Age-Dependent Vulnerability of Brain Choline Acetyltransferase Activity to Transient Cerebral Ischemia in Rats

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Male Fischer-344 rats aged 6, 12, or 24 months were subjected to four-vessel occlusion cerebral ischemia to assess age-dependent ischemic vulnerability of cholinergic and GABAergic neurons based on choline acetyltransferase (EC 2.3.1.6) and glutamic acid decarboxylase (EC 4.1.1.15) activities. Activities of both enzymes were similar (p > 0.05) in 6- (n=5) and 12- (n=5) month-old rats. Mean±SEM choline acetyltransferase activities in the cortex, hippocampus, striatum, and cerebellum of 6-month-old controls were 75±5, 123±9, 415±9, and 50±4 nmol acetylcholine/hr/mg protein, respectively, and were 20–30% lower (p<0.05) in all brain regions except the cerebellum in 24-month-old controls. Choline acetyltransferase activity was unaffected by ischemia in 6- and 12-month-old rats but was reduced by 30–60% in 24-month-old rats. Mean±SEM glutamic acid decarboxylase activities in the cortex, hippocampus, striatum, and cerebellum of 6-month-old controls were 98±8, 86±7, 144±13, and 125±9 nmol γ-aminobutyric acid/hr/mg protein, respectively, and 25–35% lower in all regions of 24-month-old controls. After 30 minutes of ischemia and 5 days of recovery, glutamic acid decarboxylase activities were reduced (p<0.05) in all brain regions and age groups. However, its activity was decreased (p<0.05 compared with age-matched controls) by 55% in the cortex and 79% in the hippocampus of 24-month-old rats compared with 30% and 45% in younger rats. Our results show that both choline acetyltransferase and glutamic acid decarboxylase activities were more vulnerable to ischemia in 24-month-old than in 6- and 12-month-old rats. (Stroke 1989;20:495–500)

The effects of ischemia on indexes of cerebral function and integrity have been partially characterized in humans and in animal models of ischemia. It is well known that neurons are more vulnerable to ischemia than are other cell types in the central nervous system and that certain neurons are more sensitive to ischemia than others. Only recently has there been any attempt to distinguish the more sensitive neurons on the basis of their neurochemical markers, especially their ability to synthesize, store, release, and respond to specific neurotransmitters. To this end, Francis and Pulsinelli measured the activities of choline acetyltransferase (EC 2.3.1.6; ChAT) and glutamic acid decarboxylase (EC 4.1.1.15; GAD) in the striatum and hippocampus of male Wistar rats subjected to transient cerebral ischemia by the four-artery occlusion method. These enzymes are responsible for the synthesis of the neurotransmitters acetylcholine and γ-aminobutyric acid (GABA), respectively, and their activities represent the integrity of their respective neurotransmitter systems. Francis and Pulsinelli reported a regional vulnerability to the effects of transient cerebral ischemia for the GABAergic marker and no effect of transient cerebral ischemia on the cholinergic marker.

Aging is also associated with many morphologic and biochemical alterations in the central nervous system, including a selective vulnerability of neuronal populations to the effects of age. Interestingly, the regions of the brain that appear most sensitive to age-related changes include those regions most susceptible to ischemic damage: the cortex, hippocampus, and striatum. Changes associated with aging may be accelerated by ischemia and vice versa as the two processes may be additive or supra-additive in their effects on the sensitive neuronal populations. It is important to note that in contrast to what was reported for transient cerebral
ischemia, the cholinergic neuronal system appears to be as sensitive, if not more sensitive, to the effects of aging than the GABAergic system.\textsuperscript{7,8} It is possible that with aging, the cholinergic as well as the GABAergic nervous systems become more sensitive to the effects of ischemia.

We describe the effects of transient cerebral ischemia by the four-artery occlusion model on the regional activities of the neurotransmitter synthetic enzymes ChAT and GAD in Fischer-344 rats of various ages. Our objective was to determine whether age affects the vulnerability of specific brain neuronal populations to the effects of transient cerebral ischemia.

