Alteration of posttraumatic ischemia in experimental spinal cord trauma by a central nervous system depressant

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Blood flow after severe experimental injury to the thoracic spinal cord was studied in cats, using a modification of the hydrogen clearance technique. Gamma hydroxybutyrate, a central nervous system depressant, was shown to markedly alter the ischemic response to injury if given during the early posttraumatic period. Other vasoactive drugs investigated had no effect on posttraumatic ischemia. Therapeutic intervention during the early posttraumatic period aimed at increasing blood flow while decreasing the metabolic requirements of the injured cord may prove of value in reversing or limiting some elements of long-tract dysfunction due to the secondary ischemic insult.

KEY WORDS • spinal cord trauma • ischemia • metabolic inhibitors • gamma hydroxybutyrate

In some instances, neurological deficit may not be fixed at the time of spinal cord trauma but could relate to pathophysiological processes occurring during the posttraumatic period. Previous studies investigating this hypothesis have been directed toward descriptions of metabolic changes,\(^3\) blood-flow alterations,\(^4,8,11,17,34,37\) microscopic patterns of tissue damage,\(^5,10\) or documentation of biochemical changes occurring after cord injury.\(^5,19,29\) Some treatment protocols have been directed toward the use of specific vasoactive amine blockers,\(^30\) although no single agent has been conclusively shown to cause progressive spinal cord pathology.\(^16\) Hypothermic irrigation of the injured cord has also been suggested as a treatment modality if begun during the early posttraumatic period.\(^1\) Whether by removing vasoactive substances or by decreasing the temperature-dependent metabolism, beneficial effects have been claimed by some investigators.\(^3,22\) The current study investigates the hypothesis that a central nervous system (CNS) depressant, gamma hydroxybutyrate (GHB), may be able to alter the ischemic response to experimental spinal cord trauma and possibly prevent any secondary injury caused by the ischemic insult.

Materials and Methods

Preparation of Animals

Measurement of blood flow using the hydrogen clearance technique is based on the detection of the current generated by the oxidation of hydrogen at the surface of a polarized platinum electrode implanted in the tissue.\(^5,31\) In order to obtain reliable and reproducible data, we have modified the technique as described in detail previously.\(^36,37\)

Adult cats were anesthetized with intraperitoneal pentobarbital and artificially ventilated with 50% \(O_2\) and 50% nitrous oxide. A cannula was inserted into the femoral vein to administer Flaxedil and drugs. A femoral arterial line was used to measure blood pressure and arterial blood gases. Respiratory endtidal \(CO_2\), \(pO_2\), \(pCO_2\), and core and epidural temperature were maintained in physiological ranges (\(pO_2\), > 90 mm Hg; \(pCO_2\), 32 to 38 mm Hg; temperature, \(37^\circ \pm 1.5^\circ\) C). A dorsal laminectomy was performed from T4–7, and the cat placed in a Kopf spinal stereotaxic holder.\(^*\) Platinum microelectrodes were placed stereotaxically in the dorsolateral funiculus to record regional spinal cord blood flow (SCBF) as previously described.\(^26\)

Experimental Groups

The cats were divided into eight groups as follows:

Group I = control (five cats)
Group II = trauma (10 cats)

\(^*\)Kopf spinal stereotaxic holder manufactured by David Kopf Instruments, 7324 Elmo Street, Tujunga, California 91042.
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Fig. 1. Group I: Control (non-trauma) series. Spinal cord blood flow (SCBF) measurements in the dorsolateral funiculus presented as percentage change against time. Average of 51 measurements was 10.99 ± 0.89 ml/100 gm/min.

Group III = control and GHB (three cats)  
Group IV = trauma and GHB (bolus) (five cats)  
Group V = trauma and GHB (bolus and infusion) (five cats)  
Group VI = trauma and haloperidol (three cats)  
Group VII = trauma and apomorphine (two cats)  
Group VIII = trauma and aminophylline and Isuprel (three cats).

The control series, Group I, consisted of five animals which had SCBF measurements obtained from two electrode sites at 30- to 45-minute intervals for 7 hours. The trauma series, Group II, consisted of 10 cats. From three to five SCBF measurements were obtained, the electrodes were removed, and a 500 gm-cm lesion (20-gm weight dropped from a 25-cm height) was inflicted at T-6.9 Electrodes were reinserted in the dorsolateral funiculus at the level of trauma and at 1 cm below trauma. Serial blood-flow measurements were then obtained for 6 hours at 30- to 45-minute intervals.

