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Hippocampal Cell Death following Ischemia: Effects of Brain Temperature and Anesthesia

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The effect of brain temperature and anesthesia on ischemic neuronal damage was studied in the hippocampal formation using the four vessel occlusion model in awake and anesthetized rats. Neuronal damage was assessed by immunocytochemistry and silver impregnation of tissue sections. The degree of ischemia was monitored by recording spontaneous and evoked electrical activity from the hippocampus and dentate gyrus in all animals. In addition, the hippocampal temperature and oxygen tension were also recorded using a chamber-type thin-film microelectrode in the anesthetized animals. Fifteen minutes ischemia in the awake animals caused greater neuronal damage and mortality of animals than 30 min ischemia in anesthetized rats. The temperature of the brain was found to drop by 4–6°C during complete forebrain ischemia in the latter group. Neuronal damage was observed infrequently in the hippocampus of these animals. When the brain temperature was kept constant at the preischemic level during 30 min occlusion, all animals died within a day, while after 15 min occlusion the majority showed an almost complete degeneration of CA1 pyramidal cells and hilar somatostatin immunoreactive neurons. Following 15 min ischemia, the awake animals showed a similar cell loss in the CA1 region and the hilus. It is concluded that, in the anesthetized animals prepared for acute recording, the decreased temperature of the brain during ischemia is a major factor in protecting neurons from damage, but that Equithesin anesthesia also has a significant protective effect. Consistent ischemic degeneration occurs in awake animals by four vessel occlusion, if the brain temperature is controlled and the completeness of ischemia is monitored by recording spontaneous and evoked electrical activity with chronic electrodes.

INTRODUCTION

The hippocampal formation is the most frequently examined region in studies of the mechanism of ischemic cell death and selective neuronal vulnerability. Ischemic degeneration in this area shows a very characteristic pattern and time course (10, 19–24, 35, 36, 39, 43). However, experiments, testing the protective effects of certain substances or surgical interventions, which require quantitative analysis of cell loss, are hampered by the great variability in the histopathological outcome of the ischemic insult. Variability occurs even in the same animal model, when physiological variables are kept under strict control (4, 10, 14, 34–36, 39, 40, 43). Substantial variation was observed in cell death among animals of the same group showing similar changes in physiological parameters in both awake (8) and anesthetized animals (13, 40) using the four vessel occlusion model of Pulsinelli and Brierley (34). Furthermore, there was also a great difference in cell death and survival between the anesthetized and awake groups. Different anesthetics, especially barbiturates (17, 42, 26), have been reported to have a protective effect on nerve cells to ischemic damage, therefore anesthesia could be a factor accounting for the observed variability. Another factor, which was recently shown to be protective as well as a variable in ischemia, is the decrease of brain temperature (6). Support of this notion was given by Okada *et al.* (30), who demonstrated that hypothermia has a powerful protective effect on the viability of neurons after long periods of anoxia in the *in vitro* hippocampal slice preparation. In the present study we analyzed the contribution of brain temperature and anesthesia to the variability in the histopathological outcome of ischemia induced by four vessel occlusion. The pattern and degree of neuronal damage in the hippocampal formation were evaluated by silver impregnation (10, 15) and by immunostaining for somatostatin (20).

MATERIALS AND METHODS

Animals and Surgical Procedures

The experiments were carried out on 49 adult Wistar rats (body weight 250–350 g, Charles River) and 17

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TABLE 1

Group no.	Anesthesia	Ischemia duration (min)	Brain temperature during ischemia	Number of animals		
				Total	Died	Oligemia
1	Awake	30	Not measured	6	6	0
2	Awake	15	Not measured	11	4	2
3	Halothane	30	5–6°C fall	11	2	2
4	Equithesin	30	5–6°C fall	17	2	2
5	Equithesin	30	Kept constant	7	6	1
6	Equithesin	15	Kept constant	14	3	2

Note. The numbers under "Died" refer to the number of animals which died during or within a day after occlusion. No histological data have been obtained from these animals. The numbers under "Oligemia" indicate the number of animals excluded from the study because of incomplete ischemia (i.e., oligemia) as judged from the EEG record (40).

Sprague–Dawley rats (250–350 g). They were divided into six groups (Table 1), and ischemia was induced by four vessel occlusion (4VO) according to Pulsinelli and Brierley (34) in each group.

