JOURNAL OF NEUROTRAUMA
Volume 14, Number 1, 1997
Mary Ann Liebert, Inc.

Extracellular Release of Serotonin following Fluid-Percussion Brain Injury in Rats

RAUL BUSTO, W. DALTON DIETRICH, MORDECAI Y.-T. GLOBUS, OFELIA ALONSO, and MYRON D. GINSBERG

ABSTRACT

Serotonin has been implicated in the pathobiology of central nervous system trauma. Using microdialysis techniques, we performed measurements of extracellular serotonin release within the traumatized cerebral cortex of rats subjected to moderate fluid-percussion (F-P) brain injury. Twenty-four hours prior to TBI, a F-P interface was positioned parasagitally over the right cerebral cortex. On the second day, fasted rats were anesthetized with 70% nitrous oxide, 1% halothane, and 30% oxygen. Under controlled physiological conditions and normothermic brain temperature (37–37.5°C), rats were injured ($n = 6$) with a F-P pulse ranging from 1.8 to 2.0 atm. Following trauma, brain temperature was maintained for 4 h at 37°C. Sham trauma animals ($n = 7$) were treated in an identical manner. Brain trauma induced acute elevations in the extracellular levels of serotonin ($p < 0.01$, ANOVA) compared to sham-operated controls. For example, serotonin levels increased from 18.85 ± 7.12 pm/mL (mean ± SD) in baseline samples to 65.78 ± 11.36 in the first 10 min after trauma. The levels of serotonin remained significantly higher than control for the first 90-min sampling period. In parallel to the increase in serotonin levels after TBI, a significant 71.1% decrease (i.e., 182.29 ± 30.08 vs 52.75 ± 16.92) in extracellular 5-hydroxyindoleacetic acid (5-HIAA) levels was observed during the first 10 min after TBI. These data indicate that TBI is followed by a prompt increase in the extracellular levels of serotonin in cortical regions adjacent to the impact site. These neurochemical findings indicate that serotonin may play a significant role in the pathophysiology of TBI.

Key words: traumatic brain injury, serotonin, microdialysis

INTRODUCTION

EXPERIMENTAL MODELS of traumatic brain injury (TBI) have been developed to investigate mechanisms underlying the pathobiology of brain injury (for review see Gennarelli, 1994). Using a parasagittal fluid-percussion (F-P) model of brain injury, our laboratory has reported the histopathological, neurochemical, metabolic, and hemodynamic consequences of this injury model (Dietrich et al., 1994a,b, 1996; Globus et al., 1995; Ginsberg et al., 1995). Most recently, we have demonstrated hemodynamic depression throughout the traumatized hemisphere 30 min after TBI (Dietrich et al., 1996). In that double-label autoradiographic study, focal platelet accumulation superimposed upon widespread hemodynamic depression was reported. In other studies, morphological evidence for platelet thrombosis has been obtained following F-P or direct cortical impact injury (Smith et al., 1969; Dietrich et

Neurotrama Research Center, Department of Neurology University of Miami School of Medicine, Miami, Florida 33101

Journal of Neurotrauma 1997.14:35-42.

al., 1994a). Serotonin is a potent vasoactive agent in the presence of endothelial injury (Holmsen, 1985; Vanhoutte and Houston, 1985; Feniuk and Humphrey, 1989) and the constricting effects of serotonin appear to be amplified by the actions of other vasoactive agents (De LaLande, 1989). Thus, platelet-derived serotonin could participate in the hemodynamic consequences of TBI.

Serotonin is a putative neurotransmitter that can also modulate the postsynaptic effects of excitatory amino acids (Nedergaard et al., 1987; Reynolds et al., 1988). In the neocortex, serotonin potentiation of excitatory amino acids has been reported. *N*-methyl-D-aspartate (NMDA), quisqualate, and glutamate depolarization are enhanced by iontophoretically or topically applied serotonin in neocortical slices from cats (Nedergaard et al., 1987). A similar facilitatory effect of serotonin on the electrophysiological response to NMDA was demonstrated in neocortical neurons (Reynolds et al., 1988). Thus, trauma-induced release of serotonin might also contribute adversely to traumatic outcome by promoting excitotoxic processes. In this regard, previous neurochemical studies using microdialysis techniques reported significant elevations in the extracellular levels of glutamate after TBI (Faden et al., 1989; Katayama et al., 1990; Nilsson et al., 1990; Palmer et al., 1993). Using midline F-P model in cats, Katayama and colleagues (1990) reported elevations in extracellular glutamate in the hippocampus after TBI. In our laboratory, we recently reported elevated levels of extracellular glutamate within the lateral cerebral cortex after parasagittal F-P brain injury (Globus et al., 1995).

The effects TBI on the extracellular levels of serotonin have not been evaluated. Thus, the purpose of this study was to document the temporal profile of serotonin release in this established model of TBI. The lateral somatosensory cortex was targeted for neurochemical analysis because of its reproducible histopathological and hemodynamic response to parasagittal F-P brain injury.

