*p*H 7.6, and dialyzed in Visking tubing against 8 liters of the same buffer, with two changes over a 48-hour period. The ADH activity was measured according to the method of Bonnishsen and Brink (*10*), which utilizes the generation of reduced nicotinamide adenine dinucleotide (NADH) as ethanol is oxidized. The enzymatic activity for each testicular homogenate at each age of rats studied is shown in Table 1. The overall total testicular ADH activity expressed per gonad pair is lowest in the least mature animals. In contrast, specific activity (ADH activity per milligram of testicular protein) is highest in the 20-day-old animals and declines progressively with increasing maturity.

The ability of these same preparations to metabolize retinol to retinal was assayed according to the method of Mezey and Holt (*9*), where the retinal generated is determined spectrophotometrically by using its known absorption maximum at 410 nm. The enzymatic activity for retinal generation by 35 to 60 percent $(NH_4)_2SO_4$ fractions of rat testicular homogenates is also shown in Table 1. The specific activity of ADH for retinal formation was also highest in the least mature preparation and declined with increasing age, which suggests that the enzyme is present in either the Leydig cells or the Sertoli cells. Finally, as shown in Fig. 1, it was demonstrated that ethanol, in concentrations as low as 1/100 of the concentration of retinol substrate and 1/10,000 of the maximum concentration found in the blood of legally nonintoxicated drinkers, inhibited retinal formation.

The results show that testicular tissue is capable of retinal formation and that, at least in vitro, testicular retinal production is inhibited by coincident ethanol oxidation. While the concentrations of retinol substrate available in testicular tissue in vivo are not at present known, the amounts of ethanol necessary for the inhibition of retinal formation appear to be well below the range of concentrations customarily found in alcoholic individuals. Specifically, it is probable that an average male alcoholic ingesting 33 ounces (~ 1 liter) of whiskey or its equivalent per day (*4*) and maintaining plasma concentrations of 50 to 150 mg per 100 ml for prolonged periods, might suppress testicular retinal production and ultimately interrupt normal spermatogenesis. Preliminary experiments dealing with this specific question have shown that chronic ethanol feeding to pair-fed rats does produce germinal cell injury in the alcohol-fed animal but not in the isocaloric control. Whether this injury is directly related to decreased retinal formation within the testes, however, has not yet been determined. Indeed, the consequences of ethanol ingestion on normal testicular function may be even more far-reaching. The presence of ADH within testicular tissue raises the possibility that ethanol ingestion may alter not only spermatogenesis but also testicular steroidogenesis as a consequence of cofactor utilization and the resultant change in the redox state of the Leydig cells.

DAVID H. VAN THIEL
JUDITH GAVALER, ROGER LESTER
Division of Gastroenterology, Department of Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania 15261

References

1. C. W. Lloyd and R. H. Williams, *Am. J. Med.* **4**, 315 (1948).
2. T. G. Morrione, *Arch. Pathol.* **37**, 39 (1944); H. S. Bennett, A. H. Baggenstass, H. R. Butt, *Am. J. Clin. Pathol.* **20**, 814 (1950).
3. L. J. Rather, *Arch. Intern. Med.* **80**, 397 (1947).
4. D. H. Van Thiel, R. Lester, R. J. Sherins, in preparation.
5. *First Special Report to the U.S. Congress on Alcohol and Health from the Secretary of Health, Education, and Welfare* (Department of Health, Education, and Welfare, Washington, D.C., 1971).
6. J. H. Leathem, in *The Testes*, A. D. Johnson, W. R. Gomes, N. L. Vandermark, Eds. (Academic Press, New York, 1970), vol. 3, pp. 188–190; S. W. Chausen, *J. Am. Med. Assoc.* **111**, 144 (1938); H. M. Evans, *Am. J. Physiol.* **99**, 477 (1932).
7. A. Steinberger and E. Steinberger, *Exp. Cell Res.* **44**, 429 (1966); E. Steinberger and A. Steinberger, in *The Gonads*, K. W. McKerns, Ed. (Appleton-Century-Crofts, New York, 1969), pp. 715–732.
8. A. J. Patek, Jr., and C. Haig, *J. Clin. Invest.* **18**, 609 (1939).
9. E. Mezey and P. R. Holt, *Exp. Mol. Pathol.* **15**, 148 (1971).
10. R. K. Bonnishsen and N. G. Brink, *Meth. Enzymol.* **1**, 495 (1955).

