γ-Aminobutyric Acid-Induced Potentiation of Cortical Hemiplegia

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A novel model of hemiplegia in young and aged rats is described. Osmotic minipumps were used to deliver a chronic (7 days), localized application of γ-aminobutyric acid (GABA) (100 μg/μl/h), to the somatomotor cortex of unrestrained rats. This resulted in an easily quantifiable, contralateral and reversible motor syndrome in both young and aged animals. In the young group, the motor deficit cleared over a 5-day period, while in the aged animals it persisted for at least a 2-week period. Control animals treated with saline-filled minipumps did not develop a long-lasting motor deficit. The GABA-induced facilitation of hemiplegia due to small motor cortex lesions and the age effects on behavioral recovery of function are discussed. Cortical inhibitory mechanisms may play a role in debilitating syndromes such as stroke or post-epileptic paralysis.

INTRODUCTION

In aged populations, ischemic cerebrovascular disease and associated hemiplegia are major causes of neurological mortality and morbidity. In patients with ischemia due to cerebrovascular disease, the major factors which correlate with high mortality are a decreased level of consciousness and the presence of hemiplegia. Moreover, if hemiplegia develops within 3 h of the onset of symptoms and persists for at least 36 h, there is a more than 90% chance that the patient will have a permanent, incapacitating motor deficit.

At the neurochemical level, vascular-induced ischemia produces differential changes in the concentration of endogenous neurotransmitters. For example, ligation of the middle cerebral artery in monkeys or the common carotid in gerbils causes significant decreases in brain dopamine but not noradrenaline. Conversely, increases in the levels of γ-aminobutyric acid (GABA) have been detected during and after ischemia in several rodent brain regions, including the cortex. The contribution of these neurotransmitter alterations to the severity of the immediate clinical deficit following stroke has, however, not been ascertained.

Currently, the medical management of hemiplegia is largely limited to supportive care. Although varying degrees of spontaneous improvement are often seen, the lack of understanding of the factors responsible for this functional recovery has restricted development of rational therapies. Various long-term symptomatic treatments including environmental enrichment, physical therapy and/or drugs have been tried without evidence of clear therapeutic effect.

The age at time of neurological insult also is a critical factor in determining the outcome following brain damage. In general, lesions acquired early in life result in less severe long-term deficits than comparable lesions incurred later in life. As is the case with neurotransmitter changes in acute ischemic disease, the mechanisms involved in the age-related factors contributing to recovery from stroke remain poorly understood.

Study of the contribution of age factors and neurotransmitter alterations to the recovery from hemiple-
gia has been impeded by lack of adequate animal models. Traditional methods used to study motor function in animals including ablation techniques, blood vessel ligation, injection of neurotoxic substances, and cryogenic or potassium-induced depression\textsuperscript{4,14} suffer from certain disadvantages including nonspecific inactivation, irreversible destruction of the area under study, and/or the transient nature of the motor deficits. Rodent models of stroke (e.g. Levine and Payan\textsuperscript{20}) using carotid ligation produce extensive infarction of the hemisphere, while more limited ligations of the middle cerebral artery (MCA) produce only short-lasting (less than 24 h) effects on behavior\textsuperscript{26}. In order to produce a sustained motor deficit, it is necessary to directly approach the motor cortex, which is located more medially than the damage produced by MCA ligation in the rat\textsuperscript{5,11,28,29}.

We have recently utilized the focal manipulation of an endogenous neurotransmitter to obtain a durable, though reversible hemiplegia in the rat. Using implanted osmotic minipumps, devices that deliver a constant amount of solutions for extended periods of time, we have been able to enhance and prolong the behavioral (hemiplegic) syndrome produced by cortical injury in rats.

GABA was selected for delivery to the motor cortex based on its known inhibitory role and upon electrophysiological studies indicating that low-dose regional GABA application had marked inhibitory effects on superficial layers of the somatosensory\textsuperscript{3} and auditory\textsuperscript{17} cortices, as revealed by evoked potential changes. These electrophysiological changes were dose-related, reversible upon washing of the cortex and were restricted to a small cortical area (3–4 mm diameter).