MATERIALS AND METHODS

General Animal Preparation

All animal procedures used were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the local Division of Veterinary Resources. Fasted male Sprague–Dawley rats, weighing 250–300 g, were initially anesthetized with Equitenzin (a mixture of nembutal, propylene glycol, ethanol, $MgSO_4$, and chloral hydrate). A 4.8-mm craniotomy was made underlying the right parietal cortex 3.8 mm posterior to bregma and 2.5 mm lateral to the midline (Zilles, 1985). A plastic injury tube (3.5 mm inside diameter) was placed over the exposed dura and bonded by adhesive. Acrylic cement was then poured around the injury tube. After the acrylic had hardened, the injury tube was plugged with a gelfoam sponge. For the microdialysis experiment, a guide cannula was attached to the skull over the parietal cortex at coordinates 5.0 mm posterior, 5.5 mm lateral to bregma and 2.0 mm ventral to the dura. The scalp was then closed surgically and the animal was returned to his home cage and allowed to recover overnight having access to food and water.

Induction of TBI

A fluid-percussion (F-P) device was used to produce brain trauma (Dixon et al., 1987; Clifton et al., 1991; McIntosh et al., 1989). The F-P device consisted of a saline-filled plexiglas cylinder that is fitted with a transducer housing and injury screw adapted to the rat's skull. The metal screw is firmly connected to the plastic injury tube of and intubated anesthetized rat (70% nitrous oxide, 1.5% halothane, and 30% oxygen). The injury is induced by the descent of the pendulum, which strikes the piston. In this study, rats underwent moderate head injury ranging from 1.8 to 2.0 atm. Brain temperature was indirectly monitored with a thermistor probe inserted into the right temporalis muscle (Jiang et al., 1991) and maintained at 37–37.5°C during the entire experimental protocol. Rectal temperature was also measured and maintained at 37°C throughout the monitoring period. Two groups of animals were studied: sham-control, nontraumatized animals (n = 7) and animals undergoing TBI (n = 6).

Microdialysis Procedure

On the day of the study, a microdialysis probe with a 2-mm membrane tip (Carnegie Medicin) was inserted through the cannula into the right parietal cortex and perfused with modified Ringer's solution at a flow rate of 1 μL/min by means of a microinfusion pump (Carnegie Medicin). The animals were maintained on 1% halothane combined with 70% nitrous oxide and 30% oxygen and were immobilized with 0.6 mg/kg, iv pancuronium bromide. Additional doses of 0.3 mg/kg were administered as needed.

During a 1.5-h pretraumatic stabilization period, the collection of three baseline samples at 10-min intervals was conducted. The microdialysis probe was then withdrawn during the production of the traumatic insult and then reintroduced for sampling. Microdialysis samplings were performed at 10-min intervals during the first 30-min posttraumatic period; thereafter, 10-min samples were obtained at 30-min intervals for up to 4 h following trauma. All samples were collected in an ice bath and then frozen and kept in −20°C until analysis. The location of the microdialysis probe was verified histologically

in each animal at the end of the experiment. Sham-operated animals were treated in an identical manner except for the traumatic impact.

Neurochemical Measurements

Concentrations of serotonin and 5-HIAA were determined by reverse-phase isocratic liquid chromatography with electrochemical detection as previously described (Globus et al., 1992; Wester et al., 1992). Briefly, the isocratic mobile phase with a flow rate of 1.0 mL/min consisted of 0.027 mM ethylenediaminetetraacetic acid (EDTA), 14.7 mM NaH_2PO_4; 30 mM Na citrate, 10 mM diethylamine with 476 mg octane sulfonic. The pH of this solution was adjusted to 3.25 and increased to a total volume of 1 L with water. Next, 30 mL of CH_3CN and 15 mL of THF were added. The separation of the compounds was accomplished by an analytical microbore column C_{18} 5 μm (150 × 1 mm) (BAS Septick). The substances are detected with a bioanalytical system consisting of solvent delivers module (BAS-PM-80), two amperometric detectors (BAS-Lc-4C), a dual glassy electrode (BAS-MF-100), and an Ag/Ag Cl reference electrode. The electrodes were set at 750 V with the detectors set at two different gains. Calibrations were run daily with three different 5-HT concentrations. The detectors were interfaced to a computer operated under control of a chromatography software package provided by a bioanalytical system.

Statistical Analysis

The effects of traumatic brain injury on serotonin and 5-HIAA levels were assessed by comparison of the values during and after trauma with baseline levels, using analysis of variance (ANOVA) followed by Bonferroni procedure. The level of significance was set at 0.05 and results given as means ± SEM.

RESULTS

Physiological Findings

Physiological findings are summarized in Table 1. Physiological variables were within normal ranges. A transient increase in systemic arterial blood pressure was routinely seen immediately after trauma with normalization within 1–2 min. Thereafter, blood pressure measurements were within control levels during the course of the subsequent microdialysis sampling.

