Central and peripheral biogenic amine effects of brain missile wounding and increased intracranial pressure

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This study was performed to ascertain the acute effects of brain missile wounding on brain-stem and hypothalamic biogenic amines in a group of cats anesthetized with pentobarbital (40 mg/kg). Brain wounding is associated with a concomitant increase in intracranial pressure (ICP); to separate the effects of elevated ICP alone from the effects of wounding, a second group of cats had ICP artificially increased from a normal level of approximately 5 mm Hg to approximately 140 mm Hg by infusion of mock cerebrospinal fluid into the cisterna magna. In both groups, significant epinephrine depletions (47% to 74%) occurred in the nucleus tractus solitarius, area A1C1, locus ceruleus, raphe nuclei, and posterior hypothalamus. Epinephrine levels were also significantly decreased in the anterior hypothalamus in the wounded cats. In addition, both brain wounding and artificially induced ICP increases caused significant decreases of norepinephrine in the posterior hypothalamus, and of serotonin, 5-hydroxyindoleacetic acid, dopamine, and homovanillic acid in the raphe nuclei. Only brain wounding, however, caused significant reductions of norepinephrine, dopamine, and homovanillic acid in the nucleus tractus solitarius and area A1C1. The plasma catecholamine levels resulting from brain wounding or artificially induced ICP increases were dissimilar only in the amount of time required to attain maximum plasma levels, with the wounded animals responding faster. It is concluded that the hypothalamic and brain-stem biogenic amine changes resulting from either brain wounding or increased ICP alone are reflective of a stress response. Brain-stem distortion caused by brain wounding did not appear to be a factor and monoaminergic systems appeared to remain intact despite a severe and eventually lethal brain injury. If the detected depletion of epinephrine and serotonin levels is associated with potentially lethal brain injury, pharmacological intervention may be possible.

KEY WORDS • intracranial pressure • brain injury • brain stem • monoamine • epinephrine • catecholamine • cat
of cats with artificially increased ICP to see whether the pattern of changes associated with missile wounding was intrinsically different from that associated with increased ICP alone. Plasma catecholamines (norepinephrine and epinephrine) were also measured both after wounding and after artificially increasing ICP.

Materials and Methods

General Surgical Procedures and Protocol

The rationale and experimental protocol for the use of the animal model in these studies have been reviewed by the Institutional Animal Care and Use Committee at the Louisiana State University Medical Center in New Orleans. The research adhered to the National Institutes of Health "Guide for the Care and Use of Laboratory Animals."

Unselected, unfasted mongrel cats (each weighing 3 to 5 kg) of either sex were used. The animals were first anesthetized with intraperitoneal pentobarbital (40 mg/kg). Additional pentobarbital (2 to 5 mg/kg) was administered intravenously if required. Arterial and venous cannulas (polyethylene 90) were implanted in the right rear leg and an endotracheal tube was inserted. The cat was mounted in a stereotactic frame and a mean arterial blood pressure (MABP) transducer was attached to the arterial cannula for physiographic recording.¢ Expired CO₂ was measured by an end-tidal CO₂/respiratory rate monitor and also recorded on the physiograph. Intracranial pressure was measured with a fiberoptic ICP transducer.† Arterial blood gas and pH levels were periodically checked using an acid-base analyzer.§ Rectal temperature was monitored and maintained via an automatic thermal heating blanket.¶ If apnea occurred and lasted more than 30 seconds during any of the experiments, the cat was attached to a small-animal respirator and artificially ventilated. At the end of the experiment the cats, while still deeply anesthetized, were rapidly decapitated using a large-animal decapitator (brain biogenic amine experiments) or given a pentobarbital overdose (plasma catecholamine experiments).

Brain Biogenic Amine Experiment Protocol

In the animals that received a brain wound, 1 sq cm of the anterior wall of the right frontal sinus was removed. The cats were wounded with a 2-mm steel sphere having an energy of 2.4 J. The missile entered the tip of the right cerebral hemisphere and tracked posteriorly angling 20° from the midline. A wound of this energy is considered severe and should eventually be expected to cause fatal apnea in 70% of the animals. All cats were sacrificed 6 minutes after the initial brain injury.