We report here the behavioral and anatomical effects of chronic infusion of the inhibitory neurotransmitter GABA to the motor cortex of young and aged rats and suggest that this model of hemiplegia may serve to investigate some aspects of the acute and chronic motor deficit seen in the human following stroke.

MATERIALS AND METHODS

Subjects

Young (6–9 months) and aged (26–30 months) male rats from the Fischer 344 strain were used. The animals were housed in groups of 5, with free access to food and water and were maintained on a standard (12:12) light–dark cycle, so that the light cycle occurred during working hours.

We chose to assess motor performance using a test which required coordinated motor control, as such a task is perhaps the most sensitive for detection of differences between young and aged rats\textsuperscript{12,35}.

The training procedure was quite simple. The animal's cage was placed at one end of a wooden beam (2.5 cm wide, 2 m long) elevated 45 cm from the table level. At the beginning of the training session, the animal was placed on the end of the beam opposite to the home cage. Initially, the rat was placed on the beam near the cage and he naturally returned to it without the need of negative reinforcement. Gradually, the rat was placed further from the cage until he returned to the cage walking over the full length of the beam.

Behavioral quantification

Motor function (see Fig. 1). We have observed that after training, the rat always maintained all four paws on the upper (2.5 cm wide) surface of the beam. When a motor deficit appeared, this pattern changed dramatically. On the day after initiation of GABA infusion, the animal was unable to run on the beam and rolled over and lay on the side contralateral to the minipump-implanted cortex. This behavior rated the maximum deficit score of 6. An animal received a '5' if it traversed the beam while dragging a hindlimb, a '4' if it fell or traversed the beam slipping off on more than half of its steps, a '3' if it traversed the beam without slipping but with the contralateral hind paw touching the lateral aspect (edge) of the beam, a '2' if the animal limped with one hindlimb ('hypotonus') and a '1' if the animal widened its base with four toes off the beam bilaterally (see Fig. 1).

The motor behavior was rated on each quarter section of the beam, and the final score was obtained by adding together the scores on each section of the beam. For instance, an animal which dragged its affected hindlimb (motor deficit = 5) over the first two quarters of the beam and made repetitive hindlimb slips (motor deficit = 4) over the final two sections of the beam, obtained a final motor score of $5+5+4+4 = 18$. The animals were required to achieve criterion performance on this test (3 consecutive days of motor
scores under 5) before minipump implantation. The motor rating was done by experimenters unaware of the minipump's content and this procedure had a high (> 95%) inter-rater reliability.

**Sensory function.** After testing posture and locomotion on the beam, the animal was returned to its cage. While in the cage, it was tested bilaterally for sensory responsiveness in three body regions: the vibrissae, the forelimbs, including the dorsal forepaw and the lateral surface of the limb, and the hindlimbs, including the dorsal hindpaw surface and the lateral aspect. The body surfaces were stimulated with a blunt needle mounted on a wood handle. A positive response was scored when the animal oriented towards the stimulus source, withdrew from the stimulus, or reacted emotionally to it (i.e. vocalization, ag-

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>SCALE</th>
<th>X</th>
<th>PERCENTAGE</th>
<th>TOTAL SCORE</th>
</tr>
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<tbody>
<tr>
<td>Unable to run</td>
<td>6</td>
<td></td>
<td></td>
<td>6 - 24</td>
</tr>
<tr>
<td>Dragging limb</td>
<td>5</td>
<td></td>
<td></td>
<td>5 - 20</td>
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<tr>
<td>Falls or &gt; 3 slips</td>
<td>4</td>
<td>(1</td>
<td>25%</td>
<td>6 - 16</td>
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<tr>
<td>≤ 3 slips and/or</td>
<td>3</td>
<td>(2</td>
<td>50%</td>
<td>4 - 12</td>
</tr>
<tr>
<td>4 toes off beam</td>
<td>2</td>
<td>(3</td>
<td>75%</td>
<td>2 - 8</td>
</tr>
<tr>
<td>(unilaterally)</td>
<td>1</td>
<td>(4</td>
<td>100%</td>
<td>1 - 4</td>
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<tr>
<td>Hypotonus (limping)</td>
<td>0</td>
<td></td>
<td></td>
<td>0</td>
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<tr>
<td>Wider sustentation</td>
<td></td>
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<tr>
<td>base (4 toes off beam, bilaterally)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>No apparent deficit</td>
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Fig. 1 Scale used for motor behavior quantification (behaviors 1 and 2 are not shown as they are not apparent in a still frame. For detailed description, see text.
gression, stereotypical behavioral). Care was taken not to give the animal any visual cues to the approaching stimulus.