Preparation of animals in groups 1 and 2. Details of methods and the physiological data have been published in Buzsáki *et al.* (8). The animals were deeply anesthetized with a mixture (4 ml/kg) of ketamine (25 mg/ml), xylazine (1.3 mg/ml), and acepromazine (0.25 mg/ml) and pairs of Polyimide-insulated 125-μg stainless-steel electrodes with 0.5 mm vertical tip separation were placed bilaterally in the angular bundles (stereotaxic coordinates: AP, −7.0 mm from bregma; L, 4.5 mm from midline; V, 3.5 mm below dura; bregma and lambda in the same horizontal plane) to stimulate the perforant pathway. Two pairs of recording electrodes with 1 mm vertical tip separation were placed bilaterally into the hippocampus, aimed at the hilus and CA1 region (AP, −4 mm; L, 2.6 mm; and V, approx 2.9, deep electrode). Following baseline recordings (EEG power spectrum analysis during theta and non-theta behaviors, evoked field potential characteristics, see details in Ref. (8)) the rats were reanesthetized and the vertebral arteries were bilaterally cauterized and split under direct visual control (44). Both carotid arteries were then exposed, and a loop, made from a 0.4-mm-diameter atraumatic silicon tube, was formed around the arteries. When the wound was closed, the end of the silicon tubes protruded between the edges of the skin (11). One day later, in the ischemia session, the rats were gently restrained and the loops of silicon tubes were tightened (for 30 min in group 1 and for 15 min in group 2). Both spontaneous and evoked (0.1 ms at 0.1 Hz, 10–50 V stimulus) hippocampal electrical activity were recorded before, during, and 2 h after the ischemic period. All six animals from the first group and four animals from the second group died soon after ischemia or from respiratory failure within 3 min of occlusion. A further two animals of the second group were excluded from the study because the EEG did not become completely flat and the amplitude of the evoked potential remained unchanged or was only slightly reduced. Recordings of spontaneous EEG and evoked potentials were also done at 3–5 h and 12–14 h after ischemia and then once every day up to 8 days.

Preparation of animals in groups 3–6. Details of methods and physiological results have been published in Freund *et al.* (13). The animals were anesthetized with Equithesin (chlornembutal, 3 ml/kg, see Ref. (9)) for surgery, and the vertebral arteries were cauterized bilaterally as described above. One day later the animals were anesthetized again by 1–2% halothane vaporized in air and administered via a nose mask (group 3) or by Equithesin (groups 4–6), and the head of the animals was fixed in a stereotaxic frame throughout the recording session. Then carotid arteries were prepared as described above for groups 1 and 2. Anesthesia was continued in each of these groups during the ischemic period and also after ischemia until the end of the recording session. Holes (approx 2 mm diameter) were drilled above the right hippocampus and angular bundle. A bipolar stimulating electrode was placed into the angular bundle at the same coordinates as for groups 1 and 2. A miniaturized thin-film electrode probe and recording device (Ottosensors Corporation, Cleveland, OH, see Refs. (32, 33)) were used to record simultaneous potential (POT), oxygen tension (O_2), and temperature (T) from the hippocampus. The serial positions of the sensors (two of each) starting from the tip of the probe were POT1, $O_2$1, T1, POT2, $O_2$2, and T2, at 300 μm from each other. Thus, the distance between the two potential sensors was 900 μm. The probe was fixed to the arm of the stereotaxic apparatus and lowered into the hippocampus at the following coordinates: AP, −3.2 mm (from the bregma); L, 2.5 mm; and V, approx 3 mm (POT1). The lower potential sensor was aimed at the hilus of the dentate gyrus, and the upper at the CA1 region. Accurate positioning of the probe has been confirmed by stimulating the perforant path with single pulses (0.2 ms, at 0.1 Hz, intensity: 10–50 V) and recording the field potentials characteristic of the different regions of the hippocampal formation (7). Following 30 min stable recording, the carotid arteries were occluded bilaterally by pulling the

silicon tubes placed around the arteries. The flattening of the EEG and the disappearance of the evoked potential were taken as evidence for complete forebrain ischemia. Body temperature was maintained at 37°C using a rectal temperature probe connected to a Homeothermic Blanket Control Unit (Harvard Apparatus). A microscope lamp, fixed about 5 cm above the head of the animals, was used to maintain the temperature of the brain in groups 5 and 6. The probe was isolated from direct heat radiation by a large sheat of aluminum foil placed between the probe and the light bulb. The light was switched on at the onset of carotid occlusion and its strength was changed gradually with a potentiometer as required for keeping the hippocampal temperature constant at the preischemic value (35–36°C). The temperature of the skull was also monitored with a thermometer and in most cases it was heated to about 37–39°C to achieve hippocampal temperatures between 35 and 36°C. In these experiments the room temperature was kept between 28 and 30°C. The duration of ischemia was 30 min in groups 3–5 and 15 min in group 6. Some animals (two in group 3, two in group 4, six in group 5, and three in group 6) died from respiratory failure during occlusion or the EEG did not recover after ischemia and the animal died within 12 h. Animals (two in group 3; two in group 4; one in group 5; and two in group 6) which did not have a flat EEG or in which the evoked potential did not disappear completely, indicating incomplete forebrain ischemia, were excluded from further studies.