**Materials and Methods**

We used 45 male Fischer-344 rats aged 6, 12, or 24 months, obtained from the contract rodent colonies of the National Institute on Aging (Bethesda, Maryland). Rats were accommodated in groups of five to seven per cage in a vivarium maintained at 23±3°C with a 12-hour light (6 AM to 6 PM Central Standard Time):12-hour dark photoperiod. Food and tap water were available ad libitum.

Using the method of Pulsinelli and Brierley,\textsuperscript{9} we induced transient cerebral ischemia in 30 rats by occluding the four major arteries supplying the brain. In this procedure, both vertebral arteries were cauterized and reversible clamps were placed around the common carotid arteries without disturbing carotid blood flow. We performed all the surgical procedures under halothane and nitrous oxide anesthesia using sterile techniques. Twenty-four hours after surgery, half of the surgically prepared rats (the ischemic group) were restrained by gloved hand, and blood flow to the brain was interrupted by closing the carotid clamps for 30 minutes. The remaining 15 surgically prepared rats (the sham group) were handled similarly but without interruption of the carotid blood flow. A third, surgically naive group was included in the study to assess the effects of surgery on the neurochemical markers. There were five rats of each age per treatment group.

Rats from each group were killed 5 days after the above procedure. Brains were rapidly removed and dissected. Samples of the cortex, hippocampus, striatum, and cerebellum were homogenized in 20 volumes (wt/vol, 1 mg/20 \( \mu l \)) of 0.32 M sucrose. ChAT activity was measured radiometrically by the method of Bull and Oderfeld-Nowak.\textsuperscript{10} Standard incubations contained approximately 50 \( \mu g \) of protein, 60 mM choline chloride (Sigma Chemical Co., St. Louis, Missouri) and 0.1 mM \([\text{14C}]\)acetyl-CoA (2–5 \( \mu ci/\text{mmol} \); Amersham Corp., Arlington Heights, Illinois). GAD activity was measured radiometrically by the method of Wilson et al.\textsuperscript{11} Standard incubations contained approximately 250 \( \mu g \) of protein, 2.5 mM \( l \)-glutamic acid (Sigma) with 5–10 \( nCi \) \([\text{14C}]\)glutamic acid (>250 \( \mu c i/\text{mmol} \); New England Nuclear Corp., Boston, Massachusetts). Enzyme activities are reported in terms of protein, assayed spectrophotometrically, with bovine plasma albumin as the standard.\textsuperscript{12}

The effects of age and treatment among the three groups were assessed by analysis of variance.\textsuperscript{13} When the value of \( F \) indicated significance, individual means were compared by Duncan’s multiple range test.\textsuperscript{13} The criterion for significance was \( p \leq 0.05 \).

**Results**

ChAT and GAD activities for all ages and treatments are shown for the cortex, hippocampus,
changes after ischemia in rats

Figure 2. Bar graphs, mean ± SEM glutamic acid decarboxylase (GAD) (top) and choline acetyltransferase (ChAT) (bottom) activity in hippocampus of five Fischer-344 rats. * different from naive and sham groups, p ≤ 0.05. Naive, sham, and ischemic GAD and ChAT activities at 24 months are significantly different from those of similarly treated younger rats, p ≤ 0.05.

Figure 3. Bar graphs, mean ± SEM glutamic acid decarboxylase (GAD) (top) and choline acetyltransferase (ChAT) (bottom) activity in striatum of five Fischer-344 rats. * different from naive and sham groups, p ≤ 0.05. Naive, sham, and ischemic GAD and ChAT activities at 24 months are significantly different from those of similarly treated younger rats, p ≤ 0.05.

striatum, and cerebellum in Figures 1–4, respectively. ChAT activity is expressed as mean ± SEM for five rats per age per group in units of nanomoles acetylcholine per hour per milligram protein. GAD activity is expressed as mean ± SEM for five rats per age per group in units of nanomoles GABA per hour per milligram protein. We observed no significant differences by region between sham and naive groups for ChAT and GAD activities for any age tested.