**Surgical procedures**

After attaining stable motor performance at criterion levels on the beam, the animals were implanted with osmotic minipumps (Alza, mod. 2001) filled with ultrafiltered (Micropore 0.22 μm) saline or GABA. These devices are rated to deliver 1 μl/h for 7 days. The minipumps were filled by a colleague according to a randomized code, so that the experimenter was unaware of the minipump contents.

The surgical procedure was developed in preliminary studies. After pretreatment with atropine methylybromide (3.5 mg/kg, s.c.), surgical anesthesia was induced with ketamine (40 mg/kg) and sodium pentobarbital (10 mg/kg) given i.p. at 10-min intervals. Intermittent open-system fluothane inhalation was used to maintain the depth of anesthesia.

The scalp was then shaved, soaked with antiseptic (merthyolate tincture) and infiltrated with local anesthetic (2% lidocaine). With the animal positioned in a stereotaxic apparatus, a midline incision was made and the muscles and fascia scraped from the bone until the skull sutures were clearly visualized. A 2-mm hole was then made near the hindlimb representation of the motor cortex5,11,28,29, 2 mm posterior to bregma and 2 mm lateral to midline. All implants were made on the left side. After opening the dura with a needle, the skull was grooved to seat a beveled cannula (PE60), and the cannula was inserted into the somatomotor region through the burr hole for 2 mm tangentially to the skull at an angle of approximately -30 degrees to the horizontal plane. The cannula had been previously filled and connected to the osmotic minipump in order to minimize the occurrence of air bubbles in the delivery system.

After insertion into the somatomotor area, the cannula was fixed to the skull with dental cement and an anchoring machine screw; the attached minipump was implanted subcutaneously in the interscapular space, and antibiotics were administered locally and systemically. In previous studies using minipumps inserted in this region, dose–response studies with different solutions of GABA (5, 10, 50, 100, 250 and 500 μg/μl/h for 7 days) revealed a dose-related increase in the motor deficit for doses in the 10–100 μg/μl/h range. The 100 μg dose was chosen for subsequent studies based both on its behavioral and regional cortical electrophysiological effects.

**Experimental design**

Four experimental groups, consisting of young and aged rats treated with either GABA or saline-filled minipumps were studied. Tests of motor and sensory functioning commenced 24 h post-surgery, when the animals showed recovery from anesthesia, and continued on a daily basis.

**Histology**

At the end of the experiment, the animals were given a lethal dose of barbiturate and perfused intracardially with 10% buffered formaldehyde. The minipump was extracted and opened to verify complete delivery of the solution. With the animal positioned in the stereotaxic apparatus, trephines were made on symmetrical lateral locations of bregma and lambda through which small stainless-steel pins were inserted. The bone and dura were removed and the lesions photographed in situ. The brain was then removed and maintained for at least 2 weeks in fresh formaldehyde solution. Nissl-stained slices, 10 μm in thickness, were then obtained for histological examination and lesion reconstruction24.

**Statistics**

The data were analyzed using an ANOVA test for repeated measures and/or the Welch–Aspin t-test (t') for significantly unequal variances when appropriate37.

**RESULTS**

**Performance prior to surgery**

Only those animals in which complete emptying of the minipump was confirmed were included in the analysis (n = 12/group in the young; n = 7 for saline and n = 6 for GABA treatments in the aged). Both young and aged animals easily learned the motor task and no significant differences in motor performance were found between the groups. Group differences in the strategy used to run the beam were, however, observed. The aged animals tended to walk slower and more cautiously than the young; they also showed higher, although nonsignificant (t' (13) =
motor scores pre-surgery than the young animals. This was due to a more frequent presentation of behavior ‘1’ — a wider sustentation base — in the aged group.