Perfusion and processing of tissue sections. Six to 7 days after the ischemia session, the animals were deeply anesthetized and perfused through the heart, first with saline (1–2 min) followed for 30 min by a fixative containing 0.1% glutaraldehyde, 4% paraformaldehyde, and 0.2% picric acid in 0.1 *M* phosphate buffer (pH 7.4). Brains were removed from the skull and postfixed in the same fixative for 1–3 h. Blocks of the dorsal hippocampus and overlying cortex were dissected, immersed into 10 and 20% sucrose (in 0.1 *M* PB) until they sank, and freeze–thawed in liquid nitrogen before being sectioned on a Vibratome at 60 μm. Alternate sections were processed for silver impregnation and for immunocytochemistry.

Silver impregnation. The procedure of Gallyas *et al.* (15) was used: briefly, 2 × 5 min in the pretreating solution (2% NaOH and 2.5% NH_4OH), 10 min in the impregnating solution (0–0.8% NaOH, 2.5% NH_4OH, 0.5% $AgNO_3$), 3 × 5 min in washing solution (0.5% Na_2CO_3 and 0.01% NH_4NO_3 in 30% ethanol), 1 min in developing solution (0.4–0.6% formaldehyde and 0.01% citric acid in 10% ethanol, pH 5.0–5.5), and 3 × 10 min wash in 0.5% acetic acid. The sections were then mounted on gelatine-coated slides, dehydrated, and covered with neutral medium (XAM).