Sham-Operated Rats

As shown in Figure 1, a mild increase in extracellular serotonin was seen in sham-operated control rats immediately after probe replacement. This increase was a consequence of 2 out of 7 control rats showing a detectable increase in serotonin after probe reinsertion. The mean value of serotonin after probe replacement was 16.57 pmol/mL of dialysate. By 20 min after the introduction of the probe, serotonin levels had normalized and remained so during the duration of the experimental protocol.

TABLE 1. PHYSIOLOGICAL DATA[a]

	Pco_2	Po_2	pH	MAP	Plasma glucose
Sham-operated					
Mean	36.6	137.6	7.45	119.2	131.2
±SD	5.1	20.6	0.03	4.9	20.7
Traumatic brain injury					
Mean	37.6	151.2	7.45	125.0	143.7
±SD	2.8	38.0	0.10	13.0	18.3

[a]Values obtained immediately prior to TBI. No significant differences between sham-operated and traumatized groups.

Traumatized Rats

The time course and changes in extracellular serotonin in the right lateral somatosensory cortex are shown in Figure 1. Following the 45-min stabilization period, small or undetectable levels of serotonin were detected during the 20-min period prior to trauma. Immediately following trauma, a significant ($p < 0.01$) increase in serotonin levels was documented. Serotonin levels (65.6 ± 27.8 pmol/mL) were greatest in the first 10-min sample fol-

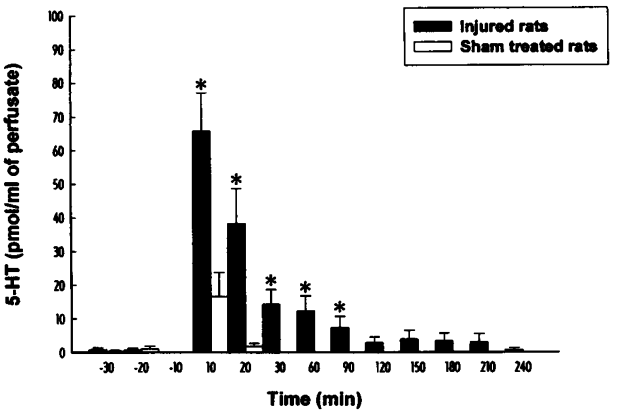

FIG. 1. Time course of changes in perfusate levels of serotonin (5-HT) from sham-operated and traumatized rats sampled from the cerebral cortex adjacent to impact site. Traumatic brain injury induced a significant increase in 5-HT levels that remained elevated up to 90 min after trauma. *Significantly different from pretraumatic values at $p < 0.05$ by analysis of variance. Values are mean ± SEM.

lowing trauma. However, serotonin levels remained significantly elevated ($p < 0.05$) up to 90 min after TBI. In addition, 2 out of 6 rats demonstrated elevated serotonin levels as late as 3.5 h after TBI. By repeated measures ANOVA, serotonin levels from the traumatized group were shown to be significantly different ($p < 0.0001$) from to the sham-operated group.

5-HIAA Response

In sham-operated control rats, an average baseline level of 141.3 pmol/mL of 5-HIAA was documented. During the last 2.5 h of the study period, 5-HIAA levels increased to 250 pmol/mL. In TBI rats, pretraumatic baseline levels of 5-HIAA were similar to sham-operated rats. Parallel to the increase in serotonin levels following trauma, a significant ($p < 0.05$) decrease in extracellular 5-HIAA levels was observed following trauma. Levels of 5-HIAA remained below baseline during the 4 h post-traumatic sampling period (Fig. 2).

DISCUSSION

Previous studies have demonstrated that various neurotransmitter systems are markedly affected following central nervous system injury (for review see McIntosh, 1993). In the present study, we describe for the first time the effects of moderate F-P brain injury on the extracellular levels of serotonin. Trauma induced an acute increase in the levels of serotonin within the lateral somatosensory cortex, a brain region that is histopathologically vulnerable in this TBI model. The rise in serotonin concentration was most severe in the first 10-min period after TBI but remained significantly elevated for 90 min after trauma.

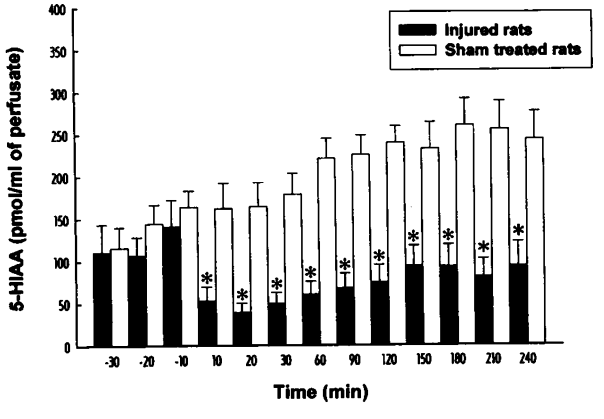

FIG. 2. Time course of changes in perfusate levels of 5-hydroxyindoleacetic acid (5-HIAA) from sham-operated and traumatized rats. Trauma induced a significant decrease in 5-HIAA concentrations in the cerebral cortex. *Significantly different from pre-traumatic values at $p < 0.05$ by analysis of variance. Values are mean ± SEM.