14 May 1974; revised 24 July 1974

Guanosine 3′,5′-Monophosphate in Sympathetic Ganglia: Increase Associated with Synaptic Transmission

Abstract. *Brief stimulation of cholinergic preganglionic nerve fibers resulted in an increase in guanosine 3′,5′-monophosphate (cyclic GMP) in the bullfrog sympathetic ganglion. When the release of synaptic transmitter was prevented by a high-magnesium, low-calcium Ringer solution, stimulation of preganglionic nerve fibers did not increase cyclic GMP in the ganglion. The increase in cyclic GMP caused by preganglionic stimulation was also blocked by the muscarinic antagonist, atropine. The data indicate that the increase in cyclic GMP is associated with synaptic transmission and support the possibility that cyclic GMP may mediate the postsynaptic action of acetylcholine at muscarinic cholinergic synapses.*

Guanosine 3′,5′-monophosphate (cyclic GMP) is distributed in a wide variety of tissues, but little is known about its functional role (*1*). Cyclic GMP can be elevated in brain by the systemic administration of oxotremorine (*2*), an agent known to affect cholinergic mechanisms in the central nervous system. Moreover, cyclic GMP concentrations in brain slices can be increased by acetylcholine (ACh) or other muscarinic cholinergic agonists (*3*, *4*). These elevations of cyclic GMP can be antagonized by atropine (*2*, *4*), which suggests that the effect may be related to the activation of muscarinic receptors. However, the functional significance of cyclic GMP in the nervous

Table 1. Effect of stimulation of preganglionic nerve fibers on the cyclic GMP content (picomoles per milligram of protein) of bullfrog sympathetic ganglia in normal and high-Mg (20 m*M*), low-Ca (0.4 m*M*) Ringer solution; and in normal Ringer solution and Ringer solution containing atropine alkaloid (free base, 10 μg/ml; from Nutritional Biochemical Co.). Stimulus frequency was 10 hertz. The number of control and of stimulated samples analyzed is *N*; each sample contained 12 ganglia pooled from six bullfrogs. The data are given as mean ± standard error.

Ringer solution	Duration of stimulation (sec)	*N*	Cyclic GMP			
			Control (pmole/mg)	Stimulated (pmole/mg)	Absolute (pmole/mg)	Percentage increase
Normal	120	5	0.85 ± 0.18	1.78 ± 0.40	0.93 ± 0.24	105 ± 12
High-Mg, low-Ca	120	5	0.45 ± 0.08	0.49 ± 0.08	0.04 ± 0.04	13 ± 9
Normal	15	8	0.71 ± 0.10	1.22 ± 0.20	0.50 ± 0.16	76 ± 22
Atropine	15	6	0.46 ± 0.06	0.45 ± 0.08	−0.01 ± 0.05	−4 ± 11

system, and, particularly, the possible relationship of this nucleotide to cholinergic transmission, require further clarification.

The sympathetic ganglion seemed well suited for an investigation of a possible role of cyclic GMP in cholinergic transmission, because the presynaptic fibers form cholinergic synapses with the neurons in the ganglion (*5*). Furthermore, in the bullfrog sympathetic ganglion, muscarinic cholinergic mechanisms are involved in the generation of slow synaptic potentials (*6, 7*), and data are available on the ionic basis of these slow potentials (*8, 9*). We therefore used the bullfrog sympathetic ganglion to investigate whether cyclic GMP content can be altered by stimulation of preganglionic nerve fibers.

The ninth and tenth paravertebral sympathetic ganglia were excised from pithed bullfrogs (*Rana catesbeiana*) and placed in a bath of oxygenated Ringer solution, together with the corresponding ganglia from the contralateral side, which served as unstimulated controls (*10*). The preganglionic nerve fibers were stimulated at a frequency of 10 hertz for various periods of time, the postganglionic response being monitored throughout the period of stimulation. Immediately after stimulation, the stimulated pair of ganglia were immersed in 0.5 ml of distilled water in a boiling water bath for 3 minutes. After six pairs of stimulated ganglia were accumulated for each chemical analysis, the samples were pooled, frozen, and stored at −70°C. The six pairs of resting contralateral ganglia were treated in an identical manner. Cyclic GMP content of the ganglia was determined by radioimmunoassay (*11*).