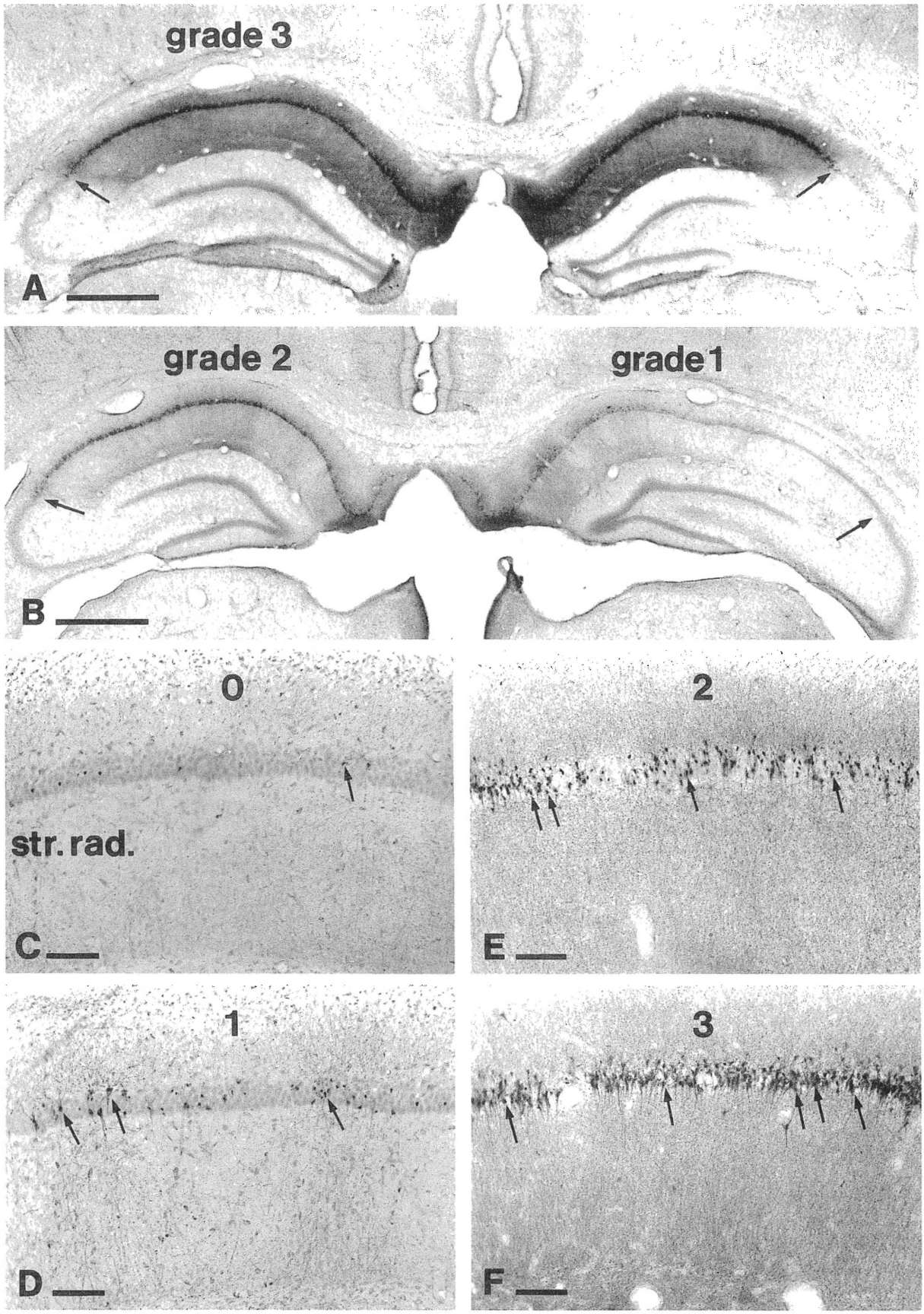
Immunocytochemistry. The steps were carried out at room temperature with constant agitation unless otherwise stated. The sections were extensively washed in 0.1 *M* sodium phosphate buffer (pH 7.4) and then in 0.05 *M* Tris-buffered saline (TBS, pH 7.4), then transferred into the blocking solution (20% normal rabbit serum, NRS). A monoclonal mouse antibody (Soma 8, see Ref. (5)) raised to cyclic somatostatin was used. The antibody recognizes the C-terminal portion of the peptide, in either cyclic or linear form. It was used in a dilution of 1 μg protein/ml TBS, for 24–48 h at 4°C. Rabbit anti-mouse IgG (Dakopatts), diluted 1:50 for 4–6 h, and monoclonal mouse PAP (Dakopatts), diluted 1:100 overnight at 4°C, were used in the subsequent steps. The sections were washed for 3 × 30 min between the antisera. All washes and antibody dilutions were done in TBS containing 1% NRS and 0.5% Triton X-100. Diaminobenzidine–tetra HCl (DAB, Sigma, 0.05% in Tris buffer) was used as a chromogen for the immunoperoxidase reaction, H_2O_2 (0.01% final concentration) was added after 25 min preincubation, and the sections were incubated in this solution for 6–8 min. The sections were washed and mounted on gelatine-coated slides, dehydrated, and covered in XAM neutral medium for light microscopy.

RESULTS

Induction and Monitoring of Hippocampal Ischemia

The electrophysiological observations in the awake and anesthetized animals have been described in detail elsewhere (8, 13). Briefly, the correct positioning of the electrodes was confirmed by recording field potentials evoked by stimulating the perforant path. When the recording electrode reached the hilus, a negative going population spike could be seen riding on a positive field EPSP. Before ischemia the awake animals exhibited the characteristic behavior-dependent EEG pattern, i.e., theta activity, or low voltage fast activity accompanied by sharp waves. In the anesthetized groups, theta activity was seen only under light halothane anesthesia when stroking the back of the animal. Following occlusion of the carotid arteries, both the spontaneous and evoked electrical activity disappeared within 1–2 min and the EEG remained isoelectric throughout the period of ischemia. Animals were excluded from further morphological studies if low amplitude EEG or evoked potentials were seen during ischemia (Table 1). The unanesthetized animals were unconscious and bilaterally unresponsive during carotid occlusion. The time required for the evoked potential and EEG to reappear varied between 4 and 25 min in animals of all groups except those in groups 1 and 5, where very little if any EEG or evoked response returned. All these animals died within 1 day.

In the anesthetized groups (groups 3–6) the local hippocampal temperature and oxygen tension were also recorded using the Ottosensors electrode probe (13, 32, 33). The hippocampal temperature was normally be-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tween 35.5 and 36.5°C before ischemia when the body temperature was kept constant at 37°C. Hippocampal temperature started to drop immediately after occlusion and reached half of the total fall within 5 min. The temperature could fall to as low as 29°C at the end of 30 min ischemia. The average drop of hippocampal temperature in groups 3 and 4 was 5–6°C. In groups 5 and 6 the local hippocampal temperature was kept constant throughout the ischemic period (see Materials and Methods). Upon release of the carotid arteries the temperature showed a sudden rise and a 0.5–1.5°C overshoot (up to 37.5°C) for 10–20 min in groups 3 and 4. In animal groups 5 and 6 the heating lamp was switched off at the same time when the carotid arteries were released, but an overshoot of 1–2°C (up to 38°C) was seen in these animals as well.