The role of neural and vascular serotonin in the pathobiology of central nervous system trauma has been recently summarized (Salzman, 1994). Several studies have reported elevated serotonin concentrations in the acutely injured spinal cord (Faden et al., 1988; Sharma et al., 1990; Zivin et al., 1976; Salzman et al., 1987a,b, 1991a,b; Lui et al., 1990). For example, Zivin and colleagues (1976), using a radioenzymatic assay, reported the accumulation of serotonin in white matter of rabbits after spinal cord compression trauma. In rats, Salzman and colleagues (1987a,b) also demonstrated an increase in serotonin following spinal cord trauma. Finally, Lui and colleagues (1990) reported elevations in 5-HT after spinal cord trauma using microdialysis.

Although serotonin has been less studied in models of brain trauma, serotonin responses have been reported after brain injury (Mohanty and Mazumdar, 1978; Singh et al., 1986; Nayak et al., 1980). For example, using a cold injury model, Pappius and Dadoun (1987) reported a widespread decrease in serotonin content and an increase in 5-HIAA 24 h after injury throughout the damaged hemisphere. In head-injured patients, Nayak and associates (1980) reported elevated levels of 5-HT in plasma. The present study represents the first documentation using microdialysis techniques of the temporal profile of elevated levels of extracellular serotonin and decreased levels of 5-HIAA following F-P brain injury.

The exact origin of the elevated serotonin levels found in the present study is unknown. Because over 90% of the body's serotonin resides in vascular sites and predominently in blood platelets (Daprada and Picotti, 1979), platelet-derived 5-HT is a potential source of the elevated extracellular levels of serotonin reported in our study. Subarachnoid hemorrhage occurs in this injury model, with a gliding contusion seen at the gray–white interface of the lateral cerebral cortex (Dietrich et al., 1994a). In addition, we have recently described, using both ultrastructural and autoradiographic strategies, venous thrombosis in this F-P model (Dietrich et al., 1994a, 1996). At 30 min after TBI, platelet accumulation was documented within pial subarachnoid spaces as well as subcortically, including the lateral external capsule and subcortical cisterns. Sites of platelet accumulation corresponded to the most severely damaged brain regions (Dietrich et al., 1994a,b). In this regard, Liu and colleagues (1990) reported elevated extracellular levels of serotonin after spinal cord trauma and noted a dependence upon proximity to sites of hemorrhage.

In the present study, the microdialysis probe was positioned within a cortical region that is histopathologically vulnerable to this traumatic insult. Three days after

parasagittal F-P brain injury, necrotic neurons are scattered throughout the lateral cortex overlying the evolving contusion (Dietrich et al., 1994b). In addition to the damaged pial surface, platelet-derived serotonin could also be released from the underlying hemorrhagic contusion. Because the blood–brain barrier (BBB) is acutely perturbed after F-P injury (Povlishock et al., 1978; Cortez et al., 1989; Dietrich et al., 1994a; Jiang et al., 1992; Tanno et al., 1992), vascular 5-HT may enter the injured brain following barrier compromise and thus be sampled by the microdialysis probe. Finally, because brain mast cells also contain serotonin (Edvinsson et al., 1977), this cell type may also be a source after TBI.

In addition to a vascular source, serotonin may also be released from presynaptic nerve endings triggered by calcium influx via voltage-operated channels (Osborne, 1982). Both glial cells and neuronal presynaptic and postsynaptic elements have high affinity serotonin uptake systems (Iverson 1974; Kimelberg and Katz, 1985). It is known that TBI leads to a massive rise in extracellular potassium (Katayama et al., 1990). Thus, during trauma-induced energy failure and membrane depolarization, extracellular serotonin levels may also increase via both neuronal release and perturbations in uptake processes.

Extracellular levels of serotonin have been reported to increase in the hippocampus and striatum following transient global ischemia (Globus et al., 1992). In that study, serotonin levels increased 4-fold compared to baseline levels during the ischemic insult and normalized within 20–30 min of reperfusion. Interestingly, the absolute increase in serotonin levels reported in the present study after TBI is higher that those reported after cerebral ischemia (Globus et al., 1992). In addition, elevated levels of serotonin appear to remain elevated longer after TBI as compared to transient cerebral ischemia. Thus, differences in the magnitude of the serotonin surge as well as the duration of elevated serotonin levels may indicate that mechanisms and sources of elevated serotonin may differ following cerebral ischemia and trauma. The mechanism underlying the 5-HIAA decrease following trauma is also not clearly understood. This decrease may reflect an attenuation of monoamine oxidase activity leading to a reduction in oxidative deamination as previously suggested after brain ischemia (Globus et al., 1992).