A separate group of cats had their ICP artificially increased by infusing mock cerebrospinal fluid (CSF) through a No. 20 spinal needle inserted into the cisterna magna. Pressure in a mock-CSF reservoir was raised by increasing air pressure within the sealed aspirator bottle by means of a 60-ml air-filled syringe connected to the CSF-reservoir bottle with appropriate tubing and fittings. By this means, the ICP was rapidly raised to between 120 and 140 mm Hg within 30 seconds and maintained at that level for 6 minutes prior to sacrifice.

Control animals for each experimental group (brain-wounded and artificially increased ICP) were anesthetized and surgically prepared identically to their respective experimental group, but were neither wounded nor had their ICP artificially increased.

Plasma Catecholamine Experiment Protocol

Separate groups of cats were used to determine the time course of the plasma catecholamine (epinephrine and norepinephrine) response. Experimental groups of three animals each consisted of a control group, those receiving a brain missile wound of either 0.9 J, 1.4 J, or 2.4 J, and those with ICP artificially increased by infusion of mock CSF into the cisterna magna. In the animals in which the ICP was artificially increased, the ICP was controlled over a 30-minute period to mimic as closely as possible the ICP response that accompanies a brain missile wound. Blood samples were taken just prior to beginning the experiment, then at 1, 3, 5, 10, 20, and 30 minutes after the injury or when the MABP began to rise sharply.

Tissue Dissection

After rapid decapitation, the cranium was opened and the brain removed and frozen by immersion in cold dichlorodifluoromethane (−40°C), then stored at −70°C for 1 to 2 days until the sampling process. The brains were subsequently brought to approximately −15°C and sliced into 3- to 5- mm thick coronal sections with reference to standard cat brain atlases.¶ Tissue samples were taken from still-frozen slices with the aid of tissue micropunches (1 to 1.5 mm diameter) or a scalpel, then placed in dry ice and stored at −70°C until assayed (1 to 2 days).

Sample Preparation

For determining of brain biogenic amine levels, samples from the left and right sides of the nucleus tractus solitarius, area A1Cl, and locus ceruleus were analyzed individually; since the raphe nuclei are not lateralized, they were analyzed as one sample. Tissue samples were

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* Transducer, Model P1000B, and physiograph, Model DMPA4A, manufactured by Narco Bio-Systems, Houston, Texas.
† End-tidal CO₂ monitor, Model IL-200, manufactured by Instrumentation Laboratory, Lexington, Massachusetts.
‡ Fiberoptic ICP monitor, Model 420, manufactured by Camino Laboratories, San Diego, California.
§ Rectal temperature monitor, Model ABL 30, Radiometer, Copenhagen, Denmark.
¶ Automatic thermal heating blanket, animal respirator, and animal decapitator manufactured by Harvard Apparatus, South Natick, Massachusetts.

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homogenized by ultrasonic disruption in 0.05 M perchloric acid containing 0.5 mM ethylenediaminetetraacetic acid (EDTA) and 20 pmol of normetanephrine as an internal standard. Following centrifugation at 40,000 G for 25 minutes the supernatant was removed for subsequent analysis by high-pressure liquid chromatography and the remaining tissue pellets were solubilized in 0.2 M sodium hydroxide and assayed for protein content using BCA protein assay reagent and bovine serum albumin as a standard. Plasma catecholamines were extracted by an alumina extraction procedure.5

High-Pressure Liquid Chromatography

Standards were obtained for liquid chromatography* and working solutions were prepared from frozen stock solutions (0.05 M perchloric acid containing 0.1 mM EDTA). Water and acetonitrile were of a grade suitable for high-pressure liquid chromatography. A high-pressure liquid chromatography system with an electrochemical detector was utilized for all analyses: the analytical column used for all assays was a 100 mm × 3.1-mm 3 μm reverse-phase column.†

For brain biogenic amines (norepinephrine, epinephrine, dopamine, and serotonin) and their metabolites (3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid) the column was kept at 40°C. The mobile phase consisted of 0.13 M monochloroacetic acid with 0.67 mM EDTA, pH 3.1, sodium octyl sulfate (170 mg/liter), and acetonitrile (2.5%). The mobile phase was pumped at 1 ml/min and maintained at 35°C. The detector potential was maintained at +800 mV versus Ag/AgCl reference electrode.