Epileptic seizures were not observed during the 6- to 7-day survival period, although the animals were not monitored during the night. The power of the EEG gradually decreased in the CA1 region in the animals of group 2 during the postischemic period (8). Recordings were not done during this period in the other groups.

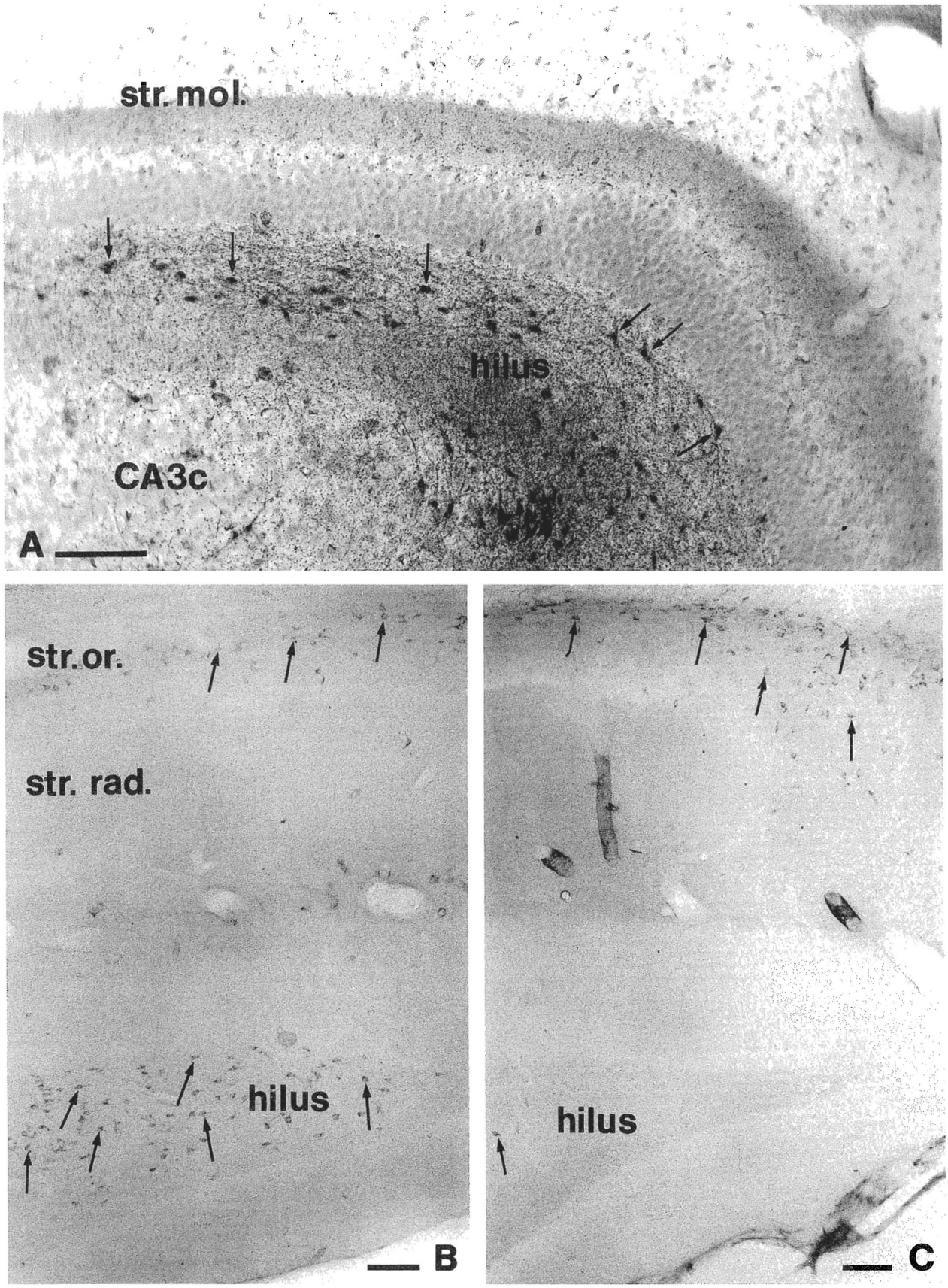
Histological Findings

In addition to the hippocampal formation, the neocortex, thalamus, neostriatum, and the substantia nigra were also analyzed from these animals and the results are described elsewhere (12). In the present study the histological analysis was restricted to the dorsal hippocampus, which in earlier studies showed a more consistent pattern of damage than the ventral regions, probably due to more residual circulation in the latter area (35, 36, 39). Alternate vibratome sections (60 μm) were processed for silver impregnation (15), a good indicator of irreversible ischemic neuronal damage (10), and for immunocytochemistry to study the loss of hilar somatostatin-containing neurons in the different models.