Several studies have investigated the consequences of pharmacologically targeting the serotonin response to central nervous system injury (Zivin and Venditto, 1984; Zivin, 1985; Faden, 1989; Salzman et al., 1987a,b, 1991a,b; Pappius et al., 1988; Sharma et al., 1989, 1990a; Puniak et al., 1991). In a spinal cord trauma model, Salzman and colleagues (1991a,b) reported that the serotonin antagonist, mianserin, improved functional recovery after spinal cord trauma. In a cold injury model, Pappius and colleagues (1988) reported that pretreatment with a serotonin synthesis inhibitor, *p*-chlorophenylalanine (PCPA), prevented serotonin accumulation and prevented a decrease in local glucose metabolism within remote brain regions. Pretreatment the 5-HT_2 antagonist ritanserin has been reported to attenuate the remote hemodynamic depression seen following thrombotic infarction (Dietrich et al., 1989). In a model of transient global ischemia, Globus and colleagues (1992) reported hippocampal CA1 neuroprotection with ritanserin treatment. Thus, it is conceivable that elevated levels of serotonin may participate in the genesis of the perfusion deficits as well as neuronal damage after TBI.

5-HT_2 serotonin receptors appear to mediate the major vascular actions of serotonin (Peroutka et al., 1991; Van Neuten et al., 1985). In the vasculature, serotonin acts upon 5-HT_2 sites in the capillary endothelium to increase permeability and produce vasoconstriction (Sharma et al., 1989). At vascular smooth muscle sites, serotonin can induce vasoconstriction effects that resemble posttraumatic sequelae seen in experimental TBI models (Lewelt et al., 1980; Dewitt et al., 1988; Dietrich et al., 1996; Yamakami and McIntosh, 1989). Importantly, the constricting effects of serotonin appear to amplify the actions of other vasoactive substances (De La Lande, 1989). Recent studies have indicated that serotonin-induced constriction of pial arterioles in piglets is markedly augmented following subarachnoid hemorrhage (Young et al., 1986; Yakubu et al., 1994). In addition, serotonin potentiates the vasoconstrictor effects of endothelin after subarachnoid hemorrhage (Alafaci et al., 1990; Yang et al., 1992). Under pathological conditions including TBI or subarachnoid hemorrhage, serotonin may therefore be a more potent vasoconstrictor and thereby participate in the perfusion deficits reported in head-injured patients (Obrist et al., 1984; Bouma and Muizelaar, 1992; Marion et al., 1991).

In conclusion, our results demonstrate that moderate TBI induces a significant increase in extracellular serotonin levels that persist for 90 min after injury. The source of serotonin release after TBI may include both vascular and neuronal components. The importance of serotonin release on the microvascular and neuronal consequences of TBI remain to be clarified. Nevertheless, these neurochemical findings implicate serotonin in the pathophysiology of TBI.

ACKNOWLEDGMENTS

We are indebted to John Perrone and Ricardo Prado for providing expert technical assistance and to Helen Valkowitz for helping to prepare the manuscript. This study was supported by USPHS Grant NS 30291.

REFERENCES

ALAFACI, C., JANSEN, I., ARBAB, M.A.-R., SHIOKAWA, Y., SVENDGAARD, N.-A.A., and EDVINSSON, L. (1990). Enhanced vasoconstrictor effect of endothelin in cerebral arteries from rats with subarachnoid hemorrhage. Acta Physiol. Scand. **138,** 317–319.

BOUMA, G.J., and MUIZELAAR, J.P. (1992). Cerebral blood flow, cerebral blood volume, and cerebrovascular reactivity after severe head injury. J. Neurotrauma 9(Suppl), S333–S348.

CLIFTON, G.L., JIANG, J.Y., LYETH, B.G., JENKINS, L.W., HAMM, R.J., and HAYES, R.L. (1991). Marked protection by moderate hypothermia after experimental traumatic brain injury. J. Cereb. Blood Flow Metab. **11,** 114–121.

CORTEZ, S.C., McINTOSH, T.K., and NOBLE, L.J. (1989). Experimental fluid percussion brain injury: Vascular disruption and neuronal and glial alterations. Brain Res. **482,** 272–282.

DAPRADA, M., and PICOTTI, G.B. (1979). Content and subcellular localization of catecholamines and 5-hydroxytryptamine in human and animal blood platelets: Monoamine distribution between platelets and plasma. Br. J. Pharmacol. **65,** 653–662.

DE LALANDE, J.S. (1989). Amplification mechanisms in peripheral tissues, in: *The Peripheral Actions of 5-Hydroxytryptamine*, J.R. Fozard (ed), Oxford University Press, NY, pp 123–146.

DEWITT, D.S., HAYES, R.L., LYETH, B.G., YUAN, X.Q., and PROUGH, D.S. (1988). Effects of traumatic brain injury on cerebral blood flow and metabolism: Autoradiographic studies. Anesthesiol. Rev. **15,** 31–32.

