Brainstem Serotonergic Neurons in Chronic Alcoholics With and Without the Memory Impairment of Korsakoff’s Psychosis

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Abstract. There are several lines of evidence to suggest that serotonergic neurons in the brain are detrimentally affected by chronic alcohol consumption. The present study aims to quantify pathological changes in brainstem regions containing serotonergic neurons in chronic alcoholics compared to age-matched non-alcoholic controls. An antibody specific for tryptophan hydroxylase was used to immunohistochemically demonstrate serotonergic neurons in serial sections of postmortem brainstem. The cases analyzed were divided into four groups on the basis of their clinical and pathological presentation: chronic alcoholics with Wernicke’s encephalopathy, chronic alcoholics with additional Korsakoff’s psychosis, non-alcoholic controls, and a single chronic alcoholic without neurological complications. There was an overall reduction in the number of serotonergic neurons in all alcoholic cases when compared with controls. All brainstem regions were affected, but the largest neuronal loss was found in areas of the medullary and caudal pontine reticular formation (reduced by 80–90%). Alcoholics with Korsakoff’s psychosis did not differ in the amount or extent of pathology from the other alcoholic cases analyzed. The data indicate that significant numbers of serotonergic neurons degenerate in chronic alcoholics. Such a loss is likely to have significant clinical consequences.

Key Words: Alcohol; Amnesia; Memory; Raphe; Serotonin.

INTRODUCTION

Biochemical analyses have indicated that the serotonergic system is affected by alcohol both transiently and, with continued insult, permanently. The changes are characterized by a marked decrease in the levels of serotonin and serotonergic markers in the cerebrospinal fluid of alcoholic patients compared with controls (1–3). These data have been reinforced by animal experiments demonstrating the downregulation of monoaminergic systems after alcohol ingestion. In rats there is a decrease in serotonin and an increase in serotonergic receptors with alcohol administration (2–5). Monoamine oxidase-A, the degrading enzyme for serotonin, is also decreased by alcohol (6, 7).

Despite the number of biochemical studies highlighting the effects of alcohol on serotonin metabolism in the brain, there have been few pathological studies of serotonergic neurons in the brains of alcoholics. A single study has counted the number of Nissl-stained neurons in a single coronal section through the dorsal raphe nucleus of two chronic alcoholics with the permanent amnesia of Korsakoff’s psychosis (KP) and five age-matched controls (8). This study found no pathological changes in this nucleus using this method. In rats, the dorsal raphe nucleus contains the majority of serotonergic neurons in the brainstem (9, 10) and is considered to be the predominant serotonergic nucleus with ascending projections. However, in humans serotonergic neurons are more widely distributed throughout the midbrain and pons (11, 12), with many regions outside the dorsal raphe nucleus contributing to the ascending serotonergic projection system (11). It is interesting to note that in Alzheimer’s disease severe pathological changes and loss of brainstem raphe neurons have been found (13–16).

Chronic alcoholics often suffer a range of clinical disorders including Wernicke’s encephalopathy (WE) and KP. Wernicke’s encephalopathy is characterized by confusion, ataxia, nystagmus, and ophthalmoplegia. These clinical signs usually respond rapidly to administration of thiamin. Alcoholics with WE or KP have similar distinctive periventricular and mammillary body lesions. This has led to these apparently clinically distinct diseases being classified in pathological terms under a single entity, the Wernicke–Korsakoff syndrome (WKS). The aim of this study was to quantify in serial sections of the brainstem the populations of serotonergic neurons in the raphe nuclei of alcoholics compared with age-matched non-alcoholic controls. Because of the disruption to serotonergic systems in conditions affecting memory, such as Alzheimer’s disease, several groups of alcoholic with different clinical pictures were studied to determine the role of serotonin in differentiating patient groups, in particular those with abnormal memory functions (i.e. WE versus KP).