For the plasma catecholamine analyses, the column and mobile phase were kept at ambient temperature. The mobile phase consisted of 0.075 M monochloroacetic acid with 0.67 mM EDTA, pH 3.0 to 3.05, sodium octyl sulfate (1 mM), and acetonitrile (1.5%). The mobile phase was pumped at 1 ml/min, and the detector potential maintained at +800 mV versus Ag/AgCl reference electrode.5

Data Analysis

All brain biogenic amine concentrations were corrected for recovery from tissue samples using the internal standard method and are expressed as nanograms/milligram of protein. Plasma catecholamine concentrations are expressed as picograms/milliliter of plasma.

Initially, the brain biogenic amine values from the right- and left-side samples of the nucleus tractus solitarius, area A1C1, and the locus ceruleus in the control and experimental groups were compared using analysis of variance (ANOVA).‡ No significant differences were seen between the right- and left-side samples of any brain area in any experimental group (all p > 0.8) and therefore right and left sample values were averaged for further statistical analyses. Comparisons between the control and experimental groups in brain biogenic amine experiments were made using ANOVA. The plasma catecholamine data were initially analyzed using ANOVA for repeated measures, and specific comparisons to the preinjury time period were made using paired t-tests.

Results

Brain Biogenic Amines

Nineteen cats were used in brain injury experiments but three cats were excluded from the study. There were technical ballistic problems with two cats, and one cat was anomalous because the skull over the frontal cortex was absent. Seventeen cats were used in artificially increased ICP experiments. Because of technical problems with rapidly increasing the ICP, five cats were excluded from further analysis.

The effects on MABP and cerebral perfusion pressure resulting from brain wounding (2.4 J) and artificially induced (unwounded) increases in ICP are displayed in Fig. 1. With either brain wounding or a rapid rise in ICP caused by mock-CSF infusion into the cisterna magna, there was an immediate (< 5 second) increase in MABP. Adequate cerebral perfusion pressure (≥ 40 mm Hg) was maintained in both groups. None of the cats in these experiments required respiratory support. No significant effects on systemic pressures occurred in the 14 cats in the control group, which were surgically prepared but were neither wounded nor subject to artificially increased ICP (data not shown).

Significant and similar decreases in the epinephrine levels in the posterior hypothalamus, nucleus tractus solitarius, area A1C1, locus ceruleus, and raphe nuclei resulted from both brain wounding and artificially induced ICP increases. Epinephrine levels were significantly decreased in the anterior hypothalamus only in wounded cats (Fig. 2).

The norepinephrine levels were significantly decreased to approximately the same extent in the posterior hypothalamus as a result of either brain wounding or artificially increasing ICP (40%, p < 0.01, and 46%, p < 0.01, respectively). The wounded cats also had significant norepinephrine decreases in the nucleus tractus solitarius (21%, p < 0.01), and the area A1C1 (33%, p < 0.01).

The serotonin and 5-hydroxyindoleacetic acid levels were significantly decreased in the raphe nucleus in both the wounded cats (30%, p < 0.05, and 22%, p < 0.05, respectively) and those with only artificially increased ICP (30%, p < 0.01, and 36%, p < 0.01, respectively).

* BCA protein assay reagent obtained from Sigma Chemical Co., St. Louis, Missouri.
† High-pressure liquid chromatography system, Model BAS 200, and BAS Phase II column manufactured by Bioanalytical Systems, Inc., West Lafayette, Indiana.
‡ SAS statistical package, developed by SAS Institute, Cary, North Carolina.
Dopamine levels were significantly decreased in the raphe nuclei either from brain wounding or artificially induced increases in ICP (37%, p < 0.01, and 27%, p < 0.05, respectively). Significant decreases in dopamine in the nucleus tractus solitarius (39%, p < 0.05) and area A1Cl (48%, p < 0.05) occurred only in wounded cats.

Concomitant decreases in homovanillic acid occurred in the same areas in which dopamine was decreased: the raphe nuclei of wounded cats (29%, p < 0.05) and those with only artificially increased ICP (32%, p < 0.01), and in the nucleus tractus solitarius (34%, p < 0.05) and area A1Cl (40%, p < 0.01) only in wounded cats. Levels of 3,4-dihydroxyphenylacetic acid were significantly decreased in the nucleus tractus solitarius (21%, p < 0.05) only in wounded cats.

