protein into the nerve parenchyma (*15*) may account for the relative independence of the disease induction from the level of antibody titer. Initially after immunization with GC, antibodies to GC consist predominantly of immunoglobulin (Ig) M. Immunoglobulin G against GC may not rise for several months (*27*). Although IgG is able to permeate into the endoneurium, IgM, a larger molecule than IgG, cannot penetrate the nonpathological nerve (*28*). It is possible that the delayed appearance of IgG against GC coincided with the induction of the demyelinative neuritis. Although GC reportedly does not induce positive skin reactions (*6*), the possible role of cell-mediated immunity cannot be excluded. However, the absence of perivenular cuffings of small lymphocytes in the early lesions in this study also supports the concept that GC-induced EAN may be primarily antibody-dependent rather than the result of tuberculin-type delayed hypersensitivity.

Possible involvement of GC in the immunopathogenesis of EAE and EAN produced by whole white matter or nerve homogenates, has been implied by the detection of antibody to GC in the serum of such animals (*6-8, 12, 29*). Antibody to GC was also found in cerebrospinal fluid of EAE animals (*30*). The present report provides evidence that GC is capable of playing a primary role in the production of a demyelinating disorder.

TAKAHIKO SAIDA
KYOKO SAIDA
SUSAN H. DORFMAN
DONALD H. SILBERBERG
AUSTIN J. SUMNER
MARGARET C. MANNING
ROBERT P. LISAK
MARK J. BROWN

Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia 19104

References and Notes

1. K. E. Åstrom, H. D. Webster, G. C. Arnason, *J. Exp. Med.* **128**, 469 (1968); R. H. M. Ballin and P. K. Thomas, *J. Neurol. Sci.* **8**, 1 (1968); R. W. Lampert, *Lab. Invest.* **20**, 127 (1969); J. M. Schröder and W. Krücke, *Acta Neuropathol. (Berlin)* **14**, 261 (1970); B. W. Waksman and R. D. Adams, *J. Exp. Med.* **102**, 213 (1955); B. H. Waksman and R. D. Adams, *J. Neuropathol. Exp. Neurol.* **15**, 293 (1956).
2. O. Abramsky, D. Teitelbaum, R. Arnon, *Eur. J. Immunol.* **7**, 213 (1977); O. Abramsky, D. Teitelbaum, C. Webb, R. Arnon, *J. Neuropathol. Exp. Neurol.* **34**, 36 (1975); S. W. Brostoff, P. Burnett, P. Lampert, E. H. Eylar, *Nature New Biol. (London)* **235**, 210 (1972); S. W. Brostoff, S. Levit, J. M. Powers, *Nature (London)* **268**, 752 (1977); M. Kadenbowski and R. A. C. Hughes, *ibid.* **277**, 140 (1979); K. Uemura, C. Tobari, S. Hirano, T. Tsukada, *J. Neurochem.* **19**, 2706 (1972).
3. M. W. Kies, in *Multiple Sclerosis*, F. Wolfgram, G. W. Ellison, J. G. Stevens, J. M. Andrews, Eds. (Academic Press, New York, 1972), p. 429.
4. P. W. Lampert and M. W. Kies, *Exp. Neurol.* **18**, 210 (1967).
5. W. T. Norton, in *Myelin*, P. Morell, Ed. (Plenum, New York, 1977), p. 161.
6. B. Niedieck, *Prog. Allergy* **18**, 353 (1975); ———, E. Kuwert, O. Palacios, O. Drees, *Ann. N.Y. Acad. Sci.* **122**, 266 (1965).
7. J. M. Oxberry and N. A. Gregson, *Brain Res.* **78**, 303 (1974); M. M. Rapport and L. Graf, *Ann. N.Y. Acad. Sci.* **122**, 277 (1965); M. M. Rapport, in *Handbook of Neurochemistry*, A. Lajtha, Ed. (Plenum, New York, 1970), vol. 3, p. 509.
8. T. Saida, D. H. Silberberg, J. M. Fry, M. C. Manning, *J. Neuropathol. Exp. Neurol.* **36**, 627 (1977).
9. T. Saida, K. Saida, S. Dorfman, M. J. Brown, R. P. Lisak, M. Manning, D. H. Silberberg, *ibid.* **37**, 685 (1978).
10. M. C. Raff, R. Mirsky, K. L. Fields, R. P. Lisak, S. H. Dorfman, D. H. Silberberg, N. A. Gregson, S. Leibowitz, M. C. Kennedy, *Nature (London)* **272**, 813 (1978); A. B. Johnson and M. B. Bornstein, *Brain Res.* **159**, 173 (1978).
11. M. Dubois-Dalcq, B. Niedieck, M. Buyse, *Pathol. Eur.* **5**, 331 (1970).
12. J. M. Fry, S. Weissbarth, G. M. Lehrer, M. B. Bornstein, *Science* **183**, 540 (1974).
13. S. Hruby, E. C. Alvord, Jr., F. J. Seil, *ibid.* **195**, 173 (1977); T. Yonezawa, T. Saida, M. Hasegawa, in *Multiple Sclerosis in Asia*, Y. Kuroiwa, Ed. (University Park Press, Baltimore, 1976), p. 225; in *International Symposium on the Aetiology and Pathogenesis of the Demyelinating Diseases*, H. Shiraki and T. Yonezawa, Eds. (Japan Science Press, Tokyo, 1976), p. 255.
14. B. H. Waksman, *J. Neuropathol. Exp. Neurol.* **20**, 35 (1961).
15. Y. Olsson, in *Peripheral Neuropathy*, P. J. Dyck, P. K. Thomas, E. H. Lambert, Eds. (Saunders, Philadelphia, 1975), p. 190.
16. Standard lipids were synthetic *N*-lignoceroyl-, palmitoyl-, and stearoyl-dihydrogalactocerebroside (prepared by Sigma following the procedure of D. Shapiro), phosphatidylethanolamine, phosphatidylcholine, and sphingomyelin.
17. E. H. Lambert, *Electroencephalogr. Clin. Neurophysiol.* **22** (Suppl.), 9 (1962).
18. After pentobarbital anesthesia, rabbits were perfused through the aorta with physiological saline, followed by 3.6 percent glutaraldehyde buffer. Sections were taken from the following portions: brain, optic nerve, brainstem, cerebellum; cervical, thoracic, and lumbar spinal cord; spinal ganglia, roots, cauda equina, brachial plexus, and sciatic and posterior tibial nerves. Paraffin sections were stained with hematoxylin-eosin and Luxol fast blue-periodic acid Schiff-hematoxylin. Epoxy sections (1 μm) were stained with toluidine blue. Electron microscopic studies were performed as described in K. Saida, T. Saida, M. J. Brown, D. H. Silberberg, A. K. Asbury, *Lab. Invest.* **39**, 449 (1978).
19. A. K. Asbury, B. G. Arnason, R. D. Adams, *Medicine* **48**, 173 (1969); J. W. Prineas, *Lab. Invest.* **26**, 133 (1972); H. Wisniewski, R. D. Terry, J. N. Whitaker, S. D. Cook, P. C. Dowling, *Arch. Neurol.* **21**, 269 (1969).
20. Rabbits were bled just before and 3 weeks after each immunization (schedules I and II) or weekly (schedule III), and at the time of the onset of clinical EAN and the autopsy.
21. J. M. Fry, R. P. Lisak, M. C. Manning, D. H. Silberberg, *J. Immunol. Methods* **11**, 185 (1976); S. H. Dorfman, J. M. Fry, D. H. Silberberg, C. Grose, M. Manning, *Brian Res.* **147**, 410 (1978).
22. R. P. Lisak, O. Abramsky, S. Dorfman, J. George, M. Manning, D. Pleasure, T. Saida, D. H. Silberberg, *J. Neurol. Sci.* **40**, 65 (1979).
23. C. R. Alving, in *The Antigens*, S. Michael, Ed. (Academic Press, New York, 1977), vol. 4, p. 1; C. R. Alving, J. W. Fowble, K. C. Joseph, *Immunochemistry* **11**, 475 (1974).
24. R. P. Lisak, R. G. Heinze, M. W. Kies, *Int. Arch. Allergy* **37**, 621 (1970).
25. K. Saida, T. Saida, M. J. Brown, D. H. Silberberg, *J. Neuropathol. Exp. Neurol.* **36**, 627 (1977); K. Saida, T. Saida, M. J. Brown, D. H. Silberberg, *Am. J. Pathol.* **95**, 99 (1979).
26. T. Saida, K. Saida, D. H. Silberberg, M. J. Brown, *Nature (London)* **272**, 639 (1978); T. Saida, K. Saida, M. J. Brown, D. H. Silberberg, *J. Neuropathol. Exp. Neurol.*, in press.
27. C. R. Alving and R. L. Richards, *Immunochemistry* **14**, 373 (1977); D. H. Conrad, C. R. Alving, G. H. Wirtz, *Biochim. Biophys. Acta* **332**, 36 (1974).
28. J. M. J. Van Lis and F. G. I. Jennekens, *J. Neurol. Sci.* **34**, 329 (1977).
29. R. A. C. Hughes and S. Leibowitz, *Immunology* **28**, 213 (1975).
30. E. Kuwert and B. Niedieck, *Nature New Biol. (London)* **207**, 991 (1965).
31. We thank Drs. A. K. Asbury, B. Zweiman, and M. Momoi for discussions. Supported by NIH grants NS11037 and NS08075, National Multiple Sclerosis Society grant 894-B, and the Kroc Foundation.