MATERIALS AND METHODS

Case Selection

Cases were collected and classified retrospectively as detailed below on the basis of clinical and pathological records from the
<table>
<thead>
<tr>
<th>Case #</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Post-mortem delay (h)</th>
<th>Brain weight (g)</th>
<th>Cause of death</th>
<th>Neuropsychological analysis</th>
<th>Diagnosis</th>
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<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>76</td>
<td>22</td>
<td>1,178</td>
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<td>—</td>
<td>normal brain</td>
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<td>2</td>
<td>M</td>
<td>58</td>
<td>14</td>
<td>1,649</td>
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<td>M</td>
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<td>normal brain</td>
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<td>4</td>
<td>M</td>
<td>70</td>
<td>11</td>
<td>1,183</td>
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<td>5</td>
<td>F</td>
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<td>6</td>
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<td>6</td>
<td>M</td>
<td>38</td>
<td>22</td>
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<td>7</td>
<td>M</td>
<td>51</td>
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<td>8</td>
<td>M</td>
<td>59</td>
<td>2</td>
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<td>9</td>
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<td>46</td>
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<td>15</td>
<td>M</td>
<td>66</td>
<td>36</td>
<td>1,233</td>
<td>grand-mal seizure</td>
<td>Korsakoff’s psychosis</td>
<td>WKS</td>
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Chronic alcoholic = alcohol consumption of > 80 g/day, WE = alcoholic Wernicke’s encephalopathy, WKS = alcoholic Wernicke-Korsakoff syndrome.

Royal Prince Alfred Hospital or the Institute of Forensic Medicine. Written consent for autopsy was obtained for hospital cases and the study was approved by the Human Ethics Committee of the University of Sydney and the Royal Prince Alfred Hospital under the New South Wales Transplantation and Anatomy Act. These cases have also been studied for the effects of chronic alcoholism on the brainstem locus ceruleus (17). Diet and alcohol consumption were detailed with the aid of telephone interviews with general practitioners and questionnaires to relatives in addition to the clinical records of the patients.

Criteria for inclusion in the study were: 1) no neurological, psychiatric or neuropathological abnormalities (e.g. stroke, Alzheimer’s disease) other than those associated with alcoholism (18); 2) post-mortem delay less than 72 hours; and 3) adequate clinical documentation of thiamin status and memory function.

Alcoholics with WKS consumed greater than 80 g of absolute alcohol per day for the majority of their adult life (range = 80-400 g ethanol/day, median consumption approximately 200 g), had clinical evidence of thiamin deficiency and had multiple admissions with documented evidence of amnesia and disorientation in the absence of an acute confusional state. Due to their memory dysfunction, these patients required permanent care. Thiamin deficiency was confirmed pathologically (WE), i.e. periventricular and mamillary body lesions (cases 12, 13, 15). In case 14 only subtle mamillary body changes were evident although periventricular lesions were present. Case 15 also had hippocampal sclerosis. These patients have been classified as WKS cases (Table 1).

Alcoholics with WE consumed greater than 80 g of absolute alcohol per day for the majority of their adult life (range = 80-400 g ethanol/day, median consumption approximately 200 g) and had clinical evidence of thiamin deficiency. These patients were independent in their daily living activities and had no documentation of permanent memory dysfunction. Three patients had neuropsychological assessment (cases 8, 9, 11). Cases 7 and 10 died during the acute episode of WE. Thiamin deficiency was confirmed pathologically (WE), i.e. periventricular and mamillary body lesions (cases 7, 8, 9, 10, 11). These patients have been classified as WE cases (Table 1).

Non-alcoholic controls consumed less than 20 g of absolute alcohol per day, although most patients consumed no alcohol (cases 1, 2, 3, 4, 5; Table 1). These patients had no evidence of thiamin deficiency or memory impairment.

One chronic alcoholic (consumed 120 g/day) with no evidence of WE or KP (case 6; Table 1), thiamin deficiency or memory impairment was also studied.