**Plasma Catecholamines**

Twenty cats were used in the plasma catecholamine experiments but, because of ballistic technical problems, five cats were excluded from the study. The effects of brain missile wounding at 1.4 J and artificially increased ICP on MABP and cerebral perfusion pressure are shown in Fig. 3. Both brain wounding and artificially increased ICP caused an immediate increase in MABP. In both groups, adequate cerebral perfusion pressure was maintained and respiratory support was not required. No systemic pressure effects occurred in the three control cats, which were surgically prepared but were neither wounded nor had their ICP artificially increased (data not shown).

The plasma catecholamines (epinephrine and noradrenaline) reached their maximum levels within 1 minute as a result of brain wounding (Fig. 4). In contrast, cats that were subjected only to an artificially rapid increase in ICP displayed a slower and more variable time course of plasma catecholamine increases (Fig. 4). The variability in the plasma catecholamine increase and the small number of animals used in these latter experiments precluded determining statistical significance in the data. Plasma catecholamine levels in the control group were unaffected throughout the 30-minute experimental period (data not shown).

Animals injured at 2.4 J responded similarly to those injured at 1.4 J, both in the resulting systemic pressures and the plasma catecholamine values. Injuries at 0.9 J evoked increases in systemic pressures and significant
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plasma catecholamine increases at 1 minute postinjury, but the values were much smaller than in animals injured at 1.4 J or 2.4 J (data not presented).

Discussion

We have delineated the effects of a severe missile wound to the brain on hypothalamic and brain-stem biogenic amine levels. Brain wounding is accompanied by a concomitant rise in ICP. Thus, we also evaluated the effect of an abrupt ICP increase (via intracisternal infusion of mock CSF) on the biogenic amine systems to discern whether the observed pattern of biogenic amine changes following wounding could best be explained by the unique effects of missile wounding or perhaps were the direct result of increased ICP. In addition, we measured the effects of these two stimuli on plasma catecholamines.

In our experiments, both brain wounding and increased ICP alone without wounding caused a significant immediate systemic pressor response. Following ICP elevation, sympathetic nervous activity may be greatly increased,23,25 inciting distinct cardiovascular responses including a dramatic increase in MABP.6,12,23,27,37 Hypothalamic and brain-stem epinephrine neurons believed to subserve vasopressor and vasodepressor functions may be activated.5,16,26,33,38

Epinephrine

Both brain-wounded cats and those with mock-CSF infusions to increase ICP showed significant decreases (47% to 74%) in epinephrine levels in the posterior hypothalamus, nucleus tractus solitarius, area A1C1, locus ceruleus, and raphe nuclei. Brain-wounded cats showed a significant epinephrine reduction (31%) in

FIG. 3. Graphs showing the effect over 30 minutes of brain missile wounding at 1.4 J in three cats (left) and cisterna magna infusions of mock cerebrospinal fluid in three cats (right) on mean arterial blood pressure (MABP), intracranial pressure (ICP), and cerebral perfusion pressure (CPP). Values are expressed as means ± standard error of the means.

FIG. 4. Graphs showing the effect of brain missile wounding (three cats, squares) and artificially induced increases in intracranial pressure (three cats, circles) on plasma epinephrine (left) and norepinephrine (right) levels for 30 minutes after brain wounding and/or elicitation of the pressor response. Values are expressed as means ± standard error of the means. Statistical significance (paired t-test): * = p < 0.05; ** = p < 0.01.
the anterior hypothalamus as well. Cats in which the ICP was raised by mock-CSF infusion also had reduced epinephrine in the anterior hypothalamus, but these reductions did not reach significance.

While brain wounding with attendant brief differences in ICP between supra- and infratentorial compartments might be expected to cause brain-stem displacement, infusion of mock CSF would not. In the latter case, the induced ICP rise would have occurred more slowly and the resultant pressure increase would have been distributed evenly within the cranium. Since most of the observed hypothalamic and brain-stem epinephrine changes occurred with increased ICP alone (without brain-stem displacement) it would appear that the ICP increase associated with brain wounding was an adequate stimulus to cause the postwounding biogenic amine changes. Possibly brain-stem displacement caused by wounding might have enhanced these changes, but brain-stem distortion would not appear to be a primary factor.