The control and GHB series, Group III, consisted of three cats. Three to five control blood-flow measurements were obtained, then a bolus of GHB, 300 mg/kg in 20 cc 5% dextrose in water (D5W), was given intravenously over 15 minutes, followed 1 hour later by an infusion of GHB (200 mg/kg/hr). The SCBF was then recorded for 6 hours from both electrode sites.

The trauma and GHB (bolus) group, Group IV, consisted of five cats prepared as in the trauma group (above). A single bolus of GHB, 300 mg/kg in 20 cc D5W over 15 minutes, was given 30 minutes after cord trauma. The SCBF was recorded for 6 hours at the level of trauma.

The trauma and GHB (bolus and infusion) series, Group V, also consisted of five cats prepared as in the above group, except that, in addition to the initial GHB bolus given 30 minutes after trauma, a continuous infusion of GHB in D5W (200 mg/kg/hr) was begun 1 hour after the bolus (1½ hours after trauma), and continued for 5 hours. Blood flow was then recorded simultaneously both at and 1 cm below the trauma site in the dorsolateral funiculus. The doses and time of administration of GHB were chosen on the basis of two other animal studies that used GHB as an anesthetic and CNS depressant, and on early results from this laboratory. Preliminary data suggested that a bolus of 600 mg/kg approached the LD50, and a dose of 150 mg/kg had no effect on SCBF. Two other animals that were given GHB (300 mg/kg) at 2 hours and 7 hours after injury also failed to demonstrate any change in posttraumatic SCBF compared to the trauma series.

Because GHB has been shown to inhibit the firing of nigrostriatal dopaminergic neurons and increase the dopamine content of that tissue, we investigated the effects of both a dopamine-receptor antagonist (haloperidol) and a dopamine-receptor agonist (apomorphine) on posttraumatic SCBF. In Group VI, three animals were treated with a bolus (0.35 mg/kg) and infusion (0.35 mg/kg/hr) of haloperidol during the same time periods as in Group V. A haloperidol dose of 0.25 mg/kg has been demonstrated to effectively block nigrostriatal dopamine-receptor sites in rats. In Group VII, two other animals were injected intraperitoneally with apomorphine (2 mg/kg) 30 minutes after trauma. A dose of 1 mg/kg has been shown to effectively stimulate nigrostriatal dopamine receptors for up to 3 hours. In both groups, SCBF was then followed for 6 hours after cord injury.

In a final group of three animals, Group VIII, aminophylline plus Isuprel (isoproterenol) were given by infusion during the same posttraumatic period. Aminophylline doses used were 3, 0.3, and 0.15 mg/kg/hr, combined with Isuprel doses of 3, 0.3, and 0.15 µg/kg/hr, respectively. The same doses of these two drugs in combination has been shown to reverse experimental basilar artery spasm.

In all groups, the cats were sacrificed by perfusion with formalin, and the cord removed for histological examination of both electrode sites and cord trauma.

Results

Control Series

The results of the control (Group I) and 500 gm-cm trauma series (Group II) have been presented in detail previously and will only be summarized here. Based on 51 sequential determinations in five cats, the normal SCBF in the dorsolateral funiculus of the thoracic cord was 10.99 ± 0.89 ml/100 gm/min with no change over a 7-hour recording period (Fig. 1). All washout curves had monoexponential decays and the recording sites were histologically confirmed to be in the dorsolateral funiculus.

Trauma Series

In the trauma series, the average of all 38 pretrauma flow measurements was 11.13 ± 1.22
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Fig. 2. Group II: Trauma series. Left: Percentage change of spinal cord blood flow (SCBF) recorded at the level of trauma with each animal compared to its own pretrauma blood flow value. The 500 gm-cm trauma was inflicted at time 0. Average values for mean systemic arterial pressure (mSAP) at 15-minute intervals before and after trauma are also shown. Vertical lines represent standard deviations. The p values compare SCBF against data from the control series during the same time after electrode implantation. Right: Similar presentation for data recorded simultaneously 1 cm below trauma site.

