10^{-7} M$ (*8*). Tertiary amine tricyclic antidepressants were more potent than secondary amine compounds, and all compounds were more potent at blocking H_1 receptors than at blocking muscarinic receptors. In other experiments, 0.1 μM atropine, which inhibited by 100 percent the cyclic GMP response to 1 mM carbamylcholine, had no effect on the response to 0.1 mM histamine. These last results indicate that muscarinic receptors were not involved in this cyclic GMP response to histamine.

Prior incubation of cells with carbamylcholine caused a rapid loss in sensitivity (desensitization) to subsequent stimulation of cyclic [^{3}H]GMP formation by this cholinergic agonist (*4*). Specific desensitization of this muscarinic receptor-mediated response was demonstrated by showing that histamine-stimulated cyclic [^{3}H]GMP formation was only slightly reduced by prior incubation with carbamylcholine. In these experiments, incubation of the cells for 30 minutes with histamine (0.1 mM) resulted in a greater than 90 percent reduction in the response to subsequent stimulation by histamine (0.1 mM) of the washed cells, whereas the response to carbamylcholine was only slightly reduced. These results indicate specific desensitization of the his-

tamine response and suggest that histamine is able to alter the concentration of H_1 receptors.

Mouse neuroblastoma clone N1E-115 cells in culture exhibit many properties of normal nerve cells (*9*). The demonstration that histamine stimulated cyclic GMP formation in this clone by activation of H_1 receptors suggests that histamine also would have this effect on some neuronal cell types in vivo. Although this point remains to be demonstrated, it has been shown that histamine stimulated cyclic GMP formation in bovine superior cervical ganglia (*10*).

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12. Twenty-five microliters each of 3.2*M* $ZnSO_4$ and 3.2*M* Na_2CO_3 were added to the 1.5-ml fraction from the cation exchange column. After sedimentation of the precipitate, the supernatant was quantitatively removed and counted for radioactivity in a liquid scintillation counter (Searle Isocap/300). The efficiency for counting tritium was 35 percent with 10 ml of Quantifluor (Mallinckrodt).
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16. I thank B. Thorpe and L. Neuroth for technical assistance. This work was supported by the May Foundation and a PHS grant from NIMH (MH 27692).

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Enhancement of Conditioned Arterial Pressure Responses in Cats After Brainstem Lesions

Abstract. *Cats were classically conditioned after the baroreceptor reflexes were abolished by bilateral placement of electrolytic lesions in the nucleus tractus solitarii. The conditioned increases in arterial pressure were more than five times larger than the responses obtained in similarly trained controls. This finding suggests that the baroreceptor reflexes actively inhibit conditioned increases of arterial pressure.*

We recently found that, in cats, lesions of the intermediate zone of the nucleus tractus solitarii (NTS) within the medulla oblongata abolish the baroreceptor reflexes (*1*) and produce labile arterial hypertension (*2*). In addition to the lability, the cats exhibit exaggerated arterial pressure responses associated with self-initiated behaviors and, in particular, with behaviors elicited by the incidental presentation of various sensory stimuli. The exaggerated vascular reactivity of these cats to sensory stimulation suggested to us that perhaps even larger and more sustained increases in arterial pressure might be produced if the cats were deprived of normal baroreceptor function by NTS lesions and then classically conditioned (Pavlovian conditioning). These procedures, entailing controlled presentation of sensory stimuli that signal the occurrence of a noxious event, have been used to produce conditioned increases of arterial pressure (*3*).

Twelve adult mongrel cats of both sexes were anesthetized and, under sterile conditions, the right common carotid artery was cannulated for the subsequent recording of arterial pressure and heart rate when the cats were awake and unrestrained (*4*). Another cannula was inserted into the right external jugular for the injection of drugs. Disk electrodes (1 cm in diameter) were implanted subcutaneously over the left flank and the right chest for the passage of electrical shock required by the classical conditioning procedure. All wires and cannulas were led through a cranial plug to a swivel that permitted free movement of the cat within the cage. The arterial cannula was connected to a strain gauge transducer for recording blood pressure and heart rate by standard methods.

After a recovery period of 2 weeks the baroreceptor reflexes were tested in all cats by measuring the reflexively mediated decreases of heart rate in response to administration of pressor doses of norepinephrine (0.5 μg/kg administered intravenously). After completion of these tests and establishment of baseline values of arterial pressure and heart rate, the cats were reanesthetized, and in six of the cats the region of the obex was exposed and electrolytic lesions were made bilaterally in the NTS (*5*). The other six cats, assigned to the control group, were similarly operated except that no current was passed after the electrodes were inserted into the NTS. Two weeks after this operation the tests of the baroreceptor reflexes were repeated and conditioning of the cats began.

Both groups were conditioned by the same procedure. The conditioning procedure was conducted for 30 daily sessions (five to seven per week). Two tones of different frequencies (2222 and 1136 Hz, referenced to a C-weighted scale) were presented randomly during each session, each tone being presented ten times. The high tones terminated with delivery, through the disk electrodes, of an electrical shock, while the low tones were not followed by a shock. The voltage level of the shock (50-Hz square wave, 10 to 36 V for 1 second) was set to elicit an increase in arterial pressure of 40 to 50 mm-Hg. At these voltage levels discomfort to the cats was minimal. The duration of the tones was gradually lengthened as training progressed. In order to expedite the formation of an association between the onset of the tone and delivery of the electrical shock, the duration of the tone was set at 10 seconds for sessions 1 to 10. In order to test the maintenance of the conditioned response, the duration of the tone was lengthened to 30 seconds for sessions 11 to 20 and to 60 seconds for sessions 21 to 30. The intertone interval varied randomly between 60 and 150 seconds.

The conditioned cardiovascular responses recorded from each cat were sampled at a rate of 50 Hz for processing by a digital computer. In order to adjust for baseline variations, each conditioned response was computed as the change relative to the average response level recorded for the 10 seconds just prior to presentation of the tone. The number of data samples was reduced to 60 values by dividing the tone period into 1-second intervals and computing the average cardiovascular response that occurred during each interval. The same procedure was followed for the 10-second period following termination of the tone. Each conditioned response for purposes of statistical comparison was reduced to the maximum response increase during the tone, the response during the 20th second of the tone, and the average re-

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