Norepinephrine and Dopamine

Norepinephrine and dopamine and its metabolites (3,4-dihydroxyphenylacetic acid and homovanillic acid) did not show consistent changes in level paralleling those of epinephrine, suggesting a dissociation between the epinephrine and the norepinephrine and dopamine systems. Although norepinephrine, like epinephrine, decreased in the posterior hypothalamus in response to wounding and ICP increase, it was unaffected by either stimulus in the anterior hypothalamus, locus ceruleus, or raphe nuclei. The fact that both epinephrine and norepinephrine decreased in the posterior hypothalamus may be of particular significance because the posterior hypothalamus supposedly mediates the vasopressor response, and application of epinephrine or norepinephrine to the posterior hypothalamus will increase MABP.19,28,30,31,42 We cannot be certain if the depletions found in our experiments were caused by increased epinephrine or by norepinephrine utilization or by decreased neuronal functioning. Likewise, we cannot definitively say whether epinephrine and norepinephrine changes represent causative vasopressor changes or attempts at vasopressor compensation.

Only brain wounding significantly reduced norepinephrine, dopamine, and homovanillic acid levels in the nucleus tractus solitarius and area A1C1. Increasing ICP alone decreased norepinephrine, dopamine, and homovanillic acid levels in these two areas, but not significantly. Since dopamine is a precursor to norepinephrine in noradrenergic neurons and because the levels of dopamine in the nucleus tractus solitarius and area A1C1 are small, the observed dopamine decreases might basically have resulted from norepinephrine reductions.

Serotonin/Raphe Nuclei

Levels of serotonin and its metabolite 5-hydroxyindoleacetic acid, as well as dopamine and homovanillic acid, were significantly reduced in the raphe nuclei in response to either brain wounding or increased ICP alone. The meaning of either decreased serotonergic functioning or dopamine depletions in the raphe nuclei relative to cardiovascular functioning is difficult to discern because discrete raphe nuclei were not sampled.

Sympathetic Response

As previously stated, the most basic effect of an increase in ICP is a massive increase in sympathetic nerve activity which causes a systemic pressor response. Generalized activation of the sympathetic nervous system is also the main component of the classic stress response, which is also associated with an increase in MABP. Both foot shock and immobilization stress have resulted in epinephrine and/or norepinephrine decreases in the hypothalamus, nucleus tractus solitarius, area A1C1, and locus ceruleus.14,24,34,36,59 Thus, from the similarity of patterns of biogenic amine responses in the hypothalamus and brain stem from either stress or increased ICP, we hypothesize that the response to increased ICP and brain wounding is a variant of a generalized stress response. Although the peripheral manifestations of an ICP increase (bradycardia instead of tachycardia and respiratory slowing instead of tachypnea) differ somewhat from those of somatic stress owing to direct brain-stem stimulation or distortion, the hypothalamic and brain-stem biogenic amine changes are remarkably similar as though the hypothalamus and brain stem are “hard wired” to react to all stress, whether it be from an increase in ICP or other source, in a specific stereotypic fashion.

Plasma Catecholamines

Both the ICP increase and brain wounding caused a rise in plasma catecholamines, although the plasma catecholamine rise was faster following brain wounding. This effect after wounding may have been the result of an abrupt brain-stem displacement activating medullary sensitive areas15,17,43 more rapidly than did increasing ICP alone by intracisternal infusions of mock CSF. In our model, a 0.9-J brain wound which increased ICP by only 10 to 15 mm Hg caused an immediate MABP rise and a large plasma catecholamine increase. By contrast, ICP had to be raised in excess of 80 mm Hg by mock-CSF infusion to elicit a systemic pressor response and plasma catecholamine increase.

The direction of applied force to the brain has been noted to affect the subsequent systemic pressor response in primates.13 In keeping with this observation, our results show that a 2.4-J brain injury applied transverse to the axis of the brain stem caused less of an MABP and plasma catecholamine rise than did a 2.4-J injury following a trajectory applied more in line with the hypothalamus/brain-stem axis (unpublished data).

Conclusions

Despite the severe brain injury and/or large increases in ICP in our experiments, none of the hypothalamic
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and biogenic amines was totally depleted. Insofar as the monoaminergic system was concerned, the pattern of a generalized stress response was preserved, indicating that the basic integrity of this system remained intact. Only selective, organized alterations occurred in the epinephrine system overall and in the serotonin system in the raphe nuclei. This suggests that a severe brain wound likely to cause early death does not do so by totally disrupting the hypothalamic and brain-stem biogenic amine system. If this system remains basically intact following severe brain injury, discrete alterations in the biogenic amine system which in the future are shown to be important may be susceptible to pharmacological therapy.

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