DIETRICH, W.D., BUSTO, R., and GINSBERG, M.D. (1989). Effect of the serotonin antagonist ketanserin on the hemodynamic and morphological consequences of thrombotic infarction. J. Cereb. Blood Flow Metab. **9,** 812–820.

DIETRICH, W.D., ALONSO, H., and HALLEY, M. (1994a). Early microvascular and neuronal consequences of traumatic brain injury: A light and electron microscopic study in rats. J. Neurotrauma **11,** 289–301.

DIETRICH, W.D., ALONSO, O., BUSTO, R., GLOBUS, M.Y.-T., and GINSBERG, M.D. (1994b). Post-traumatic brain hypothermia reduces histopathological damage following concussive brain injury in the rat. Acta Neuropathol. **87,** 250–258.

DIETRICH, W.D., ALONSO, O., BUSTO, R., PRADO, R., DEWANJEE, S., DEWANJEE, M.K., and GINSBERG, M.D. (1996). Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: A double-label autoradiographic study in rats. J. Cereb. Blood Flow Metab. **16,** 481–489.

DIXON, C.E., LYETH, B.G., POVLISHOCK, J.T., FINDLING, R.L., HAMM, R.J., MARMAROU, A., YOUNG, H.F., and HAYES, R.L. (1987). A fluid percussion model of experimental brain injury in the rat. J. Neurosurg. **67,** 110–119.

EDVINSSON, L., CEJRVOS-NAVARRO, J., LARSSON, L.I., OWMAN, CH., and RONNHBERG, A.L. (1977). Regional distribution of mast cells containing histamine, dopamine, or 5-hydroxytryptamine in the mammalian brain. Neurology **27,** 878–883.

FADEN, A.I. (1989). TRH analog YM-14673 improves outcome following traumatic brain and spinal cord injury in rats: Dose-response studies. Brain Res. **486,** 228–235.

FADEN, A.I., GANNON, A., and BASBAUM, A.L. (1988). Use of serotonin immunocytochemistry as a marker of injury severity after experimental spinal trauma in rats. Brain Res. **450,** 94–100.

FADEN, A.I., DEMEDIUK, P., PANTER, S.S., and VINK, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science **244,** 798–800.

FENIUK, W., and HUMPHREY, P.P.A. (1989). Mechanisms of 5-hydroxytryptamine-induced vasoconstriction, in: *The Peripheral Actions of 5-Hydroxytryptamine*, J.R. Fozard (ed), Oxford University Press: NY, pp. 100–122.

GENNARELLI, T.A. (1994). Animate models of human head injury. J. Neurotrauma **11,** 357–368.

GINSBERG, M.D., ZHAO, W., ALONSO, O., LOOR-ESTADES, Y., DIETRICH, W.D., and BUSTO, R. (1995). Early glucose metabolism/blood flow uncoupling following acute fluid-percussion traumatic brain injury. J. Cereb. Blood Flow Metab. **15**(Suppl), S31.

GLOBUS, M.Y.-T., WESTER, P., BUSTO, R., and DIETRICH, W.D. (1992). Ischemia-induced extracellular release of serotonin plays a role in CA1 neuronal cell death in rats. Stroke **23,** 1595–1601.

GLOBUS, M.Y.-T., ALONSO, O., DIETRICH, W.D., BUSTO, R., and GINSBERG, M.D. (1995). Glutamate release and free radical production following brain injury: Effects of post-traumatic hypothermia. J. Neurochem. **65,** 1704–1711.

HOLMSEN, H. (1985). Platelet activation and serotonin, in: *Serotonin and the Cardiovascular System*, P.M. VanHouette (ed), Raven Press, NY, pp. 75–86.

IVERSEN, L.L. (1974). Uptake mechanisms for neurotransmitter amines. Biochem. Pharmacol. **23,** 1927–1935.

JIANG, J.Y., LYETH, B.G., CLIFTON, G.L., JENKINS, L.W., HAMM, R.J., and HAYES, R.L. (1991). Relationship between body and brain temperature in traumatically brain-injured rodents. J. Neurosurg. **74,** 492–496.

JIANG, J.Y., LYETH, B.G., KAPASI, M.Z., JENKINS, L.W., and POVLISHOCK, J.T. (1992). Moderate hypothermia reduces blood-brain barrier disruption following traumatic brain injury in the rat. Acta Neuropathol. **84,** 495–500.

KATAYAMA, Y., BECKER, D.P., TAMURA, T., and HOVDA, D.A. (1990). Massive increases in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J. Neurosurg. **73,** 889–900.

KIMBELBERG, H.K., and KATZ, D. (1985). Identification of high affinity serotonin uptake into immunocytochemically identified astrocytes. Science **228,** 889–891.

LEWELT, W., JENKINS, L.W., and MILLER, J.D. (1980). Autoregulation of cerebral blood flow after experimental fluid percussion injury of the brain. J. Neurosurg. **53,** 500–511.