The mean cyclic GMP content of all resting sympathetic ganglia analyzed was 0.82 pmole per milligram of protein (*12*). Stimulation of preganglionic nerve fibers produced a significant increase in cyclic GMP content, shown in Fig. 1 as a function of the duration of stimulation. Cyclic GMP was elevated by a period of stimulation as short as 6 seconds. The increase in cyclic GMP was rapid during the first 15 to 30 seconds of stimulation, reaching a maximum concentration approximately twice that of the control. This elevated cyclic GMP content was maintained for at least 2 minutes, the longest period of stimulation tested (*13*).

The increase in cyclic GMP content of the ganglion, produced by preganglionic stimulation, raised the question

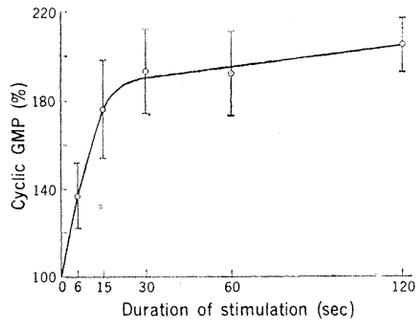

Fig. 1. Effect of stimulation of preganglionic nerve fibers on the cyclic GMP content of bullfrog sympathetic ganglia. The stimulus frequency was 10 hertz for the time periods shown. Cyclic GMP content is expressed as percentage (mean ± standard error) of the value in unstimulated contralateral control ganglia. Five to eight experimental samples and control samples were analyzed in duplicate for each point; each sample contained 12 ganglia pooled from six bullfrogs. The preganglionic nerves were stimulated between the eighth and ninth ganglia, with rectangular pulses 1 msec in duration and 20 volts in amplitude, by using bipolar electrodes in a mineral oil pool.

whether the increase was associated with impulse conduction along the nerve fibers or with synaptic transmission. Ringer solution containing high Mg and low Ca is known to prevent transmitter release from nerve endings in the sympathetic ganglion (*7*), as well as at other synaptic junctions (*14*). In contrast, nerve impulses are conducted in the presence of high-Mg, low-Ca Ringer solution (*15*). To determine whether an increase in cyclic GMP would be observed if no synaptic transmitter were released, we studied the effect of preganglionic nerve stimulation on ganglionic cyclic GMP content in high-Mg, low-Ca Ringer solution (*16*). As shown in Table 1, the increase in cyclic GMP content that occurred with 2 minutes of stimulation in normal Ringer solution was not observed in ganglia stimulated for the same period of time in high-Mg, low-Ca Ringer solution. This result suggests that the increase in cyclic GMP in the sympathetic ganglion is associated with synaptic transmission, rather than with impulse conduction along the preganglionic nerve fibers.

Since ACh is known to be released in amphibian sympathetic ganglia by stimulation of preganglionic nerves (*5*), we studied whether an ACh receptor might be associated with the increase in cyclic GMP. Slow synaptic potentials are mimicked by the iontophoretic administration of ACh (*7, 17*) and blocked by the administration of atropine (*6*) in frog sympathetic ganglia. It is generally accepted that atropine is a competitive antagonist of ACh at muscarinic receptors (*18*). We therefore used atropine to determine whether muscarinic receptors might be involved in the increase in cyclic GMP following stimulation of preganglionic nerve fibers. Stimulation for 15 seconds at 10 hertz approximates the number of impulses usually used to elicit the slow synaptic potentials in physiological experiments (*8, 9*). After the ganglia were stimulated for 15 seconds in normal Ringer solution, the cyclic GMP content was 176 percent of the control value. In the presence of atropine (10 μg/ml), however, preganglionic stimulation for 15 seconds did not produce a significant change in the cyclic GMP content of the ganglion (Table 1). Nicotinic cholinergic transmission through the ganglion (*19*), monitored by recording the postganglionic action potential, was not affected by the atropine. The results with atropine suggest that the activation of muscarinic receptors is responsible for the increase in cyclic GMP, and that neither nicotinic cholinergic transmission in sympathetic ganglion cells nor the associated propagation of impulses along the axons from these cells is responsible for the increase in cyclic GMP.