Histological data were not obtained from the animals in groups 1 and 5 since they all died within 1 day following ischemia. The mortality was highest in these groups, followed by the awake animals occluded for only 15 min (group 2), and then by the anesthetized constant temperature group with 15 min occlusion (group 6). These mortality data already indicate that both intraischemic brain temperature and anesthesia have an effect on the sensitivity of certain vital areas to ischemia.

Silver impregnation and semiquantitative analysis of neuronal damage. Neurons were considered as irreversibly damaged if they were shrunken and strongly argyrophilic. Some neurons, especially the so-called pyramidal basket cells in the dentate gyrus, and some other types of interneurons occasionally showed a different type of argyrophilia. They were not shrunken and silver deposits were confined to small discrete granules in their cell bodies and proximal dendrites. These neurons were not considered to undergo irreversible degeneration. Somata of intact neurons appeared dark yellow, if impregnation was performed at the optimal pH. A large number of degenerating (i.e., shrunken, argyrophilic) neurons were always seen in the CA1 region (Fig. 1) and in the hilus (Fig. 2) of the dentate gyrus in animals of groups 2 and 6 and in some hemispheres in animals of groups 3 and 4. The degree of CA1 damage was assessed in these and also in Nissl-stained or osmium-treated sections, and a score was determined for each hemisphere according to Block and Pulsinelli (2). Hemispheres were scored 0, normal; 1, up to 10%; 2, 10–50%; 3, more than 50%, of CA1 neurons irreversibly damaged (Fig. 1). The results are presented in Table 2. The greatest damage was seen in the awake animals, where the hippocampus in most hemispheres showed grade 3 degeneration. The average cell loss was somewhat lower in the Equithesin-anesthetized, constant temperature animals (group 6); nevertheless, the majority of hemispheres showed grade 2 or 3 damage. Less degeneration was seen in the anesthetized groups (3 and 4) in which the temperature of the brain was allowed to drop (usually 4–6°C) during occlusion. In the group anesthetized with Equithesin (group 4), only one animal showed grade 3 damage and another grade 2 while the rest (11 animals) appeared normal or mildly effected. The scores of the different groups were statistically compared (Table 2). The awake group differed significantly ($P < 0.001$) from groups 3 and 4, but not from the group with constant brain temperature ($P > 0.01$), whereas group 6 differed significantly ($P < 0.001$) from the other Equithesin-anesthetized group in which the temperature was allowed to drop (group 4). This indicates that lowered temperature during ischemia is a major factor in protecting neurons in the anesthetized animals, but also shows that Equithesin anesthesia provides additional protection. Halothane anesthesia is also protective (significant difference from awake animals: $P < 0.001$), but to a smaller extent. The absolute brain temperature and the relative drop during occlusion were similar in the halothane- and Equithesin-anesthetized groups.

FIG. 1. Different grades of neuronal damage are shown in silver-impregnated sections from animals exposed to 15 min ischemia under Equithesin anesthesia while brain temperature was kept between 35.5 and 36.5°C. The grading was determined according to Block and Pulsinelli (2), i.e., grade 3, more than 50% (A and F); grade 2, 10–50% (B and E); grade 1, up to 10% (B and D) of the pyramidal cells are irreversibly damaged in the CA1 region. Silver-impregnated sections cut from different levels of the dorsal hippocampus were used for this semiquantitative analysis. Summary of data are presented in Table 2 for six different groups, which are compared using the Mann–Whitney *U* test. Arrows in A and B label the CA1–CA3 border region. (C–F) Higher magnification light micrographs of the CA1 region from animals with different grades of degeneration (indicated at the top of each figure). Irreversibly damaged, argyrophilic pyramidal cells are labeled with arrows. Scales: A and B, 1 mm; C–F, 100 μm.


str. mol.
hilus
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Somatostatin immunoreactivity. The pattern of ischemic neuronal damage in the different groups of animals was studied by the analysis of somatostatin-immunoreactive (SS) neurons (Figs. 2B and 2C). The loss of SS neurons in the hilar area has been confirmed in all animals which showed, in adjacent sections, at least grade 1 or 2 damage of CA1 pyramidal cells. Animals with an intact CA1 region did not seem to have a loss of SS cells from the hilus, although quantitative analysis has not been performed. The density of SS-immunoreactive neurons in stratum oriens of the CA1-3 regions was not effected by ischemia, even in animals with massive destruction of the CA1 pyramidal cells (Figs. 2A and 2B). There was no apparent difference among the four groups of animals in the loss of hilar SS-immunoreactive neurons. The silver-impregnated sections showed that the majority of hilar neurons degenerated, thus they likely included other cell types, most notably the hilar mossy cells, in addition to the SS-immunoreactive neurons (Fig. 2A).