Material Preparation

Brains were removed at autopsy and the weight and volume were determined prior to fixation by immersion in 15% buffered formalin for 2 weeks. Brain weight and volume were re-measured after fixation. The brainstem was removed at the level of the superior colliculus and sectioned transversely at 5 mm intervals after detachment of the cerebellum. The cerebrum was embedded in 3% agarose and cut into 3 mm coronal slices using a rotary meat slicer. This allowed detailed macroscopic examination of cases. Standardized sections were taken for histological examination to identify WKS cases and to exclude other disorders such as Alzheimer’s disease.

Brainstem slices were cryoprotected in 30% sucrose in 0.1 M Tris-HCl buffer, pH 7.4, for 2-4 days and cut at 50 µm on a Leitz freezing microtome. Every fifteenth section was stained using immunohistochemical techniques to locate serotonin-synthesizing neurons using a monoclonal antibody (anti-PH8) which recognizes tryptophan hydroxylase in formalin-fixed human
material (19, 20). Subsequent sections were stained using buffered cresyl violet (0.5% in acetic buffer, pH 5.3) in order to establish total neuron counts. These sections were also used to quantify locus coeruleus neurons (17). Routine stains (hematoxylin and eosin, luxol fast blue and modified Bielschowsky silver stain counterstained with Congo red) were carried out for the identification of other pathological abnormalities.

Immunohistochemistry

Tissue sections were pretreated with 3% hydrogen peroxide in 50% ethanol to block endogenous peroxidase activity and were preincubated in 10% normal horse serum in Tris-HCl buffer, pH 7.4. The sections were incubated in mouse anti-PH8 (diluted 1:2,000), followed by washing in Tris-HCl buffer. PH8 labeling was localized using the avidin-biotin peroxidase (ABC) technique by incubation with biotinylated anti-mouse IgG (1:100) followed by ABC (1:200). Finally, the peroxidase complex was visualized with 0.05% diaminobenzidine hydrochloride and 0.01% hydrogen peroxide in Tris-HCl buffer for 10 minutes. Sections were washed, mounted onto gelatinized slides, dehydrated, cleared and mounted in DPX. Specificity of the immunohistochemical reaction was tested by substitution of the primary antibody with normal horse serum. No peroxidase reaction was observed in these control sections.

The PH8 antibody is a monoclonal antibody to phenylalanine hydroxylase (extracted from monkey liver) which recognizes the enzyme’s active site for amino acids and has been shown to bind to purified tyrosine hydroxylase and tryptophan hydroxylase (21, 22). In human tissue, the antigenic site for tyrosine hydroxylase is masked by formalin fixation (19, 20). Thus, the immunohistochemical reaction is specific for tryptophan hydroxylase as it can be abolished by preabsorption with the antigen (and not related enzymes) and there is no phenylalanine hydroxylase in the brain (19). Therefore, PH8-positive structures contain the serotonin-synthesizing enzyme and, consequently, serotonin (19).

Although both neuronal cell bodies and fibers were stained with the PH8 antibody, only cell bodies and proximal dendrites were consistently stained regardless of the pregonal state and postmortem delay. In control patients with a postmortem delay of less than 14 hours, all structures were labeled with the PH8 antibody. In patients with longer postmortem delays, there was a reduction in the numbers of smaller fibers visualized, which is consistent with previous reports on the degradation of pre-synaptic terminals (23). In addition, the staining of fine dendrites was variable. The analysis was therefore restricted to quantifying the PH8-positive neurons only, which under our experimental conditions appear to stain consistently in the postmortem time frame (see 20). Thus, neuronal cell bodies with peroxidase reaction product within the cytoplasm were selected for quantitation.