4 January 1979; revised 12 March 1979

GABA Receptor Control of Parasympathetic Outflow to Heart: Characterization and Brainstem Localization

Abstract. *Blockade of γ-aminobutyric acid (GABA) receptor function by direct microinjection of the GABA receptor antagonist bicuculline into the nucleus ambiguus of the brainstem produced a marked, dose-related depression of heart rate and blood pressure which was mediated by the vagus nerve. This effect was not obtained in other regions of the brainstem and was reversed by the GABA receptor agonist muscimol. These data indicate that the nucleus ambiguus may be the site of a GABA receptor-mediated inhibition of vagal outflow.*

The recent availability of muscimol, a potent and selective agonist for γ-aminobutyric acid (GABA) receptors (*1*) has spurred investigations into the role of GABA receptors in specific brain regions for the control of such diverse functions as food intake (*2*), motor activity (*3*), synthesis of various neurotransmitters (*4*), and release of pituitary hormones (*5*). Moreover, the combined use of muscimol and the specific GABA receptor antagonist bicuculline (*1*), has allowed the exploration of the effects of both augmentation and reduction in GABA receptor function in various brain systems. Recently, evidence has emerged suggesting that GABA receptors might also be involved in the central autonomic control of cardiovascular function (*6, 7*), but the role and anatomical site (or sites) of such a GABA influence have not been characterized. Antonaccio and co-workers have reported that muscimol, administered into the cerebral ventricles, decreased heart rate and blood pressure and that this effect of muscimol was reversed by the GABA antagonists bicuculline and picrotoxin (*6*). In contrast, we have reported that bicuculline and picrotoxin, administered via the verte-

0036-8075/79/0608-1106$00.50/0

bral artery, produced decreases in heart rate and blood pressure which could be reversed by administering muscimol (*7*). It appears that the GABA agonist-induced bradycardia is caused by an inhibition of sympathetic outflow (*6*) whereas the GABA antagonist-induced bradycardia is caused by stimulation of parasympathetic function, mediated by vagal nerves innervating the heart (*7*). Consistent with the latter observation is the finding that the benzodiazepines, agents thought to potentiate GABA function (*8*), also inhibit reflex vagal bradycardia (*9*). Furthermore, initial observations have suggested that the GABA receptors that influence sympathetic cardiovascular activity are located in the forebrain (*6*) whereas those involved in parasympathetic cardiac function are located in the brainstem (*7*). Thus, it is possible that GABA may be an important neurotransmitter in central autonomic pathways and that it may have critically different actions depending upon the location of the GABA-receptive neurons.

In the present study, we sought to demonstrate a relationship between GABA receptors and cardiac parasympathetic function by determining the specific anatomical site (or sites) where GABA receptor-mediated influences on vagal outflow could occur. Of particular interest to us was the nucleus ambiguus, since several electrophysiological studies have indicated that this nucleus may contain the cell bodies of preganglionic vagal neurons projecting to the heart (*10*). Furthermore, cells in the nucleus ambiguus have been reported to contain significant amounts of GABA (*11*). By monitoring heart rate and using a microinjection technique to apply bicuculline methiodide and muscimol into this nucleus as well as into other brainstem nuclei associated with central vagal pathways, we were able to identify a selective response to these compounds which was specifically localized to the region of the nucleus ambiguus.