Cyclic GMP in nervous tissue can be elevated by the administration of ACh or muscarinic cholinergic agonists (*3, 4*), but it has not previously been demonstrated that the change in cyclic GMP is associated with synaptic transmission. The data reported here demonstrate that the cyclic GMP content of nervous tissue can be significantly increased by stimulation of presynaptic cholinergic axons. The increase in cyclic GMP produced by stimulation of preganglionic cholinergic nerve fibers was antagonized by high Mg and low Ca concentrations and by atropine, which suggests that the increase is associated with synaptic transmission and involves the activation of muscarinic postsynaptic receptors. In support of this conclusion, either ACh or bethanechol, a muscarinic agonist, can increase the content of cyclic GMP in slices of bovine sympathetic ganglia, and this response is antagonized by atropine (*20*). Moreover, by using a histochemical procedure for localizing cyclic GMP (*21*), evidence has been obtained that the ACh-induced accumulation of cyclic GMP occurs in the postganglionic neurons (*22*).

Stimulation of cholinergic preganglionic axons not only increases cyclic GMP but also generates slow synaptic potentials in the bullfrog sympathetic ganglion (*6, 7*). The fact that both responses are mediated by the activation of muscarinic receptors suggests that the generation of the slow synaptic potential or potentials may be related to the increase in cyclic GMP. Compatible with this possibility, administration of dibutyryl cyclic GMP to rabbit (*23*) or bullfrog (*24*) sympathetic ganglia produces a transient hyperpolarization followed by a depolarization of the ganglia. In view of these data, it seems reasonable to hypothesize that ACh, released from presynaptic nerve terminals, activates muscarinic receptors on postsynaptic neurons, causing an increase in cyclic GMP in the neurons, and that the increase in cyclic GMP results in a depolarization of the membrane, that is, a slow excitatory postsynaptic potential. It is possible that a cyclic nucleotide is also involved in the generation of the slow inhibitory postsynaptic potential.

FORREST F. WEIGHT
Laboratory of Neuropharmacology, National Institute of Mental Health, St. Elizabeths Hospital, Washington, D.C. 20032
GARY PETZOLD
PAUL GREENGARD
Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

References and Notes

1. N. Goldberg, R. O'Dea, M. Haddox, in *Advances in Cyclic Nucleotide Research*, P. Greengard and G. Robison, Eds. (Raven, New York, 1973), vol. 3, pp. 155–223.
2. J. A. Ferrendelli, A. Steiner, D. McDougal, D. Kipnis, *Biochem. Biophys. Res. Commun.* **41**, 1061 (1970).
3. J.-F. Kuo, T.-P. Lee, P. Reyes, K. Walton, T. Donnelly, P. Greengard, *J. Biol. Chem.* **247**, 16 (1972).
4. T.-P. Lee, J.-F. Kuo, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3287 (1972).
5. S. Nishi, H. Soeda, K. Koketsu, *J. Neurophysiol.* **30**, 114 (1967).
6. S. Nishi and K. Koketsu, *ibid.* **31**, 109 (1968); B. Libet, S. Chichibu, T. Tosaka, *ibid.*, p. 383; T. Tosaka, S. Chichibu, B. Libet, *ibid.*, p. 396.
7. F. Weight and A. Padjen, *Brain Res.* **55**, 225 (1973).
8. F. Weight and J. Votava, *Science* **170**, 766 (1970).
9. F. Weight and A. Padjen, *Brain Res* **55**, 219 (1973).
10. The composition of the Ringer solution was: 100 m*M* NaCl; 2 m*M* KCl; 1.8 m*M* $CaCl_2$; 16 m*M* tris(hydroxymethyl)aminomethane chloride (tris Cl), *p*H 7.2; and glucose, 1 g/liter. The ganglia were equilibrated for approximately 1 hour in normal Ringer solution or in Ringer solution containing either high Mg and low Ca or atropine. All experiments were at room temperature (26° ± 1°C).
11. Samples were thawed, sonicated, and centrifuged. Protein in the precipitate was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)]. Cyclic GMP in the lyophilized supernatant was determined by the method of A. Steiner, C. Parker, and D. Kipnis [*ibid.* **247**, 1106 (1972)] except that the antigen-antibody complex was precipitated by the addition of 4.0 ml of a solution containing trichloroacetic acid (5 percent) and Na_2WO_4 (0.25 percent), *p*H 2.0. Analyses were performed on triplicate portions, including one with internal standard. Cyclic GMP in resting or stimulated ganglia could be completely hydrolyzed by beef heart phosphodiesterase.
12. The value of 0.82 ± 0.07 pmole per milligram of protein (mean value ± standard error) was obtained from analyses of 51 pooled samples of 12 ganglia each (ninth and tenth ganglia from six frogs). The experiments were conducted between October and May in two successive years.
13. Cyclic AMP was also increased by preganglionic stimulation; for example, stimulation for 2 minutes at 10 hertz increased the cyclic AMP content to 782 ± 85 percent ($N = 5$) of the control value.
14. R. Rubin, *Pharmacol. Rev.* **22**, 389 (1970).
15. B. Katz, *The Release of Neural Transmitter Substances* (Thomas, Springfield, Ill., 1969).
16. The composition of the high-Mg, low-Ca Ringer solution was: 100 m*M* NaCl; 2 m*M* KCl; 20 m*M* $MgCl_2$; 0.4 m*M* $CaCl_2$; 4 m*M* tris Cl, *p*H 7.2; and glucose, 1 g/liter. The effect of high Mg and low Ca on impulse conduction was studied in six control experiments by recording the preganglionic action potential between the ninth and tenth ganglia. Neither the threshold nor the maximal amplitude of the preganglionic action potential was appreciably altered by 1 hour in the high-Mg, low-Ca Ringer solution. Furthermore, the stimulus strength used was more than twice that required to maximally activate the C fibers.
17. K. Koketsu, S. Nishi, H. Soeda, *Life Sci.* **7**, 741 (1968).
18. L. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, New York, 1970).
19. J. Blackman, B. Ginsborg, C. Ray, *J. Physiol. (Lond.)* **167**, 355 (1963)
20. J. Kebabian, A. Steiner, P. Greengard, *J. Pharmacol. Exp. Ther*, in press.
21. E. Fallon, R. Agrawal, E. Furth, A. Steiner, R. Cowden, *Science* **184**, 1089 (1974).
22. J. Kebabian, A. Steiner, F. Bloom, P. Greengard, in preparation.
23. D. McAfee and P. Greengard, *Science* **178**, 310 (1972).
24. N. Busis and F. Weight, unpublished observations.