Analysis

Using the midbrain–pontine junction as a reference point, the number of PH8-positive neurons in the serotonergic nuclei were compared quantitatively at equivalent levels. Neurons were counted by eye at 100× magnification with the aid of an eyepiece graticule in a high quality binocular microscope (Olympus BHM2). The number of neurons per section for each region in each case was entered into a spreadsheet program (Microsoft Excel) on an Apple Macintosh personal computer for further calculations and graphic display. The total number of serotonergic neurons in each nucleus was estimated for each case by multiplying the total numbers of neurons counted by 15 (the number of sections in the series). No mathematical corrections were made for counting cell fragments for two reasons: to compare neuronal counts of these regions with those previously published (11, 12, 16, 24) and because recent publications suggest that such procedures only partially correct for over-counting rather than improve accuracy (25). There were no statistical differences (Student’s t-test) between counts made by two investigators. In all cases with very low cell numbers, the immunohistochemical protocol was repeated on an adjacent series of sections and consistent results were obtained. The effects of sex, diagnosis and cause of death on the total number of neurons were analyzed using the computer program SuperANOVA (Abacus) which calculated one-, two- and three-way analyses of variance with protected t-tests. Only the diagnosis had a significant effect on the total number of neurons (see Results). The effects of age, brain weight and postmortem delay on the total number of neurons were analyzed using the computer program StatView (Abacus) which calculated correlations and simple and multiple linear regressions. There were no significant correlations or regressions for group data or combined data. The lack of correlation between the number of PH8-positive cells and postmortem delay indicates that the tryptophan hydroxylase within the cell body is resistant to autolysis within this postmortem interval.

RESULTS

Serotonergic neurons in the brainstem can be differentiated into two groups according to their principal target. The midbrain and pontine groups project rostrally to the forebrain while the medullary groups project caudally (26). The midbrain and pontine groups can be further subdivided morphologically into the dorsal and median raphe nuclei. Dorsal raphe axons are fine and varicose, while median raphe axons are thick and non-varicose and terminate on specific target cells (26). Both groups of fibers are mixed in the medial forebrain and are distributed to the telencephalon and diencephalon. Therefore, serotoninergic neurons in the midbrain (dorsal raphe), pons (median raphe) and medulla were considered separately.

Midbrain Pathology

Consistent pathological changes were seen in several midbrain regions in all alcoholic cases. Vascular pathology was found in periaqueductal areas (Fig. 1A), ranging from petechial hemorrhages often involving the third and fourth cranial nuclei (case 7) to microhemorrhages involving the medial lemniscus and dorsal raphe nucleus (case 9). Gliosis and increased vascularity were observed in the inferior colliculi in most cases (Fig. 1B, C). Neuronal loss and gliosis were seen in the trochlear nucleus in cases 8, 9, 11, 12.

The Serotonergic Dorsal Raphe Nucleus: The dorsal raphe is a well-defined heterogeneous group of neurons...
Fig. 1. Photomicrographs of brainstem pathology in WE and WKS patients compared to controls. A. Many patients with WE and WKS had damage to periaqueductal regions. Examples of petechial hemorrhages (large arrows), endothelial proliferation (small arrows), and a larger hemorrhage with some artificial tearing (star) can be seen in this hematoxylin and eosin-stained section from case 7. Aq = aqueduct. B & C. Heavy glial infiltrate was commonly seen in the inferior colliculi of alcoholic WE and WKS patients. The right inferior colliculi of case 15 (B) and case 3 (C) are shown for comparison. Note the aggregation of glial nuclei throughout the deep layers of the colliculus in case 15 (darker region) compared to the same region in case 3. Scale in C applies to both photomicrographs of cresyl violet-stained sections. D & E. Bilateral neuronal loss of the dorsomedial segment of the principal inferior olivary nucleus was also a common feature in alcoholic WE and WKS patients. Photomicrographs of this region at mid-olivary levels from case 15 (D) and case 4 (E) are shown for comparison. Loss of neurons and increased gliosis in D are indicated by arrows. Remaining neurons were often hyperchromatic (compare olivary neurons in D and E). Scale in D applies to both photomicrographs of cresyl violet-stained sections.