ml/100 gm/min, which correlates well with the value from the control group (above). A 500 gm-cm injury at T-6 consistently produced an initial hypertensive response lasting 3 to 5 minutes, followed by a gradual fall in blood pressure to a level 30 to 40 mm Hg lower than the pretrauma blood pressure. The mean systemic arterial blood pressure thereafter remained depressed for the duration of the experiment. Figure 2 left presents the average SCBF measurements obtained in 10 cats recorded at the level of trauma. Shown as percentage change in flow, SCBF did not differ significantly from normal for over 1 hour after injury, but thereafter was significantly reduced (p < 0.005) compared to the baseline established in the control series (Fig. 1). Below SCBF is a graph of blood-pressure changes before, during, and after trauma. The relationship between blood pressure, autoregulation, and ischemia was investigated in a previous study. It was found that autoregulation was maintained for 1 to 2 hours after experimental spinal cord injury and was then lost coincident with the onset of ischemia. At 1 cm below the trauma site (Fig. 2 right), SCBF did not fall to a level of ischemia statistically different from control values until over 2 hours after trauma, despite the severe posttraumatic hypotension.

Control Series with GHB (Bolus and Infusion)

The data obtained in non-traumatized animals treated with a 300 mg/kg bolus of GHB followed in 1 hour by a continuous 200 mg/kg/hr infusion of GHB (Group III) is shown in Fig. 3. It is evident that there was a rapid and significant increase in SCBF when compared to the control series (p < 0.005). After 3 hours, however, despite continuous infusion of GHB, blood flow returned to normal and remained at that level for the duration of the experiment. During GHB administration there was no change in pCO2, pO2, pH, core or epidural temperature, or blood pressure to account for this finding. The maintenance of these physiological parameters during GHB administration has been confirmed by MacMillan. All washout curves remained monoexponential and the electrode placement was confirmed histologically.

Trauma with GHB (Bolus)

Figure 4 presents the data obtained in animals treated with a single bolus of GHB (300 mg/kg) 30 minutes after a 500 gm-cm injury (Group IV). Initially, there was an increase in SCBF lasting less than 3 hours (p < 0.005) and then a reduction in blood flow to ischemic levels. After 3 hours, the degree of
FIG. 3. Group II: Control (non-trauma) and GHB (300 mg/kg bolus and 200 mg/kg/hr infusion). Each cat's blood flow is compared to its own pre-drug control flows, and is graphed as percentage change SCBF. Vertical lines represent standard deviations, and p values compare SCBF against data from the control series (Fig. 1) during the same time period.

FIG. 4. Group IV: Trauma and GHB (bolus of 300 mg/kg) given 30 minutes after a 500 gm-cm injury. Same presentation as in Fig. 2 left; p values compare spinal cord blood flow (SCBF) to data from the trauma series (Fig. 2 left) during the same time after cord injury.
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Fig. 5. Group V: Trauma and GHB (bolus and infusion). Typical washout curve of flows pretrauma (A), and 1 hour (B) and 6 hours posttrauma (C) from one animal. Also shown are their semilog transpositions demonstrating monoexponential decays. The spinal cord blood flow (SCBF) values were 11.17, 12.83, and 10.50 ml/100 gm/min, respectively. D: Histological section of the recording site from which these curves were obtained. The arrow indicates the electrode tract. The cord shows the typical changes of posttraumatic central hemorrhagic necrosis.

ischemia was the same as in the group without GHB (p < 0.5). The hypertensive-hypotensive response to cord injury was not altered by the GHB administration, nor were there any detectable changes in pCO₂, end-tidal CO₂, pH, temperature, or mSAP.

Trauma with GHB (Bolus and Infusion)

Figure 5 presents the pretrauma blood flow, and 1-hour and 6-hour posttrauma blood flow with their respective semilog monoexponential transpositions from one animal in Group V. Blood-flow values were 11.17, 12.83, and 10.50 ml/100 gm/min, respectively. The recording site from which these flows were obtained (Fig. 5D) shows the typical features of hemorrhagic necrosis in the central gray matter and scattered petechial white matter hemorrhages.¹⁰