LIU, D., VALADEZ, V., SORKIN, S.L., and McADOO, D.J. (1990). Norepinephrine and serotonin release upon impact injury to rat spinal cord. J. Neurotrauma **7,** 219–227.

MARION, D.W., DARBY, J., and YONAS, H. (1991). Acute regional cerebral blood flow changes caused by severe head injury. J. Neurosurg. **74,** 407–414.

McINTOSH, T.K. (1993). Novel pharmacologic therapies in the treatment of experimental traumatic brain injury: a review. J. Neurotrauma **10,** 215–261.

McINTOSH, T.K., VINK, R., NOBLE, L., YAMAKAMI, I., SOARES, H., and FADEN, A.L. (1989). Traumatic brain injury in the rat: Characterization of a lateral fluid-percussion model. Neuroscience **28,** 233–244.

MOHANTY, S., and MAZUMDAR, S. (1978). Role of serotonin in human cerebral edema and contusion. Indian J. Med. Res. **67,** 1029–1032.

NAYAK, A.K., MOHANTY, S., SINGH, R.K., and CHANSOURIA, J.P. (1980). Plasma biogenic amines in head injury. J. Neurol. Sci. **47,** 211–219.

NEDEGAARD, S., ENGBERG, I., and FLATMAN, J.A. (1987). The modulation of excitatory amino acid response by serotonin in the cat neocortex in vitro. Cell. Mol. Neurobiol. **7,** 367–379.

NILSSON, P., HILLERED, L., PONTEN, U., and UNGERSTEDT, U. (1990). Changes in cortical extracellular levels of energy-related metabolites and amino acids following concussive brain injury in rats. J. Cereb. Blood Flow Metab. **10,** 631–637.

OBRIST, W.D., LANGFITT, T.W., JAGGI, J.L., CRUZ, J., and GENNARELLI, T.A. (1984). Cerebral blood flow and metabolism in comatose patients with acute head injury. J. Neurosurg. **61,** 241–253.

OSBORNE, N.N. (ed). (1982). *Biology of Serotonergic Transmission.* John Wiley: NY, pp. 1–522.

OSTERHOLM, J.L., BELL, J., MEYER, R., and PYENSON, J. (1969). Experimental effects of free serotonin on the brain and its relation to brain injury. J. Neurosurg. **31,** 408–421.

PALMER, A.M., MARION, D.W., BOTSCHELLER, M.L., SWEDLOW, P.E., STYREN, S.D., and DeKOSKY, S.T. (1993). Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. J. Neurochem. **61,** 2015–2024.

PARFENOVA, H., SHIBATA, M., and LEFFLER, C.W. (1993). Subarachnoid blood causes pial arteriolar constriction in newborn pigs. Stroke **24,** 1729–1734.

PAPPIUS, H.M., and DADOUN, R. (1987). Effects of injury on the indoleamines in cerebral cortex. J. Neurochem. **49,** 321–325.

PAPPIUS, H.M., DADOUN, R., and McHUGH, M. (1988). The effect of p-chlorophenylalanine on cerebral metabolism and biogenic amine content of traumatized brain. J. Cereb. Blood Flow Metab. **8,** 324–334.

PEROUTKA, S.J. (1991). The molecular pharmacology of 5-hydroxytryptamine receptor subtypes, in: *Serotonin Receptor Subtypes: Basic and Clinical Aspects*, S.J. Perutka (ed), Wiley-Liss: NY, pp. 65–80.

POVLISHOCK, J.T., BECKER, D.P., SULLIVAN, H.G., and MILLER, J.D. (1978). Vascular permeability alterations to horseradish peroxidase in experimental brain injury. Brain Res. **153,** 223–239.

PUNIAK, M.A., FREEMAN, G.M., AGRESTA, C.A., VAN NEWKIRK, L., BARONE, C.A., and SALZMAN, S.K. (1991). Comparison of a serotonin antagonist opioid antagonist, and TRH analog for the acute treatment of experimental spinal trauma. J. Neurotrauma **8,** 193–203.

REYNOLDS, J.N., BASKYS, A., and CARLEN, P.L. (1988). The effects of serotonin on N-methyl-D-aspartate and synaptically evoked depolarization in rat neocortical neurons. Brain Res. **456,** 286–292.

SALZMAN, S.K. (1994). The role of neural and vascular serotonin in secondary central nervous system trauma, in: *The Neurobiology of Central Nervous System Trauma*, S.K. Salzman and A.I. Faden (eds), Chapter 18, Oxford University Press: NY, pp. 182–192.

SALZMAN, S.K., HIROFUJI, E., KNIGHT, P.B., LLADOS-ECKMAN, C., BECKMAN, A.L., and WINOKUR, A. (1987a). Treatment of experimental spinal trauma with thyrotropin-releasing hormone: Central serotonergic and vascular mechanisms of action. CNS Trauma **4,** 181–196.