ChAT activity in 6-month-old sham rats was greatest in the striatum (415 ± 19), followed by the hippocampus (123 ± 9), cortex (75 ± 5), and finally, the cerebellum (50 ± 4). ChAT activity in 24-month-old sham rats in the striatum (337 ± 40), hippocampus (85 ± 5), and cortex (50 ± 6) was significantly lower than that of 6-month-old sham rats (p < 0.05). There were no age differences observed for ChAT activity in the cerebellum.

Five days after transient cerebral ischemia, ChAT activity in all four regions of 24-month-old ischemic rats was significantly reduced compared with that of age-matched sham or naive rats (p < 0.05). ChAT
activity following ischemia (compared with that of age-matched shams) was reduced from 50±6 to 17±8 in the cortex, from 85±5 to 47±5 in the hippocampus, from 337±40 to 165±42 in the striatum, and from 57±4 to 35±2 in the cerebellum. Transient cerebral ischemia had no effect on regional ChAT activity in 6- or 12-month-old rats.

GAD activity in 6-month-old sham rats was highest in the striatum (144±13), followed by the cerebellum (125±9), cortex (98±8), and lastly the hippocampus (86±7). GAD activity in 24-month-old sham rats in the cerebellum (100±10), striatum (98±8), cortex (66±4), and hippocampus (64±4) was significantly lower than that in 6-month-old sham rats.

GAD activity was significantly lower in the ischemic group than in the sham or naive groups in each region and at each age tested \( (p<0.05) \); the reduction of GAD activity following ischemia averaged 45%. The effects of ischemia were uniform among age groups in the striatum and cerebellum, with GAD activity averaging 59% and 45% of age-matched sham values, respectively. However, the effects of ischemia in the cortex and hippocampus were not uniform among age groups. Transient cerebral ischemia in 6-month-old rats reduced GAD activity (compared with that of age-matched shams) in the cortex by 30%, from 98±8 to 69±7, and in the hippocampus by 45%, from 86±7 to 47±6. In contrast, transient cerebral ischemia in 24-month-old rats reduced GAD activity in the cortex by 53%, from 66±4 to 30±8, and in the hippocampus by 78%, from 64±4 to 14±8.

**Discussion**

Our findings demonstrate age differences in regional ChAT and GAD activities in the brains of Fischer-344 rats. These differences are partially consistent with earlier reports. Ours is the first report of age-related decrements in regional GAD activity in Fischer-344 rats. For example, we observed an age-related decrease in ChAT activity in the cortex, hippocampus, and striatum but not in the cerebellum of Fischer-344 rats. Although similar age-related reductions of ChAT activity have been observed in the cortex, striatum, and hippocampus of Fischer-344 rats,14-16 reports of a lack of age-related changes in ChAT activity are also present in the literature.14-16 The reasons for these inconsistencies in age effects for ChAT are not known. However, factors such as differences in the age of the rats tested, their sources, parent colonies, handling and assay procedures, environmental factors, and individual variations may, at least in part, be responsible for the differences observed.

The effects of transient cerebral ischemia by four-artery occlusion on markers of the cholinergic and GABAergic systems of rats we reported also agree in part with the effects reported by others. Francis and Pulsinelli4 reported that transient cerebral ischemia depressed activity of the GABAergic marker, GAD, in the striatum but not in the hippocampus and had no effect on activity of the cholinergic marker, ChAT, in either region. Recently, Kakihana et al19 reported depression of a cholinergic marker, specifically the rate of acetylcholine synthesis, in the cortex following transient cerebral ischemia in male Wistar rats approximately 2 months of age. We used more mature 6-month-old Fischer-344 rats and reported depression in GAD activity in the striatum and hippocampus and no effect of ischemia on ChAT activity. The cause of the discrepancies between these reports regarding the effects of...
transient cerebral ischemia is not apparent. Factors that may have contributed include differences in the strain and age of the rats used, methodologic differences, differences in the severity of the ischemic episode, and experimental variability.