The other regions of the hippocampal formation were resistant to damage in all groups of animals. The only exception was a group of pyramidal cells at the tip of the CA3 region which extends deep into the hilus of the dentate gyrus (CA3c). These pyramidal cells were often damaged and appeared to be approximately in the same phase of degeneration as the CA1 pyramidal cells. In Fig. 2A only a small number of argyrophilic CA3c pyramidal cells have undergone degeneration, but occasionally the whole segment of CA3c extending into the hilus was damaged. Interneurons in stratum radiatum and oriens of the CA1-3 regions and interneurons in stratum moleculare and granule cells in the dentate gyrus appeared to be intact (Fig. 2A).

DISCUSSION

The four vessel occlusion method that was used in the present study produces a variable degree of ischemia in the rat brain (34, 39, 40). (A) The ischemia can be mild, and in these animals the EEG shows residual neuronal activity. (B) It can be severe in the forebrain, resulting in a flat cortical EEG. (C) In addition to the forebrain, it also can be severe in the brain stem, leading to respiratory failure. Animals from group B were selected for histological examination as in earlier studies (34, 39). However, in our experiments the spontaneous and evoked electrical activity, as well as the local oxygen tension and temperature (in the acute animals), were recorded from the same hippocampal areas where the histological analysis was carried out.

TABLE 2

Degree of Ischemic Damage in the CA1 Region of the Hippocampus as Determined by a Semiquantitative Analysis of Shrunken Argyrophilic Neurons in Silver-Impregnated Sections

Group	Mean	SEM (n)	Statistical comparison		
2	2.70	±0.21 (10)	Group 2		
3	1.29	±0.24 (14)	$P < 0.001$**	Group 3	
4	0.65	±0.18 (26)	$P < 0.001$**	$P < 0.05$*	Group 4
6	1.94	±0.25 (18)	$P < 0.05$*	$P < 0.05$*	$P < 0.001$**

Note. Grade 0, normal; grade 1, up to 10%; grade 2, 10–50%; grade 3, more than 50% of pyramidal cells irreversibly damaged. All animals in groups 1 and 5 died during or within a day after ischemia and were not analyzed histologically. The different groups (n = the number of hemispheres) are statistically compared using the nonparametric Mann–Whitney U test (one-tailed).

A substantial fall in brain temperature has been demonstrated when ischemia was induced in animals prepared for acute recordings under general anesthesia. This 4–6°C drop in temperature has a significant protective effect on the hippocampus, confirming earlier observations by Busto *et al.* (6). We also showed that Equithesin anesthesia provides an additional protection, probably due to its barbiturate component (17, 26, 42), whereas halothane is relatively ineffective in reducing ischemic cell death.

Technical considerations. The recording of spontaneous and evoked electrical activity with deep hippocampal electrodes is a sensitive way for monitoring the degree of ischemia. Both in anesthetized and in awake animals the spontaneous EEG and evoked potentials disappeared within 1–2 min, if ischemia was complete. Occasionally the EEG record showed residual activity in spite of a disappearance of evoked potentials, suggesting that this activity was volume conducted from other brain areas, but in the hippocampus the ischemia was complete. In the awake animals, brain temperature was not recorded, but the fall in temperature during occlusion is likely to be much smaller than in the acute animals with their scalp and skull open. Recording of brain temperature in the acute animals was also a good indicator of the exact onset and time course of reperfusion. The electrode probe was 0.5 mm wide and produced a cut when inserted into the hippocampus. Occasionally, groups of

FIG. 2. Degeneration of hilar neurons. (A) Silver-impregnated section from a rat in group 6. Note that almost all neurons in the infragranular region of the hilus as well as some pyramidal cells at the tip of the CA3c region are strongly argyrophilic (arrows). Axonal degeneration is seen within the hilus and in the proximal one-third of the molecular layer (str.mol.). (B, C) Sections immunostained for somatostatin from a control animal (B) and from an ischemic animal in group 6 (C). Note that in the normal animals the hilus and stratum oriens of the CA1–CA3 regions contain large numbers of somatostatin-immunoreactive neurons (e.g., arrows in B). After ischemia the hilar somatostatin neurons degenerate, whereas those in stratum oriens of the CA1–CA3 regions remain intact (arrows in C). Scales: A–C, 100 μm.

intact pyramidal cells were found near the electrode track, even if the rest of CA1 was completely degenerated. This could be explained by a protective effect of gliosis around the track or deafferentation. However, this apparent protection was limited to a 300- to 400-μm-long segment of stratum pyramidale distributed around the electrode and did not influence the grading of damage.