8 July 1974; revised 16 August 1974

Electrophysiological Correlates of Meaning

Abstract. *The use of context-sensitive symbols offers an appropriate methodology for investigating the representation of meaning in the brain. This approach revealed that late components of frontal, but not occipital, evoked potentials reflect the change of meaning of a symbolic stimulus when it appears in different temporal contexts.*

Although scientists agree that the human brain is the organ of the body responsible for the elaboration of meaning, little is known of the neuropsychological mechanisms involved. We report here that neuronal activity in the frontal lobes, as evidenced by changes in the wave form of evoked potentials recorded from this area, is indicative of a change in the meaning of a stimulus.

Several experiments have suggested that the wave shape of stimulus-locked potentials may reflect a change in meaning. For example, John *et al.* (*1*) demonstrated consistent differences in the late components of visual evoked potentials (VEP's) induced by two very similar stimuli, a square and a rotated square (diamond), irrespective of the stimulus size. However, the interpretation of such experiments is difficult because the experimental procedure involves a change in the physical stimulus as well as a change in meaning. Physical attributes of a stimulus change the wave form of VEP's (*2, 3*) so, in order to avoid confounding the meaning change with the physical stimulus change, it is necessary to keep the latter constant.

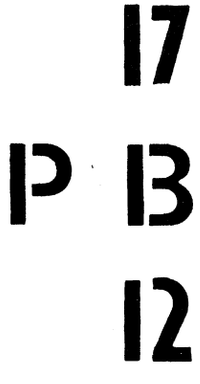

Fig. 1. Stimuli used in experimental procedures. Central stimulus is ambiguous.

It is possible to alter the meaning of a constant stimulus by adding a new association using a conditioning procedure, for example, pairing a visual stimulus with an auditory click (*4*). However, any change in the VEP as a result of this may reflect enhanced arousal or attention rather than the meaning change per se. Modification of VEP's by such variables as expectancy, affect, uncertainty, or attentional state have been demonstrated in many situations (*5*). The conditioning procedure not only brings about a change in meaning but may also have a quantitative effect on one or more of these state variables. The difficulties inherent in equating both the physical stimulus and state variables may be circumvented by the use of a symbolic stimulus that has two or more distinct meanings depending on the context in which it is presented.

The central symbol in Fig. 1 can be interpreted as "B" or "13," depending