Cats were anesthetized with α-chloralose (70 to 80 mg per kilogram of body weight, intravenously) and artificially ventilated with room air. The femoral artery was catheterized for recording blood pressure and limb leads were placed on the extremities for recording the electrocardiogram. Both were monitored on a Beckman Dynograph recorder. Rectal temperature was maintained between 36° and 38°C by intermittent warming of the cat with radiant heat. Cats were then mounted in a stereotaxic instrument and the dorsal surface of the lower brainstem was exposed by a limited occipital craniotomy. Coordinates taken from Berman (*12*) were used to locate the nucleus under study. The shaft of a 26-gauge Quincke-Babcock spinal needle that had been cut from its base and filed smooth was guided stereotaxically at a 42° angle into the nucleus area. This needle was attached to a 10 μl Unimetrics syringe by PE 20 tubing. Drugs were dissolved in 0.9 percent saline and administered by infusing (Sage infusion pump) at rates ranging from 0.02 μl/min for 5 minutes to 0.1 μl/min for 1 minute to give a total volume of 0.1 μl of infusate. For control infusions, saline alone was used. At the termination of each experiment 5 to 10 μl of 10 percent formalin was infused into the injection site followed by 1 μl of concentrated methylene blue dye. The animal was then killed and the brain was removed and placed in 10 percent formalin. Frozen 25-μm sections were cut and mounted on slides. From these sections, the cannula track was identified and verification of the injection site was made.

Microinjections of the GABA antagonist bicuculline methiodide were made into the following nuclei thought to comprise part of the central pathways influencing vagal outflow to the heart: nucleus ambiguus, external cuneate nucleus, and the regions of nucleus tractus solitarius and dorsal motor nucleus of the vagus. Injections into only one of these nuclei—namely, nucleus ambiguus—produced a pronounced slowing in heart rate. Significant effects were observed with doses as little as 1 ng of bicuculline methiodide, and doses between 4 and 20 ng of bicuculline methiodide produced reliable dose-related decreases in sinus rate ranging from 27 to 60 beats per minute (mean values). Slowing in heart rate produced by these injections lasted from 2 to 76 minutes with the largest doses producing the longest duration of response. In addition, maximal bradycardia was often accompanied by sinus arrest and junctional escape rhythms. Finally, injection of an equivalent volume of saline into nucleus ambiguus had no significant effect on heart rate (*13*).

Injection of bicuculline methiodide into the external cuneate nucleus and into the regions of nucleus tractus solitarius and dorsal motor nucleus of the vagus failed to produce any significant slowing in heart rate. Bicuculline methiodide (5 to 10 ng) was also infused into a series of sites within a coronal plane passing through the nucleus ambiguus. The experimental results obtained are depicted in Fig. 1 and indicate that responses to bicuculline methiodide are limited to a discrete area corresponding to the nucleus ambiguus. Microinjections made at a site as little as 0.5 mm from this nucleus usually yielded negative results.