Lesion effects

Animals with either saline or GABA infusion developed a similar syndrome in the initial 24 h after the surgery, consisting of a bilateral motor deficit which cleared by the second or third day post-implantation. This indicates that the initial syndrome is due to the cannula-induced somatomotor lesion combined with the post-anesthetic state. Placing reactions and sensory deficits followed the same time course and did not show clear evidence of lateralization. In all experimental groups, we found that the most sensitive and long-lasting sign of unilateral motor dysfunction was abnormal paw placing on the lateral aspect of the beam. This behavior was comparable in both groups prior to the surgery.

Effects of GABA infusion

All animals with saline minipump implants returned to pre-implantation motor performance levels, usually by the second post-operative day. In contrast, the GABA-treated animals exhibited motor impairments that persisted in the young group for up to 5 days post-implantation \((F_{1,22} = 20.00, P < 0.001, \text{ see Fig. 2}), and in the aged rats for at least 2 weeks post-surgery \((F_{1,11} = 64.48, P < 0.001, \text{ see Fig. 3})\). The motor deficits observed in both age groups ranged from a motionless animal, with no placing reactions apparent on the first post-implantation day, to more lateralized deficits on subsequent days. These lateralized deficits ranged from a dragging, parietic hindlimb, to discrete abnormalities in paw placing. In both GABA-treated groups, this hemi-motor syndrome remained consistently lateralized to the contralateral hind paw.

Diminished response to sensory stimulation and absence of placing reactions were observed in the first 2 or 3 days post-surgery in all treatment groups. These abnormalities were more variable as the animals recovered from the surgery and became rare by the third or fourth day after the implantation as evidenced by the animal’s normal appearing orientation and reaction to sensory stimulation.

Histology

Fig. 4 shows the extent and location of the cortical lesions produced by the cannula implantation itself in young animals. These lesions averaged (in length, width and depth) 3.3, 1.2 and 1.9 mm in saline-treated and 2.2, 1.5 and 1.8 mm in the GABA-treated animals. None of these differences were significant. There was also no difference in lesion size in GABA-treated young and aged animals (see Fig 5).

The microscopic examination of the Nissl-stained
slices showed preservation of neuronal integrity and comparable morphological features in the inner edge of the lesioned area in both the GABA- and saline-treated animals.

DISCUSSION

The localized, chronic administration of minute amounts of GABA to the rat's somatomotor cortex resulted in a syndrome of hemiplegia in both young and aged rats which lasted longer than that produced by the trauma of the cannula itself. In contrast, saline infusion did not produce a comparable chronic motor deficit. The GABA effects on motor performance resemble those reported by others using more extensive motor cortex ablations\(^8,10,23\). The observed effects were not due to simple destruction of nervous tissue, since the lesions produced by either GABA or saline-filled cannulae were comparable in size. Furthermore, aspirations of motor cortex similar in size to those produced by the minipump's cannula do not produce a long-lasting motor deficit (D.M. Feeney and T. Schallert, personal communication), suggesting that the observed motor deficit is due to drug diffusion into a region surrounding the perfusion site.

Anatomically, all lesions were located in the hindlimb representation of the rat's motor cortex\(^5,11,28\), and the behavioral syndrome exhibited by the GABA-treated animals correlates well with this localization. The response to somatic stimulation showed abnormalities ranging from marked sensory loss on the contralateral hind limb on the first day af-
ter surgery, to recovery by the third day post-surgery as evidenced by the orientation of the animal to sensory stimulation.

All animals, independent of treatment or age, evidenced some degree of recovery. The aged group, however, never attained pre-surgery motor performance values, at least for the 2-month follow-up period examined, suggesting that the recovery mechanisms present in old animals were unable to completely compensate for the consequences of the lesion. These observations parallel clinical and experimental reports of prolonged deficits in aged organisms and provide behavioral and pharmacological support for the theory that aged brain may be more susceptible to GABAergic influences. Indeed, Bowen et al. have reported an increase of GABA receptors during aging in human brain.