occupying the midbrain and pontine central gray matter. It contains the largest population of serotonergic neurons in the brainstem (12). The PH8-immunoreactive neurons visualized in the control cases in the present study were similar in morphology and distribution to those previously described (12). In contrast, there was a marked reduction (averaging 60%; Table 2) in the number of serotonergic neurons in the dorsal raphe nucleus in all alcoholic cases (Fig. 2). Neuronal counts along the rostrocaudal extent of the nucleus revealed that the reduction

Fig. 2. Photomicrographs of PH8-immunoreactive (A, C, E, G) and cresyl violet-stained (B, D, F, H) sections of the dorsal raphe nucleus from control case 2 (A, B), alcoholic case 6 (C, D), alcoholic WE case 9 (E, F), and alcoholic WKS case 13 (G, H). The number of serotonin-synthesizing and Nissl-stained neurons was reduced in all alcoholics. There was also a reduction in the visualization of serotonergic fibers in alcoholic cases. Dotted line represents the midline, 4 = trochlear nucleus, Aq = aqueduct. Scale in H applies to all photomicrographs.
Dorsal Raphe Nucleus
chronic alcoholic

Graphs of the bilateral distribution of PH8-immunoreactive neurons in serial 50 μm sections throughout the dorsal raphe nucleus. Comparisons are made between controls and a single alcoholic without WE or WKS, controls and alcoholic WE patients, and controls and alcoholic WKS patients. Bars represent the standard deviation of the control mean. All alcohols had significantly reduced numbers of neurons at most levels through the dorsal raphe nucleus.

was found throughout the nucleus, with no evidence of focal loss (Fig. 3). Statistical analysis of the distributions and total neuronal numbers revealed that the neuronal loss was significant in all alcohols compared with controls (F\(_{3,14} = 27.0, p = 0.0001\)) and no significant differences were found between any alcoholic group (protected t-test, p > 0.05). The loss of PH8-positive neurons was reflected by an overall loss of Nissl-stained neurons with the proportion containing PH8 immunoreactivity remaining constant in alcoholic cases compared with controls (60–65% of Nissl-stained neurons were immunoreactive). This provides evidence of neuronal loss rather than a loss of immunoreactivity or a reduced amount of tryptophan hydroxylase within the neurons.

Pontine Pathology

The median raphe nucleus in all alcoholic cases was gliotic. Increased vascularity was also a feature in both the midline and paramedian raphe. Petechial hemorrhages were seen in the median raphe nucleus in cases 7 and 10.

The Serotonergic Median Raphe Nucleus: The median raphe lies ventral to the pontine part of the dorsal raphe nucleus extending some 12–14 mm caudally from the decussation of the superior cerebellar peduncles (11). The median raphe nucleus in our controls had two distinct regions as previously described (11): a serotonergic-rich midline zone bordered by a more diffuse neuronal population in the paramedian zone. There was a marked reduction in the number of serotonergic neurons in these regions in alcoholic cases (Fig. 4). Quantitation along the rostrocaudal extent of the median raphe nucleus revealed that this reduction (averaging 65%; Table 2) was greatest in the regions containing the highest numbers of serotonergic neurons in controls (Fig. 5). The neuronal loss was significant in all the alcoholic groups when compared with controls (F\(_{3,10} = 19.2, p = 0.0002\)). There was no significant difference between any alcoholic group (protected t-test, p > 0.05). There was a greater loss of serotonergic neurons in the midline compared with the paramedian region (Table 2) with the ratio of serotonergic neurons to the total number seen with cresyl violet remaining constant between the groups, indicating neuronal loss rather than a loss of immunoreactivity. Despite this, the ratio of midline to paramedian serotonergic neurons did not differ significantly between any group (approximately 65%).

The Serotonergic B9 Group: The B9 group of neurons lies ventrolateral to the median raphe and occupies the supracalaminiscal region (11). The morphology and distribution of serotonergic neurons in controls was similar to that previously described (11). In the alcoholic cases, there was an overall reduction (approximately 75%; Table 2) in the number of serotonergic neurons with no obvious focal loss (Fig. 5). This neuronal loss was significant in all alcoholic groups (F\(_{3,10} = 40.0, p = 0.0001\); protected t-test, p > 0.05).