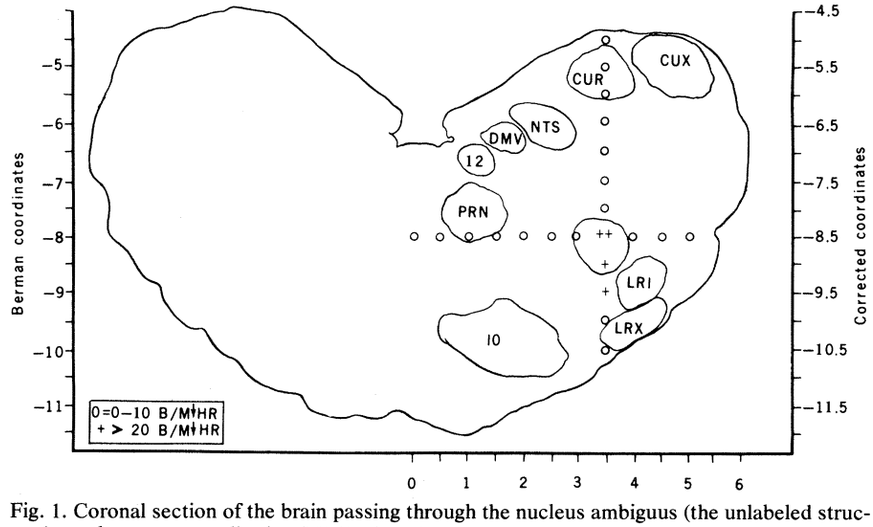

Fig. 1. Coronal section of the brain passing through the nucleus ambiguus (the unlabeled structure), nucleus tractus solitarius (*NTS*), dorsal motor nucleus of the vagus (*DMV*), external cuneate nucleus (*CUX*), cuneate nucleus, rostral division (*CUR*), hypoglossal nucleus (*12*), paramedian reticular nucleus (*PRN*), inferior olivary complex (*IO*), lateral reticular nucleus, internal division (*LRI*), and lateral reticular nucleus, external division (*LRX*). Data shown by the symbols ○ and + represent results of injections of bicuculline methiodide (5 to 10 ng) made at 0.5-mm intervals along vertical (one experiment) and horizontal (one experiment) axes passing through the nucleus ambiguus. Circles represent areas where no significant bradycardia was produced (decrease of less than 10 beats per minute) whereas plus signs represent sites where significant bradycardia was produced (greater than 20 beat-per-minute decrease). The axis at the left represents stereotaxic coordinates taken from the atlas of Berman (*12*). The axis at the right represents corrected stereotaxic coordinates as determined by examination of histological sections.

A representative experiment depicting the heart rate and arterial blood pressure effects of two doses of bicuculline methiodide injected into nucleus ambiguus is depicted in Fig. 2. As can be seen, 4 ng of bicuculline methiodide produced an immediate reduction in heart rate; this was associated with a fall in blood pressure. Effects on both rate and pressure reached a nadir 5 minutes after the start of injection and persisted. Administration of 20 ng produced an even greater reduction in rate and pressure. Once the effects of the larger dose were attained, an injection of the specific GABA receptor agonist, muscimol, was made. This agent given in a dose of 2 ng caused a slight reversal of the rate and pressure responses induced by bicuculline methiodide. Administration of a second and larger dose of muscimol (10 ng) completely reversed the negative chronotropic and hypotensive effects produced by bicuculline methiodide. In other experiments microinjection of 20 ng of bicuculline methiodide alone produced effects lasting for 60 minutes. Microinjection of 2 to 10 ng of muscimol alone had no effect on either heart rate or blood pressure. The bicuculline-induced depression of heart rate and arterial pressure was abolished by bilateral transections of the cervical vagus.

In two separate experiments, GABA synthesis was inhibited by the microinjection of 70 μg of isoniazid (*8*) (in a total volume of 0.5 μl infused over 5 minutes) into nucleus ambiguus. The effect of isoniazid resembled that produced by bicuculline, with a 45-beat-per-minute decrease in heart rate in one cat and a 70-beat-per-minute decrease in the second. A lower dose of isoniazid (10 μg) produced a slightly less marked effect. In contrast to the rapid onset of bradycardia observed after GABA receptor blockade, the decrease in heart rate observed after inhibition of GABA synthesis was observed only after a latency of 20 to 30 minutes from the time of isoniazid infusion. This delay is most likely a reflection of the time course of GABA depletion after isoniazid which reaches a maximum after 30 minutes (*8*). In one experiment, the bradycardia produced by isoniazid was found to be completely reversed by 10 ng of muscimol infused into the nucleus ambiguus. Reversal of the isoniazid-induced bradycardia began immediately after muscimol infusion, and heart rate returned to normal baseline level within 20 minutes.