Other reports investigating the differences in functional recovery after cortical lesions in young and aged animals have produced contrasting results. In a study attempting to correlate age with the serum-lesion effect in which better recoveries are seen when the lesions are accomplished in successive stages as opposed to a single stage, Walbran ablated the somatosensory cortex and found similar deficits in tactile discrimination in 1-month-old and 19-months-old animals, either after 1- or 2-stage lesions. Moreover, 1-stage lesions were less deleterious in the aged group than in the young. In animals with frontal cortex lesions, Stem and Firl found that bilateral ablation of that region in aged rats produced impairments in a shock avoidance paradigm without significant deficits in a spatial alternation task, in young animals, however, deficits in both tests were seen. More recently, LeVere failed to find differences in recovery of a brightness discrimination task among young and aged rats with visual cortex ablations. Our results also showed functional recovery in both young and aged rats, but with clear distinctions between groups. Pharmacological vs anatomical approaches to produce cortical inactivation may account, at least in part, for the observed differences.

We have no direct evidence on the extent of GABA diffusion from the minipump site. However, recent studies have shown that drugs administered intracerebrally by means of osmotic minipumps have comparable patterns of distribution. For example, tritiated muscimol or fast green dye uniformly spread from 2.5 to 5.5 mm in subcortical (thalamus and striatum) structures. Likewise, labelled dopamine, methotrexate and antipyrine microperfusion into the diencephalon of rabbits shows a steep decline in drug concentration (approximately 2 orders of magnitude) at 5 mm distance from the cannula's tip. Similar figures have been reported for intracortically administered catecholamines. Further diffusion studies are necessary to determine the exact extent of the GABA spread in our chronic preparation. We do know, however, that acute topical administration of the amino acid to the cortical surface of the brain produces inhibition in a circumscribed, 3–4 mm diameter, area, which is similar to the diffusion ranges reported for other neurotransmitters.

In a rat model of brain ischemia, substantial increases in GABA levels during an ischemic episode have been reported. These changes lasted for 30 min with subsequent gradual recovery to control values 2 h thereafter. Although both GABA- and saline-infused animals developed lesions of comparable size, the faster recovery observed in the saline-treated group indicates that enhancement of local inhibitory mechanisms can delay functional recovery.

The young GABA-treated animals recovered by the fifth post-implantation day at a time when GABA was still being delivered into the somatomotor region. Several alternatives are available to explain this progressive recovery from hemiplegia while the amino acid was still being infused, including: a desensitization phenomenon, previously described at the cellular level, and/or changes in pharmacokinetic clearance of the infused agent, and/or receptor down-regulation ("fade"), reported to exist during constant infusions. The long-lasting effects of GABA infusion in the old animals does, however, suggest that the amino acid may have irreversible effects on lesioned, aged brain.

Animals with extensive motor cortex ablations (up to 10 mm) recovered faster than the GABA-infused animals, supporting the notion that partially functioning GABA-treated cortex impedes the development of functional recovery by distant brain systems. These results may be compared to the effects of hippocampal lesions on scopolamine-induced memory dysfunction in which scopolamine produces memory deficits only in animals with partial hippocampal damage, having no effects in animals with
more extensive lesions\(^30\). These results indicated that a partially functioning hippocampus interfered with the ability of other brain systems to assume memory function.

The motor syndrome observed after GABA infusion is reminiscent of another type of reversible paralysis frequently observed in humans: post-epileptic paralysis (Todd phenomenon). This condition has been proposed to be due to exhaustion, anoxia, or to the release of an inhibitory neurotransmitter inducing hyperpolarizing effects on the region involved in the paroxysmal activity\(^6\). This ‘paralysis’ can affect any area of the cortex as indicated by the variety of transient deficits ranging from monoparesis to aphasia reported in the literature, suggesting a common inhibitory mechanism. The existence of a region of ‘surround inhibition’ adjacent to epileptic foci has been proposed\(^25\). The widespread distribution and well-known inhibitory action of GABA in the cortex make it a likely candidate for involvement in this phenomenon.

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This rat model of hemiplegia may be helpful for the study of the acute and chronic mechanisms involved in recovery from brain lesions and may be useful for screening of pharmacological agents that might facilitate these recovery processes. An animal model of long-lasting dysfunction due to cortical neurotransmitter manipulation is desirable, since motor deficits can be obtained which more closely parallel the time course of motor deficits observed in humans than those obtained by current animal models of hemiplegia.

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