Serotonergic Neurons in the Pontine Reticular Formation: In the reticular formation of the pons serotonergic neurons are located lateral to the median raphe, forming a diffuse network (11). As for the B9 group, the reticular formation showed no focal neuronal loss (Fig. 5), yet there
TABLE 2
Statistical Analysis of Neurons

<table>
<thead>
<tr>
<th>Region</th>
<th>Nuclei</th>
<th>Control</th>
<th>Alcoholic</th>
<th>%</th>
<th>WE</th>
<th>%</th>
<th>WKS</th>
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<tr>
<td>Midbrain</td>
<td>DR</td>
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<td>41,790</td>
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<td>35,415</td>
<td>10,180</td>
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<td></td>
<td>(63 ± 16)</td>
<td>(100)</td>
<td>(69 ± 11)</td>
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<td>(100 ± 12)</td>
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<td>(63 ± 12)</td>
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<td>18,970</td>
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<td>(100 ± 17)</td>
<td>(81)</td>
<td>(100 ± 12)</td>
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<td>(100 ± 8)</td>
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<td>total MnR</td>
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<td></td>
<td>B9</td>
<td>27,495</td>
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<td>92,160</td>
<td>23,170</td>
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<td>103,685</td>
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The estimated number of tryptophan hydroxylase-immunoreactive neurons (mean ± SD) and the percentage reduction (%) in alcoholics by diagnostic group in each analyzed region. The proportion of tryptophan hydroxylase-immunoreactive neurons within the dorsal and midline median raphe nuclei is given in parentheses (mean ± SD).

WE = alcoholic Wernicke's encephalopathy, WKS = alcoholic Wernicke–Korsakoff syndrome, DR = dorsal raphe nucleus, MnR = median raphe nucleus, RF = reticular formation.

was a significant reduction (approximately 65%; Table 2) in neuronal numbers throughout the nuclei in all alcoholic cases compared with controls (F3,10 = 28.8, p = 0.0001; protected r-test, p > 0.05).

Medullary Pathology

Gliosis and neuronal loss were seen in the dorsomedial regions of the inferior olives in cases 7, 8, 13. This varied in both extent and severity (Fig. 1D, E). Increased vascularity and gliosis were consistent findings in the floor of the fourth ventricle in all alcoholics and were seen in the dorsal vagal, hypoglossal and solitary nuclei in several alcoholic cases.

Serotonergic Midline Regions: There are three separate groups of midline serotonergic neurons in the medulla oblongata: the rostral raphe magnus which spreads laterally over the dorsal pole of the inferior olive, the caudal raphe obscurus, and the raphe pallidus located ventral to the pyramids throughout the entire length of the medulla (24). A similar distribution of serotonergic neurons was seen in our control cases. There was a marked reduction in the number of midline serotonergic neurons in all alcoholic cases (averaging 78%; Table 2) which was most severe in the raphe pallidus and obscurus nuclei (Figs. 6, 7). The neuronal loss was significant in all the alcoholic groups when compared to controls (F3,10 = 83.7, p = 0.0001). There was no significant difference between any alcoholic groups (protected r-test, p > 0.05).

Serotonergic Neurons in the Lateral Reticular Formation: The lateral reticular formation of the medulla oblongata contains a large population of serotonergic neurons in rostral regions (24). The analysis of this region showed a substantial loss (averaging 83%; Table 2) of serotonergic neurons throughout the medulla (Fig. 7), although in some cases of WE the most rostral regions were spared. This significant loss (F3,10 = 48.9, p = 0.0001) in all alcoholics (protected r-test, p > 0.05) was proportionally greater than the loss seen in the midline raphe regions (Table 2).