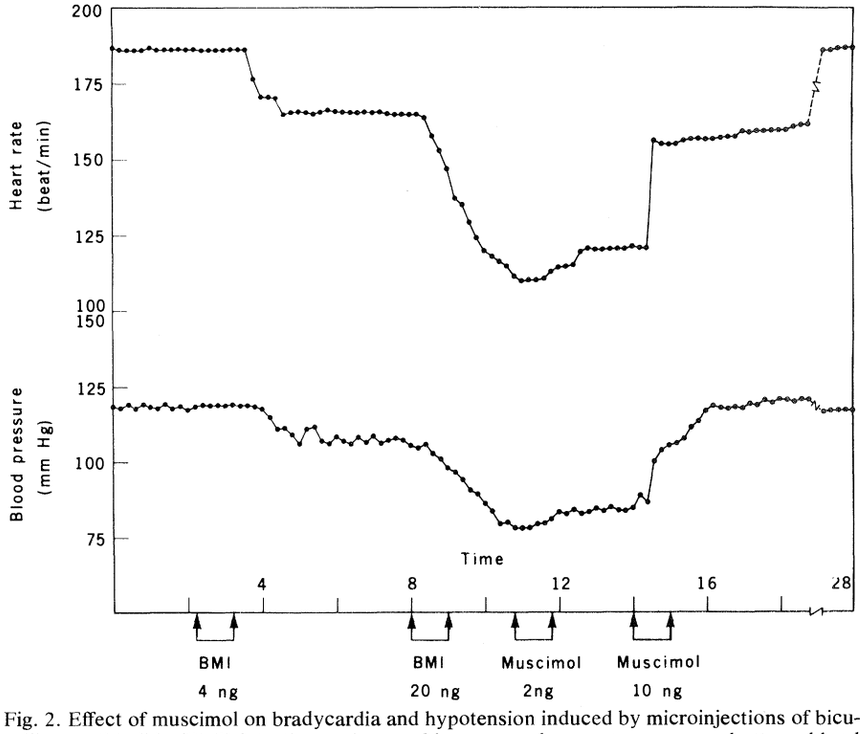

Fig. 2. Effect of muscimol on bradycardia and hypotension induced by microinjections of bicuculline methiodide (*BMI*) into the nucleus ambiguus; top, heart rate response; bottom, blood pressure response. All drugs were injected in volumes of 0.1 μl over 1 minute as indicated by the arrows on the horizontal axis. As can be seen, 4 and 20 ng of bicuculline methiodide produced increasing degrees of bradycardia and hypotension. Muscimol (2 ng) microinjected into the nucleus ambiguus arrested and partially reversed the falling heart rate and arterial pressure. A larger dose (10 ng) produced sharp increases in both parameters and resulted in a return to the control baseline values within 15 minutes after administration.

To assess the specificity of the response of nucleus ambiguus to bicuculline and isoniazid, the glycine antagonist strychnine hydrochloride was injected into the same sites where bicuculline or isoniazid were found to elicit a pronounced degree of cardiac slowing. This was done in three animals with molar amounts of strychnine up to twice the molar amounts of bicuculline. Amounts ranging between 10 and 60 ng produced variable heart rate effects. A dose of 10 ng of strychnine decreased rate by 10 beats per minute in one cat, while a dose of 60 ng increased rate by 19 beats per minute in the second cat and decreased rate by 25 beats per minute in the third.

Our present findings with microinjections of bicuculline methiodide, isoniazid, and muscimol thus confirm and extend those from our earlier experiments with vertebral arterial injections of bicuculline, picrotoxin, and muscimol. Our observation that nucleus ambiguus is the site where drugs affecting GABAergic transmission influence parasympathetic cardiac function is consistent with the proposal that this nucleus contains the cell bodies of the vagal projection to the heart (*10*).

Specificity of the muscimol reversal of the bicuculline- or isoniazid-induced bradycardia and hypotension was indicated by our previous observation that the bradycardia and hypotension induced by clonidine is not reversed by muscimol but instead can be reversed by the α-adrenergic receptor antagonist piperoxan (*7*). Conversely, piperoxan did not antagonize the bicuculline-induced bradycardia and hypotension, which suggests that the GABA receptor response we have described is not mediated by α-adrenergic neurons.

Although muscimol antagonized bicuculline- or isoniazid-induced decreases in heart rate and blood pressure, muscimol alone had no effect when placed in the nucleus ambiguus. Such a situation would be expected if the vagal outflow from the nucleus ambiguus is normally maintained in an inhibited or inactive state. This in fact appears to be true in the cat, as indicated by electrophysiological recordings made from cardiac vagal units (*14*). Therefore, in our experiments it was not until the parasympathetic response was activated by bicuculline or isoniazid that an inhibitory action of muscimol could be demonstrated in the nucleus ambiguus.