The SCBF at the level of trauma and 1 cm below trauma, as followed for 6½ hours in these five animals, is shown in Fig. 6. The SCBF of each animal is represented as percentage change from its own pretrauma control flow. The 500 gm-cm injury was produced at time 0, the GHB bolus was given at 30 minutes and the GHB infusion begun at 1½ hours.
after trauma. It is evident that at the level of trauma (Fig. 6 left) there was a significant hyperemia in four animals, and only a 15% fall in SCBF in the fifth animal. By the end of the sixth hour, however, blood flow was returning to normal values in all animals. The single cat with the non-hyperemic response (black circles) had both a higher pretrauma blood flow (15 ml/100 gm/min) and a slightly lower pCO₂ (31 mm Hg) than the other animals. One cm below trauma (Fig. 6 right), four animals maintained blood flow in the normal range (±15% of pretrauma SCBF) while only one animal demonstrated hyperemia. The two animals with a 15% fall in blood flow despite GHB (asterisks and diamonds) had no difference in any physiological parameter.

The average of all SCBF measurements recorded at trauma and below trauma, respectively, in these GHB-treated animals is shown in Fig. 7. The SCBF is presented as percentage change in flow against time, with each animal compared to its own pretrauma controls. Standard deviation and two-tailed Student’s t-test for each point are shown compared to the trauma series (Fig. 2). At the level of trauma (Fig. 7 upper) all blood-flow determinations including those within the first hour, were significantly different from those in the control trauma series (Group II) (p < 0.01 or better). As long as the GHB infusion was continued, blood flow remained normal or elevated, and no post-traumatic ischemia occurred in this 6-hour time period. Figure 7 lower demonstrated that 1 cm below trauma, blood flow was not statistically different from that in Group II for nearly 2 hours, but thereafter demonstrated significant hyperemia. At no time during the bolus or infusion of GHB was there any change in the physiological parameters we have referred to. We were aware of the possible association between increased blood flow and increased gray and white matter hemorrhages, but were not able to make a definite association between the two parameters in this study.

Trauma with Dopamine-Receptor Agonists and Antagonists

Figure 8 presents the SCBF data obtained in five animals treated with either haloperidol (a dopamine-receptor blocker) or apomorphine (a dopamine-receptor stimulator). At no point in either treatment protocol was there any significant difference between the trauma and either the trauma-haloperidol (Fig. 8 left) or trauma-apomorphine (Fig. 8 right) groups (p < 0.5 at all points).

Trauma with Aminophylline and Isuprel

Figure 9 presents the data recorded at trauma in three animals treated with aminophylline and Isuprel (a phosphodiesterase inhibitor and a β-adrenergic stimulator, respectively). The greater doses of both
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Fig. 7. Group V: Trauma and GHB (bolus and infusion). *Upper:* Summation of data from Fig. 6 left showing percentage change of spinal cord blood flow (SCBF) recorded at the level of trauma. The GHB bolus and infusion time markers are so indicated. Vertical lines indicate standard deviations, and p values (Student's t-test) compare SCBF against values obtained from the control trauma series (Group II) during the same time period (Fig. 2 left). *Lower:* Similar presentation for data recorded simultaneously 1 cm below trauma.

Discussion

We have previously demonstrated that ischemia is the predominant vascular response in the dorsolateral white matter to severe cord injury and that there is a consistent time delay between injury and demonstrable ischemia. The delay in onset of decreased blood flow suggests that therapeutic intervention within the first 60 to 90 minutes after trauma may prove of value in reversing or limiting some elements of long-tract dysfunction due to the secondary ischemic insult. The current study suggests that GHB may alter the ischemic response to trauma.
Gamma hydroxybutyrate, an analog of gamma aminobutyric acid (GABA) which is known to extend inhibitory actions on some neural systems, has been shown to act as a CNS depressant with specific suppressant action on nigrostriatal dopaminergic neurons and on spinal cord reflexes. It can cross the intact blood-brain barrier and has been found in normal mammalian brain. The half-life of an anesthetic dose of GHB is about 1 hour and a single dose can abolish the righting reflex in rats for 3 hours. While its precise mechanism is unknown, two effects have been identified: it inhibits the release of dopamine from dopaminergic neurons resulting in an increase in local tissue dopamine content, and it is also a potent CNS depressant, although its mechanism in this respect is completely unknown. It has been used clinically as an anesthetic agent and it has been investigated for possible beneficial effects in head-injured patients. In one study, at a relatively low dose of 60 mg/kg, GHB decreased cerebral metabolic rate (CMRO₂) 27% and cerebral arteriovenous O₂ difference by 33% with no effect seen on cerebral blood flow (CBF). The same study however found that if a barbiturate was used, a comparable decrease in CMRO₂ was associated with a fall in CBF. Relative to the barbiturates, therefore, GHB was able to lower cerebral metabolism while maintaining adequate CBF. The physiological and pharmacological mechanisms by which these effects are mediated are unknown.