SALZMAN, S.K., HIROFUJI, E., LLADOS-ECKMAN, C., MacEWEN, G.D., and BECKMAN, A.L. (1987b). Monoaminergic response to spinal trauma: Participation of serotonin in posttraumatic progression of neural damage. J. Neurosurg. **66,** 431–439.

SALZMAN, S.K., MENDEZ, A.A., DABNEY, E.W., et al. (1991a). Serotonergic response to spinal distraction trauma in experimental scoliosis. J. Neurotrauma **8,** 45–54.

SALZMAN, S.K., PUNIAK, M.A., LIU, Z.-J., MAITLAND-HERIOT, R.P., FREEMAN, G.M., and AGRESTA, C.A. (1991b). The serotonin antagonist mianserin improves functional recovery following experimental spinal trauma. Ann. Neurol. **30,** 533–541.

SHARMA, H.S., DEY, P.K., and OLSSON, Y. (1989). Brain edema blood brain barrier permeability and cerebral blood flow changes following intracarotid infusion of serotonin: Modification with cyproheptadine and indomethacin, in: *Pharmacology of Cerebral Ischemia 1988*, J. Krieglstein (ed), CRC Press: Boca Raton, FL, pp. 317–323.

SHARMA, H.S., and OLSSON, Y. (1990a). Edema formation and cellular alterations following spinal cord injury in the rat and their modification with p-chlorophenylalanine. Acta Neuropathol. **79,** 604–610.

SHARMA, H.S., WESTMAN, J., OLSSON, Y., JOHANSSON, O., and DEY, P.K. (1990b). Increased 5-hydroxytryptamine immunoreactivity in traumatized spinal cord. Acta Neuropathol. **80,** 12–17.

SINGH, S., SINGH, P.M., PRASAD, G.C., and UDUPA, K.N. (1986). Response and action of 5-hydroxytryptamine in experimental head injury. Indian J. Exp. Biol. **24,** 503–507.

SMITH, D.R., DUCKER, T.B., and KEMPE, L.G. (1969). Experimental in vivo microcirculatory dynamics in brain trauma. J. Neurosurg. **30,** 664–672.

TANNO, H., NOCKELS, R.P., PITTS, L.H., NOBLE, L.J. (1992). Breakdown of the blood-brain barrier after fluid percussive brain injury in the rat: Part 1: Distribution and time course of protein extravasation. J. Neurotrauma **9,** 21–32.

VANHOUTTE, P.M., and HOUSTON, D.S. (1985). Platelets, endothelium and spasm. Circulation **72,** 728–734.

VAN NEUTEN, J.M., JANSSENS, W.J., and VANHOUTTE, P.M. (1985). Serotonin and vascular smooth muscle, in: *Serotonin and the Cardiovascular System*, P.M. Vanhoutte (ed), Raven Press: NY, pp. 95–103.

WESTER, P., DIETRICH, W.D., PRADO, R., WATSON, B.D., and GLOBUS, M.Y.-T. (1992). Serotonin release into plasma during common carotid artery thrombosis in rats. Stroke **23,** 870–875.

YAKUBU, M.A., SHIBATA, M., and LEFFLER, C.S. (1994). Subarachnoid hematoma attenuates vasodilation and potentiates vasoconstriction induced by vasoactive agents in newborn pigs. Pediatr. Res. **36,** 589–594.

YAMAKAMI, I., and McINTOSH, T.K. (1989). Effects of traumatic brain injury on regional cerebral blood flow in rats as measured with radiolabeled microspheres. J. Cereb. Blood Flow Metab. **9,** 117–124.

YANG, B.C., NICHOLS, W.W., LAWSON, D.L., and MEHTA, J.L. (1992). 5-hydroxytryptamine potentiates vasoconstrictor effect of endothelin-1. Am. J. Physiol. **262,** H931–H936.

YOUNG, H.A., KOLBECK, R.C., and SCHMIDEK, H.H. (1986). Hemorrhage-induced alterations of rabbit basilar artery reactivity and sensitivity to 5-HT. Neurosurgery **19,** 502–506.

ZILLES, L. (1985). *The Cortex of the Rat. A Stereotaxic Atlas.* Springer-Verlag: Berlin.

ZIVIN, J.A. (1985). Cyproheptadine reduces or prevents ischemic central nervous system damage. Neurology **35,** 584–587.

ZIVIN, J.A., and VENDITTO, J.A. (1984). Experimental CNS ischemia: Serotonin antagonists reduce or prevent damage. Neurology **34,** 469–474.

ZIVIN, J.A., DOPPMAN, J.L., and REID, J.L. (1976).Biochemical and histochemical studies of biogenic amines in spinal cord trauma. Neurology **26,** 99–107.

Address reprint requests to:
Raul Busto
Department of Neurology (D4–5)
University of Miami School of Medicine
P.O. Box 016960
Miami, FL 33101

Journal of Neurotrauma 1997.14:35-42.