Further work is required to determine the source of the neuronal GABA (*11*) in the nucleus ambiguus, since very little is known about the neurotransmitter path-

ways in the brainstem which are involved in central regulation of parasympathetic function. In previous experiments we found that the response to GABA antagonists was not altered by a midcollicular transection (*7*), which suggests that the mechanisms involved in this response are located within the hindbrain. Whether GABA is released from the terminals of neurons intrinsic to the nucleus ambiguus, or from neurons projecting to this nucleus from more distant sites in the brainstem, it appears likely that GABA, by interacting with receptors on neurons in the nucleus ambiguus, may inhibit parasympathetic outflow to the heart.

JOSEPH A. DIMICCO
KAREN GALE
Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, Washington, D.C. 20007
BETTY HAMILTON
Department of Anatomy, Georgetown University Schools of Medicine and Dentistry
RICHARD A. GILLIS
Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry

References and Notes

1. G. A. R. Johnston, in *GABA in Nervous System Function*. E. Roberts, T. H. Chase, D. B. Tower, Eds. (Raven, New York, 1976), p. 395; S. J. Enna, J. F. Collins, S. H. Snyder, *Brain Res.* **124**, 185 (1977); K. Beaumont, W. S. Chilton, H. I. Yamamura, S. J. Enna, *ibid.* **148**, 153 (1978); P. Krosgaard-Larsen, G. A. R. Johnston, D. A. Curtis, C. J. A. Game, R. M. McCulloch, *J. Neurochem.* **25**, 803 (1975).
2. L. Grandison and A. Guidotti, *Neuropharmacology* **16**, 533 (1977).
3. J. Scheel-Kruger, A. R. Cools, W. Honig, *Eur. J. Pharmacol.* **42**, 311 (1977); J. Scheel-Kruger, J. Arnt, G. Magelund, *Neurosci. Lett.* **4**, 351 (1977).
4. K. Gale and A. Guidotti, *Nature (London)* **263**, 691 (1976); G. Zsilla, D. L. Cheney, E. Costa, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **294**, 251 (1976); K. Fuxe, T. Hokfelt, L. Agnati, O. Johansson, A. Ljungdahl, M. Perez de la Mora, *Adv. Biochem. Psychopharmacol.* **16**, 47 (1977).
5. L. Grandison and A. Guidotti, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 555 (1978); E. Vijayan and S. M. McCann, *ibid.*, p. 555; M. Simonovic, H. Y. Meltzer, V. S. Fang, *ibid.*, p. 555.
6. M. J. Antonaccio and D. G. Taylor, *Eur. J. Pharmacol.* **46**, 283 (1977); M. J. Antonaccio, L. Kerwin, D. G. Taylor, *7th International Congress of Pharmacology* (Paris, 1978), Abstr. 2068. Similar results were recently reported by B. Delbarre and D. Senon [*11th Collegium Internationale Neuro-psychopharmacologicum (CINP) Congress* (Vienna, 1978), p. 39] after intracerebroventricular application of diazepam.
7. J. A. DiMicco, B. L. Hamilton, R. A. Gillis, *J. Pharmacol. Exp. Ther.* **203**, 64 (1977); J. A. DiMicco and R. A. Gillis, *ibid.*, in press; *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 687 (1978).
8. A. Guidotti, in *Psychopharmacology: A Generation of Progress*, M. A. Lipton, A. DiMascio, K. F. Killam, Eds. (Raven, New York, 1978); E. Costa, A. Guidotti, G. Toffano, *Br. J. Psychiatry*, in press; E. Costa, A. Guidotti, C. C. Mao, in *GABA in Nervous System Function*, E. Roberts, T. H. Chase, D. B. Toug, Eds. (Raven, New York, 1976), p. 413.
9. C. H. Hoekman and K. E. Livingston, *Neuropharmacology* **12**, 319 (1973); J. A. Quest, L. S. Freer, J. R. Kunec, R. A. Gillis, *Life Sci.* **21**, 659 (1977).
10. C. G. Gunn, G. Sevelius, M. J. Puiggari, F. K. Myers, *Am. J. Physiol.* **214**, 258 (1968); R. M. McAllen and K. M. Spyer, *J. Physiol. (London)* **258**, 187 (1976); H. I. Chen and C. Y. Chai, *Am. J. Physiol.* **231**, 454 (1976); N. K. Achari, C. B. B. Downman, W. V. Weber, *J. Physiol. (London)* **197**, 35P (1968); F. R. Calaresu, A. A. Faiers, G. J. Mogenson, *Prog. Neurobiol. (N.Y.)* **5**, 1 (1975).
11. Y. Miyata, K. Obata, Y. Tanaka, M. Otsuka, *J. Physiol. Soc. Jpn.* **32**, 377 (1970).
12. A. L. Berman, *The Brain Stem of the Cat* (Univ. of Wisconsin Press, Madison, 1968).
13. The rate of infusion was found to be critical, as infusion of saline at rates higher than those used in the present study often elicited profound bradycardia. These artifacts, presumably due to mechanical stimulation [see H. I. Chen and C. Y. Chai in (*10*)], were excluded in the present study by (i) placing the cannula at least 5 minutes before drug injection, and (ii) testing the effects of saline infusion at the same rates used for drug injection.
14. D. L. Kunze, *J. Physiol. (London)* **222**, 1 (1972).
15. Supported by grant NS-12566 from the Public Health Service.