Silver impregnation (15) has been shown to be a very sensitive method for visualizing irreversibly damaged neurons after ischemia (10). This method reveals degenerating cells with their dendritic arbors and axons. As discussed by Crain *et al.* (10), the appearance and time course of development of argyrophilia in shrunken neurons correlate well with a stage of electron-dense degeneration, as seen in electron microscopic preparations, that is most likely to be irreversible (18, 23, 24, 31). The distribution of shrunken argyrophilic neurons in the postischemic brain found in the present study agrees closely with the pattern described using other sensitive stains (24, 31, 39, 43).

General pattern of vulnerability in the hippocampal formation. The most vulnerable neurons were found in the hilus and in the CA1 region as described in earlier studies. The majority of the damaged hilar cells are the somatostatin-containing neurons (20), but other hilar cell types (e.g., the mossy cells) are probably also among the degenerating neurons since in several animals there were hardly any intact neurons left in the hilar region (just below the granule cell layer). The tip of the CA3 region extending into the hilus (CA3c) was also frequently damaged, but other parts of CA3 were remarkably resistent. The CA1 region, especially its subicular end, was at least as vulnerable as the hilus since hemispheres showing hilar damage without CA1 cell death have not been found in any of the groups. The most resistent neurons were the granule cells, and interneurons in strata radiatum and oriens of CA1-3, and in stratum moleculare of the dentate gyrus (10, 24, 31, 38, 39, 43).

Effect of brain temperature and anesthesia. Our results confirm and extend those of Busto *et al.* (6) that hypothermia during ischemia has a powerful protective effect on neurons. This finding helps to explain several controversial results and the greater vulnerability of the hippocampus in awake animals implanted with chronic recording electrodes. The variability in intraischemic temperature could be a major reason for the great variability of ischemic damage found among identically treated individual animals, although there may be individual differences in the severity of ischemia as well (4, 14, 31, 39, 43). The protective effect of hypothermia on irreversible hypoxic damage of hippocampal neurons has also been demonstrated *in vitro* (30). Clinical (27, 29, 37, 41) and *in vivo* experimental data (1, 25, 28) are also available showing increased viability of the brain if temperature is lowered during ischemia or hypoxia. The exact mechanism of this powerful protective action of hypothermia is unknown. Possible explanations include decreased membrane failure (1), temperature-sensitive modulation of changes in ion channel, neurotransmitter receptor function, or in neurotransmitter synthesis and release (see Ref. (6)). The latter explanation is supported by a recent study of Busto *et al.* (6a), who demonstrated that hypothermia reduces the ischemia-induced release of glutamate.

Hypothermia during ischemia alone, however, cannot fully explain why anesthetized animals had substantially less degeneration than awake rats. The constant temperature group (group 6) with 15 min ischemia showed a somewhat lower average grade of damage than awake rats (difference significant at $P < 0.05$). These results suggest that anesthesia (Equithesin) may provide additional protection. This is supported by the finding that group 4 animals anesthetized with Equithesin showed significantly less damage than group 3 animals anesthetized with halothane ($P < 0.05$). The protection by Equithesin anesthesia may be due to its barbiturate component since barbiturates have been shown to decrease ischemic cell death (17, 26, 42). Barbiturates were even more effective in combination with hypothermia (16), but the mechanism of this combined effect is unknown. It is conceivable that the hypothermia-induced decrease in glutamate release (6a) coupled with another protective mechanism acting mostly on the postsynaptic side (e.g., by delaying ischemic depolarisation and decreasing calcium influx) would provide additive protection.

It is concluded that a 4–6°C fall of brain temperature during ischemia can provide a powerful protection of the brain from irreversible ischemic injury. Such a decrease in temperature during neurosurgery or cardiac arrest could be effectively applied without risking major functions of other systems. Our results also suggest that the intraischemic brain temperature should be monitored in studies of ischemic neuronal damage in all models, if reproducibility is a requirement.

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