DISCUSSION

The present study documents the pathology found in the raphe nuclei of chronic alcoholics. The results show that all of the alcoholic cases analyzed had significantly reduced numbers of tryptophan hydroxylase-immunoreactive neurons in all regions of the brainstem compared to controls, despite differences in the clinical and neuropathological presentation of these alcoholics. In addition, the results suggest that serotonergic neurons degenerate and die rather than just lose their capacity to produce serotonin, since equivalent cell loss and gliosis were seen in Nissl-stained preparations. Because this loss was found in all the alcoholic cases regardless of clinical complications, we suggest that it is prolonged chronic alcohol abuse that affects the serotonergic neuronal population of the brain.

Serotonergic neurons in caudal brainstem regions were more affected than those located rostrally. In addition, the largest reductions were found in the serotonergic neurons located in the reticular formation. Where different cell types could be analyzed (only 63% of neurons in the dorsal raphe nucleus are serotonergic), there was a reduction in all cell types as indicated by the constant proportion of serotonergic neurons. Thus, although serotonergic neurons are dramatically affected, other neuronal populations in the raphe regions are also reduced. Such
neurons are thought to be interneurons related to the internal functioning of these nuclei (27).

Comparison Between Alcoholic Groups

The alcoholic cases analyzed all had extensive histories of chronic alcohol consumption (>80 g/day for >20 years) and could be divided into three groups: alcoholics with WE, alcoholics with WE and KP (WKS), and a single alcoholic without the symptoms of WE or KP. All alcoholics with WE had documented evidence of thiamin deficiency, with the majority having all three cardinal signs (acute confusion, eye disorders, and ataxia). In addition, all alcoholic WE cases had mamillary body lesions.
The single chronic alcoholic without clinical or pathological evidence of thiamin deficiency had a long history of alcohol abuse (80–100 g/day), but had maintained an adequate diet. He died of cardiac arrest and, although his drinking caused some personal conflicts in his family relationships, neither his wife or employer had noticed social difficulties. In contrast, all alcoholics with KP had been institutionalized because of their mental dysfunction. Despite these different clinical presentations, the loss of serotonergic neurons within the brainstem of these alcoholic cases was similar in both extent and topography. This indicates that serotonergic neurons are particularly susceptible to degeneration after chronic exposure to alcohol. To our knowledge there are no other regions of the brain where such consistent damage has been documented in alcoholics, particularly in patients without WE (18).

Our results contradict some observations made on two alcoholic patients with KP (8). Single Nissl-stained sections through the dorsal raphe nucleus were analyzed and no neuronal loss was found in these alcoholics when compared with five age-matched controls (8). It is difficult to reconcile this with the data in the present study, particularly when noting the number of neurons counted per section (mean of 36.8 ± 4.6 neurons/16 μm section in controls [8]). In the control cases in the present over 1,000 neurons/50 μm section were found in the dorsal raphe nucleus throughout the majority of the midbrain while approximately 200 neurons/50 μm section were seen bilaterally in the pons. The topography of the area sampled by Mayes and colleagues (8) is not depicted nor explained in the text and the references cited for the delineation of the dorsal raphe nucleus are not specific for that nucleus. Examination of these references suggests that the dorsal tegmental nucleus in the pontine central gray matter was sampled (28) instead of the dorsal raphe nucleus. The dorsal tegmental nucleus does not contain serotonergic neurons (26) and may therefore be unaffected in such
cases. In the present study the overall sampling regime for quantitation, the larger number of cases, and the specific staining regime for tryptophan hydroxylase strongly suggest that serotonergic neurons are destroyed by chronic alcohol exposure.

The biochemical evidence of significantly reduced levels of serotonin and its metabolites in the cerebrospinal fluid of alcoholics compared with controls corroborates the pathological evidence of serotonergic disruption in chronic alcoholics (1–3). Such testing in alcoholic patients is problematic because of differences between the transient and chronic effects of alcohol on the metabolism of serotonin (29). After a single dose of alcohol, serotonin is increased transiently but decreased in the longer term. Such a metabolic profile is thought to produce a dependence, where alcohol is repeatedly sought to compensate for the alcohol-induced depletion in serotonin (29). It is of interest to note that selective destruction of serotonergic neurons in rats with 5,6-dihydroxytryptamine increases ethanol intake (30, 31) and that alcohol-prefering rats have lower levels of both serotonin and its metabolites (32).