29 August 1978; revised 18 December 1978

Alteration of Cortical Orientation Selectivity: Importance of Asymmetric Input

Abstract. *Rearing cats so that each of the two eyes sees stripes of a different orientation alters the orientation preference of visual cortex cells. This result can be obtained by rearing the cats in striped cylinders or with goggles attached to their faces, but a tighter control of orientation preference is achieved by the goggles.*

In normal cats the orientation preferences of single units in visual cortex are uniformly distributed (*1*). Blakemore and Cooper reported that rearing animals in horizontally or vertically striped cylinders altered this distribution (*2*). Most of the cells in the visual cortex preferred stimuli with the orientation seen during rearing. This finding has been replicated in both Blakemore's (*3*) and Pettigrew's (*4*) laboratories, but Stryker and Sherk have failed to replicate it (*5*). Stryker and Sherk found a uniform distribution of preferred orientations in animals reared in striped cylinders. Hirsch and his colleagues have altered the distribution of preferred orientations by rearing cats wearing striped goggles; one eye saw vertical stripes and the other eye saw horizontal stripes (*6*). This procedure controlled the orientation preferences of cortical cells with great precision so that even cells preferring diagonals were extremely rare. Stryker *et al.* (*7*) confirmed and extended this result.

Because there have been no detailed reports comparing cylinder- and goggle-reared animals from the same laboratory, it is difficult to determine whether goggle rearing is, in fact, substantially more effective at shaping cell properties than cylinder rearing. There are at least two important differences between the two rearing procedures. (i) In animals reared with goggles, the orientation of stripes on the retina is practically constant, the only changes being those due to torsional eye movements. In contrast, in animals reared in cylinders, the orientation of stripes on the retina changes when the animal tilts his head or when he looks at the distant top or bottom of the cylinder. (ii) The effects of symmetric input (both eyes seeing the same orientation) versus asymmetric input (the stripes seen by each of the two eyes are perpendicular) have not been investigated systematically. In particular, all cylinder experiments have used symmetric binocular input, while goggle experiments have used both types.

In the experiments described in this report we have compared goggle and cylinder rearing under conditions of symmetric and asymmetric binocular input. We reared four groups of animals (i) goggle reared, one eye seeing horizontal and one eye seeing vertical (HV goggle), (ii) goggle reared, both eyes seeing horizontal (HH goggle), (iii) cylinder reared, one eye seeing horizontal, one eye seeing vertical (HV cylinder), and (iv) cylinder reared, both eyes seeing horizontal (HH cylinder).

All animals were put into a dark room at about the time of natural eye opening. At about 3 weeks of age stripe rearing began. For 1 hour per day, the animals wore goggles or were placed in a striped cylinder. All but one of the cats was reared with alternating occlusion. For HV cats this meant that on alternate days one eye was exposed and the animal saw horizontal stripes or the other eye was exposed and the animal saw vertical stripes. One HV goggle animal was reared with simultaneous exposure; one eye saw horizontal stripes at the same time as the other eye saw vertical stripes.

When a cat was used in more than one recording session, it was usually maintained in the dark before the second and subsequent experiments (ten experiments). In six experiments, the animals received daily exposure to the goggles or cylinders between experiments.

Control data were obtained from ani-

0036-8075/79/0608-1109$00.50/0