The four-artery occlusion model of ischemia was originally characterized by Pulsinelli and Brierley9 using Wistar rats. Subjected to 30 minutes of occlusion, Wistar rats demonstrated severe neuronal damage 72 hours later in the hippocampus and striatum and less severe damage in the anterior and posterior areas of the cortex and cerebellum. Using four-artery occlusion in Fischer-344 rats, evidence in our study suggests that the neuronal damage associated with ischemia occurs in each of the four regions tested. Although no estimate of the severity of neuronal damage can be made directly, indirect assessment of neuronal damage, specifically the reduction in enzyme activity, is possible. Based on the reduction in GAD activity following transient cerebral ischemia, it appears that there are GABAergic neurons sensitive to ischemic damage in each region and at each age tested. Further, as the magnitude of GAD activity reduction was greater in the hippocampus and cortex of 24-month-old rats than in younger rats, it seems that aging of the GABAergic neurons in these regions is associated with an enhanced sensitivity to ischemic damage. Similarly, as the reduction in ChAT activity following transient cerebral ischemia was observed only in 24-month-old rats, it would appear that aging of the cholinergic system is also associated with enhanced sensitivity to ischemic damage. There is additional indirect evidence that the difference in vulnerability is due to differences in neuronal sensitivity to ischemic damage and not simply the result of age-related differences in blood flow disruption. If neuron vulnerability to ischemic damage were based only on the disruption of blood flow and not on the neuron's sensitivity to ischemia, then the patterns of sensitivity between the two markers would be similar. The regions of the brain most sensitive to ischemic damage in 24-month-old rats are different for ChAT and GAD, as determined by the magnitude of enzyme activity reduction. For ChAT, the most sensitive region was the cortex, followed by the striatum, the hippocampus, and finally the cerebellum. For GAD, the most sensitive region was the hippocampus, followed by the cerebellum, the cortex, and finally the striatum.

While our study results are preliminary, they do provide valuable insight into the effects of ischemia on the aging brain. To our knowledge, ours is the first report of age-related vulnerability of cholinergic and GABAergic markers to transient cerebral ischemia. In 24-month-old rats but not in 6- or 12-month-old rats, ChAT activity was very sensitive to transient cerebral ischemia. Transient cerebral ischemia's effects on GAD activity in the hippocampus and cortex of 24-month-old rats were greater than those in younger rats. The loss of ChAT and GAD activity after four-vessel occlusion possibly represents the death of cholinergic neurons caused by the transient cerebral ischemia. In a related study, Gibson et al20 reported changes in neurotransmitter synthesis during mild hypoxia in older rats. They reported that the combination of aging and hypoxia reduces the brain's ability to synthesize acetylcholine and, to a lesser extent, GABA from glucose. These findings considered together with ours support the hypothesis that aging selectively reduces the brain's ability to adapt to the metabolic insults associated with ischemia. However, it should be noted that neither Gibson et al20 nor we addressed the reversibility of these changes or the aged brain's ability to recover from these effects.

It is not possible from these studies to ascertain why an age-related vulnerability to transient cerebral ischemia occurs. It is possible that the neurons sensitive to transient cerebral ischemia as a function of age were already compromised by another age-related event. Further studies will be necessary to characterize the significance of these differences in the aged brain's response to transient cerebral ischemia. Altered neurotransmitter function may be responsible for some of the age-related motor and cognitive functional impairments observed in normal aging and age-related pathologies. Specific changes in central GABAergic and cholinergic neuronal systems have been associated with many of these functions.21-23

References

KEY WORDS • cerebral ischemia, transient • enzymes • rats
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