Comparison with other Neurodegenerative Diseases

The loss of serotonergic neurons has been previously described in both Parkinson's and Alzheimer's diseases (16, 33–35). Only the serotonergic neurons within the midline median raphe nucleus and midline medullary raphe are affected in patients with idiopathic Parkinson's disease (35); substantial neuronal loss is seen in the pons while intracytoplasmic inclusions are common in the medulla oblongata. This selective loss in a single region contrasts markedly with the loss of PH8-positive neurons throughout the brainstem of chronic alcoholics. The pattern of serotonergic cell loss in patients with Alzheimer's disease is dependent on their clinical symptoms (16); patients with no other neurological complications have substantial serotonergic cell loss and pathology while other cases appear to be unaffected. This differential loss in patients with Alzheimer's disease also contrasts markedly with the overall loss of serotonergic neurons in all chronic alcoholics regardless of their clinical symptoms.

These comparisons suggest that brainstem serotonergic neurons are vulnerable to a variety of degenerative changes in a selective and distinctive manner. The overall loss of serotonergic neurons throughout the brainstem of alcoholics has not been previously described in other neurodegenerative disorders. This adds weight to the idea that serotonergic neurons are particularly susceptible to degeneration after chronic exposure to alcohol.

Significance of Raphe Lesions

All serotonergic nuclei were analyzed in the present study. The dorsal and median raphe nuclei contain the highest concentrations of serotonin-synthesizing neurons...
in humans (12) and provide the majority of the serotonin innervation to the forebrain (26). These nuclei, together with the pontine reticular formation, are thought to affect hippocampal slow wave activity (36, 37), cortical sensory processing (38, 39), as well as striatal motor functions (36, 40). The loss of the ascending serotonin system is likely to affect the neural regulation of consciousness, as has been previously suggested in alcoholics (1, 41). In contrast, medullary serotonergic neurons innervate diencephalic, brainstem, cerebellar, and spinal regions (27) and are thought to influence somatomotor and autonomic functions, particularly pain modulation (42),
cardiorespiratory integration (42–46), and motor reflex sensitivity (47). It is interesting to note that desensitization to pain, cardiorespiratory problems, and decreased reflexes are common complications of chronic alcoholism. Many of these symptoms have been attributed to the common peripheral and autonomic neuropathies found in alcoholic patients (18). Since the medullary serotonergic system is nearly completely abolished in such cases, central neural control of these functions is also likely to be severely impaired and may significantly contribute to these complicating symptoms.

Several studies have suggested that disruption of the ascending serotonergic pathways contributes to the memory impairment of KP (1, 48). The present study would not support this contention, because the loss of serotonergic neurons was not more severe in patients with KP. However, unlike other alcoholics, alcoholics with KP have been shown to have substantially reduced numbers of cholinergic neurons in the basal forebrain (49). Thus, two major cortical influences would be substantially impaired in these patients. Recent animal studies have shown that the cortical release of acetylcholine is regulated by serotonin (50) and that disruption of serotonergic pathways potentiates cortical acetylcholine release which changes neocortical electrical activity (51), thus affecting the animal’s mood and activity (50, 51). It is therefore possible that the loss of the serotonergic amelioration for a compromised cholinergic system in patients with KP may combine to produce amnesia, although the reduction in cholinergic neurotransmission has itself been postulated as the precipitating event (2, 4, 49). Further studies are necessary to clarify these issues. It has been suggested that disruption of the ascending noradrenergic system also contributes to the amnesia of KP (2). We have previously shown that the noradrenergic locus ceruleus is not significantly affected in any of these alcoholistic cases (17), suggesting that its contribution to the symptoms of KP is likely to be secondary to other major pathologies.

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