In this series of experiments, GHB exerted a significant effect upon blood flow in both the normal and traumatized spinal cord. A single dose produced a dramatic increase in posttraumatic SCBF that lasted 2 to 3 hours, thereafter returning to its normal posttraumatic ischemic pattern (Fig. 4). If a continuous infusion of GHB was begun 1 hour after the single dose, the ischemic response was altered for the duration of the drug infusion. This effect was seen both at the trauma site and 1 cm below the site of trauma.
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Because of GHB's inhibitory effect on dopaminergic neurons, we attempted but failed to modify the ischemic response with a dopamine-receptor blocker reported to have no activity as a CNS depressant (haloperidol). A dopamine-receptor stimulator (apomorphine) similarly failed to alter the ischemic response to trauma. This suggests that the effect of GHB on SCBF was not related to dopamine-receptor stimulation or block. However, to confirm the inability of either dopamine-receptor stimulators or blockers to alter posttraumatic ischemia, a larger series is needed.

Several studies have attempted to quantify alterations in vasoactive substances occurring after both cerebral ischemia24,26 and experimental spinal cord injury.19,20,29,30 Increased levels of norepinephrine,30 histamine,19,29 serotonin,6 and dopamine19,29 have all been reported after cord injury but results have frequently been contradictory and no single substance has been conclusively linked to posttraumatic blood-flow alterations.7,10,16 Although both hypothemic irradiation1,22,40 and the use of specific vasoactive amine blockers have their respective proponents,6,25,30 no treatment protocol to date has been shown to alter cord pathology.10 It is entirely possible that the combination of several vasoactive substances, all released in small amounts after cord injury, could contribute to the ischemia. It has been suggested that small amounts of one vasoactive substance will potentiate the vasocnstrictive properties of other substances.5,15,27,28,30,38,41

Attempts to increase posttraumatic blood flow should not, therefore, be directed at single vasoactive blocking agents. Several studies in cerebral ischemia14,16 and spinal cord trauma have suggested that a crucial time period of 1 to 2 hours exists after the insult, during which irreversible biochemical,6,10 vascular,6,37 electrophysiological,2,10 and pathological processes are occurring.9 If, as suggested by several investigators,10,37 the ischemic response to cord injury constitutes a secondary insult, then attempts to increase blood flow during the early posttraumatic period may prove of value in reversing some elements of long-tract dysfunction.

The mechanism of GHB-induced hyperemia is unknown. By acting as a CNS and metabolic depressant, GHB may be interfering with the release or activation of several vasoactive substances, possibly through depressed enzymatic activity. MacMillan26 has recently demonstrated that GHB, like the barbiturates,16,38,45 is able to normalize tissue levels of high-energy phosphates in hypoxic rat brain and decrease postischemic lactate accumulations. A similar mechanism in the ischemic spinal cord could account for the maintenance of blood flow in our GHB-treated traumatized cords. Gamma hydroxybutyrate may also be acting as a direct vasodilator; however preliminary studies in our laboratory show no effect of GHB on experimental vasospasm, nor did aminophylline and Isuprel, a popular vasodilating regime,12 have any effect on posttraumatic spinal cord ischemia.

The concept of increasing blood flow while decreasing metabolic activity, thereby protecting ischemic tissue from secondary injury, is not new.12,38,49 In several clinical and laboratory studies, metabolic inhibitors, especially barbiturates, have demonstrated the ability to minimize neural dysfunction caused by an ischemic insult.12,38,45 The barbiturates, however, cause a decrease in blood flow.12 Other studies suggest that the early return of blood flow to ischemic cortex, especially white matter, can restore lost electrical activity, normalize metabolic activity, improve synaptic transmission, and improve neurological function.21,43,44 A similar situation may exist in the ischemic spinal cord. By decreasing the metabolic requirements of the injured tissue and simultaneously increasing blood flow, GHB may prove of value in reversing or limiting some elements of long-tract dysfunction.

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